The modulation of methamphetamine-induced behaviours by oxytocin in the nucleus accumbens core and subthalamic nucleus

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List of Abbreviations

µl	microlitre
µm	micrometre
ACN	acetonitrile
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	analysis of variance
AVP	arginine vasopressin
cAMP	cyclic adenosine monophosphate
cm	centimetre
CORT	corticosterone
CPP	conditioned place preference
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
DA	dopamine
DAT	dopamine transporter
DBS	deep brain stimulation
DSM-IV	Diagnostic and Statistical Manual Fourth Edition
g	gram
GABA	gamma-aminobutyric acid
GHB	ϒ-hydroxybutyrate
GPi	internal segment of the globus pallidus
H	hour
icv	intracerebroventricular
i.p.	intraperitoneal
ir	immunoreactive

IU	international units
IVSA	intravenous self-administration
MDMA	3,4-methylenediozymethampehtamine
METH	methamphetamine
min	minute
mg	milligram
ml	millilitre
mm	millimetre
mRNA	messenger ribonucleic acid
ng	nanogram
nl	nanolitre
nmol	nanomol
NAc	nucleus accumbens
OTR	oxytocin receptor
OXY	oxytocin
PBS	phosphate buffered saline
PFA	paraformaldehyde
PFC	prefrontal cortex
pg	picogram
pmol	picomol
PVN	paraventricular nucleus
S	second
s.c.	subcutaneous
SDR	steroid displacement reagent
SON	supraoptic nucleus
SNr	substantia nigra pars reticulata
STh	subthalamic nucleus

TFA	trifluoracetic acid
TPBS	tris phosphate buffered saline
TPBSm	tris phosphate buffered saline with merthiolate
VEH	vehicle
VTA	ventral tegmental area

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Statement of Originality and Ethical Approval

I, Sarah Jane Baracz, declare that that this submission is my own work and does not represent the work or view of others, except where acknowledged in the text. This thesis consists of three experimental chapters that have either been submitted or published in peer-reviewed journals as well as a general introduction, an additional experimental chapter, and discussion. The experimental manuscripts are submitted in journal format whilst the general introduction, chapter 5, and discussion follow APA formatting guidelines. No part of this thesis has been submitted for a higher degree to any other university or institution.

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Co-Author Declaration

We, the undersigned, acknowledge that this work represents that of Sarah Baracz, and where appropriate, information regarding co-author contribution is accurately provided.

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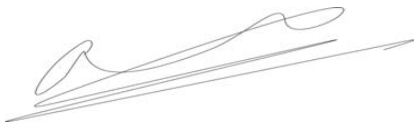

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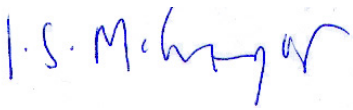

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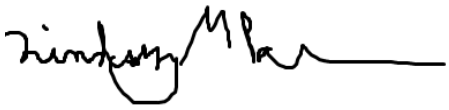

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Abstract

The psychostimulant methamphetamine (METH) is an addictive illicit drug, which is commonly abused on a global scale. Repeat administration of the drug is associated with a range of long-term adverse effects and effective pharmacotherapies for METH dependence are currently lacking. The neuropeptide oxytocin has been identified as a potential pharmacotherapeutic agent due to the ability of systemic administration of this peptide to modulate METH-related reward and METH-seeking behaviour. This modulation is thought to occur through the attenuation of dopamine activity and release. Recent findings identified the nucleus accumbens (NAc) core and subthalamic nucleus (STh) as key regions involved in oxytocin modulation of acute METH-related reward. However, little is known about the mechanisms by which oxytocin modulates METH-related behaviours, which in turn limits a thorough understanding of the implications of using oxytocin as a pharmacotherapy for METH dependence. Taken together, this thesis aimed to examine the ability of oxytocin to modulate METH-related reward and relapse to METH seeking within the NAc core and STh through the utilisation of animal models of reward and addiction, incorporating pharmacological, cellular, and biochemical investigation.

In the first experimental chapter of this thesis (Chapter 2), oxytocin modulation of dopamine-related reward in the STh was investigated using the conditioned place preference paradigm. Following a single conditioning session, male Sprague Dawley rats formed a place preference for the context paired with a microinjection of dopamine (100 nmol/side) into the STh (200 nl/side) and this preference was blocked by the co-administration of oxytocin (0.6 pmol/side). In addition, the inhibitory effect of oxytocin on dopamine place preference formation was reversed by the concurrent administration of desGly-NH_2d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (3 nmol/side), a selective oxytocin receptor (OTR) antagonist into the STh. These findings suggest that oxytocin modulated dopamine-related reward within the STh through the OTR.

Considering the detrimental impact that METH abuse has on the individual, the following two chapters examined the effect of exogenous oxytocin on METH reinforcement. More specifically, the ability of oxytocin to modulate relapse to METH-seeking behaviour when microinjected into either the NAc core (Chapter 3) or STh (Chapter 4) was examined using the drug-primed reinstatement model of intravenous drug self-administration. Chapter 3 determined in male Sprague Dawley rats that oxytocin (0.5 pmol, 1.5 pmol, 4.5 pmol/side) microinjected into the NAc core (500 nl/side) reduced METH-primed reinstatement (1 mg/kg, i.p.) and that the co-administration of the selective OTR antagonist used in Chapter 2 surprisingly had a non-specific effect on reinstatement to METH-seeking behaviour. When oxytocin was locally administered into the STh (200 nl/side; Chapter 4), the highest dose tested (3.6 pmol/side) decreased reinstatement to METH-seeking behaviour. Similar to the findings reported in Chapter 3, co-administration of the selective OTR antagonist did not specifically reverse the inhibitory effect of oxytocin on reinstatement to METH-seeking behaviour. The findings of Chapters 3 and 4 suggest that oxytocin mediated METH-primed reinstatement through the activation of receptors beyond the OTR.

In light of the findings from Chapters 3 and 4, and the lack of reporting on the role of the OTR in oxytocin attenuation of psychostimulant-induced behaviours, the final experimental chapter (Chapter 5) primarily investigated whether there are cellular changes to the endogenous oxytocin system in the NAc core and STh, as well as changes to oxytocin plasma levels following chronic METH intravenous self-administration (IVSA) and after behavioural extinction. Male Sprague Dawley rats that self-administered METH had higher oxytocin plasma levels, and decreased OTR-immunoreactive fibres in the NAc core than yoked saline rats. After behavioural extinction, oxytocin plasma levels remained elevated, OTR-immunoreactive fibre density increased in the STh that exceeded baseline yoked control levels, and a trend towards normalisation of OTR-immunoreactive fibre density to baseline-yoked levels was evident in the NAc core in rats that were previously experienced at METH IVSA compared to yoked controls. These findings demonstrate that the oxytocin system, both

centrally within the NAc core and STh, and peripherally through plasma measures, are dysregulated following METH abuse.

Chapter 6 discusses implications for the research findings of this thesis and future research directions. The results of this thesis show a direct modulatory role of oxytocin at the NAc core and STh on METH administration. These effects may incorporate the activity of oxytocin at receptors other than the OTR as previous studies have demonstrated interactions with the amino acids glutamate and gamma-aminobutyric acid (GABA) to mediate METH-related behaviours. Further, oxytocin is known to act through the V1a receptor of the structurally similar neuropeptide arginine vasopressin to modulate prosocial and autonomic effects, suggesting that V1a receptors may be involved in regulating psychostimulant abuse. As such, a more complex interplay incorporating dopamine, glutamate, GABA and vasopressin in oxytocin modulation of METH reward and abuse is discussed. Lastly, a regulated endogenous oxytocin system has been proposed to increase resilience to addiction. This may be through the regulation of the circuits impacted by drug abuse. Intranasal oxytocin administration may then help replenish depleted oxytocin levels in drug-addicted individuals, potentially reducing engagement in drug-seeking and taking behaviours.

In conclusion, the results of this thesis demonstrate that oxytocin modulation of METH reward and abuse incorporates the NAc core and STh. In terms of reward, oxytocin attenuates dopamine-driven reward in the STh through activation of the OTR. Oxytocin administration to either the NAc core or STh reduced relapse to METH-seeking behaviour however, additional receptors to the OTR were involved. Regardless of the weak effect of OTR antagonism to alter oxytocin reductions in METH-seeking behaviour, the density of OTR-ir fibres in the NAc core and STh were differentially affected by METH IVSA and following a period of extinction, despite a constant increase in blood plasma levels of oxytocin. These results provide insight into the neurobiological processes of oxytocin and it's receptor in regulating METH abuse, with the NAc core and STh as primary brain substrates. Future studies should determine additional receptor interactions by oxytocin in these and

other brain regions affected by chronic METH exposure. Overall, the current body of research has important implications for the development of oxytocin-based compounds for pharmacological treatment of METH abuse and dependence.

Chapter One: Introduction

Drug addiction is a chronic disorder characterised by compulsive drug seeking and drug taking behaviour despite the associated adverse consequences (Koob, 2008). Drugs become valued over previously important aspects of the addicts life, resulting in a narrowed focus on acquiring and administering drugs (Hyman, Malenka, & Nestler, 2006). Even though some individuals may be able to completely cease drug administration, for others, this is a relapsing disorder where despite the myriad of problems surrounding drug use, as well as attempts to engage in treatment, they typically revert back to their previous drug taking behaviours (American Psychiatric Association, 2013).

Methamphetamine (METH) is a commonly abused and highly addictive illicit drug. Globally, amphetamine-type stimulants (excluding ecstasy) were estimated to have been used by 34 million individuals in 2011, making it the second most commonly used illicit drug following cannabis (United Nations Office on Drugs and Crime, 2013). Usage rates in Australia are one of the highest worldwide, whereby 66% of regular drug users reported administering METH within the last 6 months, and 20% reported it was their drug of choice (Australian Crime Commission, 2013).

The prevalence of METH use worldwide is related to its relatively low cost and ease of production. METH is synthetically produced in clandestine laboratories using various chemical precursors, with the most recent being pseudoephedrine (Brzeczko, Leech, & Stark, 2013; United Nations Office on Drugs and Crime, 2010). The pseudoephedrine reduction method results in a high purity METH product, which has allowed the production of large amounts of the drug in one production cycle (Meredith, Jaffe, Ang-Lee, & Saxon, 2005). In addition, unlike cocaine and opium that rely on crop growth, production of METH is not restricted to particular geographic locations (United Nations Office on Drugs and Crime, 2010). Together with METH being a relatively cheap illicit substance to purchase (Stafford & Burns, 2013), this highlights the ease with which METH can be produced and sold on a global scale.

METH abuse and addiction does not only impact the individual, it is also a serious public health concern. The cost of METH abuse in Australia is substantial as it includes health care, hospital-based treatment, arrests, seizure of products domestically and at Australian borders, custodial care of children, and cleaning up of laboratory sites (Australian Institute of Health and Welfare 2011; Brzeczko et al., 2013). Currently, there are no widely accepted effective pharmacotherapeutic treatments for METH dependence (Ciketic, Hayatbakhsh, Doran, Najman, & McKetin, 2012). The significant impact this drug has on both the addicted individual and the community highlights the need for the development of more effective pharmacological treatment strategies.

1.1. Methamphetamine

1.1.1. Pharmacology

METH (N-methyl-O-phenylisopropylamine) is a synthetically produced cationic molecule, which is derived from the psychostimulant amphetamine (alpha-methyl-phenethyl-amine; (Zorick, Rad, Rim, & Tsuang, 2008). Both molecules are similar in structure, although METH is more lipophilic, increasing its ability to cross the blood-brain barrier. This allows a higher concentration of METH to enter the central nervous system, which greatly increases its potency and addictive potential when compared to amphetamine (Rose & Grant, 2008; Shoblock, 2003; Zorick et al., 2008).

Methamphetamine can be administered in a number of ways; it may be snorted, smoked, injected or ingested (Elkashef et al., 2008). The route of administration impacts on the bioavailability of METH. When injected, smoked or inhaled, the bioavailability of METH is relatively high, with the subjective effects of the drug rapidly experienced within 10 to 20 minutes following administration (Cruickshank & Dyer, 2009). Oral administration results in substantially lower bioavailability and a slower peak in experiencing the subjective effects (45

minutes to 1.5 hours; (Rose & Grant, 2008). The subjective effects of METH have been reported to occur for approximately 8 to 12 hours following administration, which is in keeping with the long 12-hour half life of the drug (Meredith et al., 2005). Methamphetamine is largely metabolised in the liver through enzymatic degradation to produce the metabolites amphetamine, 4-hydroxymethamphetamine and norephedrine (Cruickshank & Dyer, 2009; Rose & Grant, 2008). Within 24 hours of administration, approximately 70% of the dose is excreted in urine along with its metabolites (Cruickshank & Dyer, 2009). Methamphetamine can typically still be detected in the urine or blood approximately 2 to 3 days after administration (Cruickshank & Dyer, 2009).

1.1.2. Mechanisms of action following acute administration

The highly addictive properties of METH are attributable to its effect on monoamine neurotransmission. Following administration in humans and non-human mammals, METH rapidly and sustainably increases the concentration of dopamine (DA), serotonin and noradrenaline that is released into the synapse (Rothman et al., 2001; Zorick et al., 2008). As an inverse agonist, METH is able to increase extracellular levels of the monoamines by three mechanisms: i) by reversing the vesicular monoamine transporter-2, which results in the release of intravesicular monoamines from storage vesicles into the cytosol, ii) by reversing the DA, serotonin and noradrenaline transporters, causing the release of the monoamines from the cytosol into synapses, and iii) by inhibiting monoamine oxidase to impede metabolism of the monoamines, which also increases the levels to be released (Elkashef et al., 2008; Zorick et al., 2008). As METH reverses or inhibits mechanisms that regulate synaptic clearance of monoamines, a strong and sustained effect on these monoamines is produced.

1.1.3. Subjective, physiological, and cognitive effects of acute methamphetamine ingestion

The resultant increase in monoamine activity, coupled with stimulation of the sympathetic nervous system following acute METH administration is associated with various subjective, physiological, and cognitive effects. Typically, users describe experiencing a "rush" or "high", which is characterised by euphoria, increased energy, improved confidence and self-esteem, hyper-sexuality, reduced fatigue and appetite suppression (Elkashef et al., 2008; Zorick et al., 2008). Improvements in cognitive processing, such as increased sustained and divided attention, and improved reaction time can also be experienced following METH use, where it is often consumed to assist with engaging in tedious activities for extended periods of time (Cruickshank & Dyer, 2009; Looby & Earleywine, 2007).

Positive subjective effects are not always experienced by METH-naive individuals. By contrast, the hyper-excited state can result in anxiety and insomnia (Elkashef et al., 2008). Additionally, paranoia, aggression, depression, irritability, hallucinations, and delusions of parasitosis have been reported (Darke, Kaye, McKetin, & Duflou, 2008; Elkashef et al., 2008; Rose & Grant, 2008). Further, the physiological symptoms typically experienced following acute METH administration are adverse and include tachycardia, hypertension, tachypnoea, elevated body temperature and diaphoresis (Rose & Grant, 2008; Zorick et al., 2008).

1.1.4. Complications surrounding chronic methamphetamine use

Chronic use of methamphetamine is associated with serious and pronounced neurological and physical health problems, cognitive deficits, and psychiatric symptoms in addition to dependence on the drug. Such complications often require medical attention, which is

reflected by the substantial number of hospital admissions for difficulties associated with METH use. The most recent Australian report indicates that in 2003 - 2004, 824 individuals were admitted to a New South Wales hospital for medical reasons relating to METH administration (Drabsch, 2006). The number of admissions for psychiatric reasons is also high, with 437 users admitted in 2002 - 2003 for psychosis symptoms (Drabsch, 2006). Considering the serious nature and range of difficulties associated with METH use, they will be covered in more detail in the following sections.

1.1.4.1. Methamphetamine dependence

Dependence on METH is characterised by compulsive and uncontrolled use despite the associated harms, tolerance, withdrawal, and typically relapse to compulsive drug taking (Darke et al., 2008; Rose & Grant, 2008). Tolerance to the acute effects of METH is often evident when users transition from less efficient routes of administration to more efficient routes such as injecting or smoking, using more frequently, using higher doses and using crystalline METH; the more potent form of the drug, in an attempt to experience the initial "high" (Darke et al., 2008). The change in METH use related to the development of tolerance is associated with the degree of dependence on METH, whereby higher rates of dependence have been reported by users who predominantly use the crystalline form as well as principally smoke or inject METH (McKetin, Kelly, & McLaren, 2006). Withdrawal from METH use is characterised by craving, fatigue, lethargy, depression, anxiety, anhedonia, increased appetite, changes to sleep patterns and psychomotor slowing or agitation, where the severity of the withdrawal symptoms is dependent on the duration and frequency of METH use (Darke et al., 2008; Rawson, 2013). Typically, the more severe withdrawal symptoms last for approximately one to three weeks, although more subtle symptoms such as anhedonia can persist for several months to one year (McKetin, Kaye, Clemens, & Hermens, 2013; Rawson, 2013; Rose & Grant, 2008).

1.1.4.2. Overdose and neurotoxicity

The symptom presentation of a METH overdose can vary substantially and incorporates both physical and psychological symptoms. In terms of the medical problems, nausea, vomiting, profuse perspiration, tremors, shortness of breath, chest pain, heart palpitations, and seizures can be experienced (Darke et al., 2008; Hamamoto & Rhodus, 2008). Psychological symptoms that have been reported include agitation, high levels of anxiety, paranoia, hallucinations and suicidal ideation (Darke et al., 2008). Fatal METH overdoses commonly result from pulmonary oedema, pulmonary congestion, brain haemorrhage, ischaemic stroke, seizure, ventricular fibrillation, and acute cardiac, respiratory, or renal failure (Cruickshank & Dyer, 2009; Darke et al., 2008; Hamamoto & Rhodus, 2008).

Methamphetamine is typically administered in a "binge and crash" pattern, where once the immediate euphoric effects abate, more METH is administered, further increasing the concentration of METH in the bloodstream (McKetin et al., 2013). Moreover, with increasing METH use, tolerance to the euphoric effects develops (Darke et al., 2008). As such, higher doses of METH are administered in this binge and crash pattern to experience the acute subjective effects. Altogether this mode of administration with escalating doses of METH can produce neurotoxic effects (McCann & Ricaurte, 2004). Methamphetamine neurotoxicity has been examined in humans using various brain imaging techniques or in post-mortem brain tissue, where the degeneration of monoamine systems, dysregulation of energy metabolism, oxidative stress markers and structural changes have been identified (Krasnova & Cadet, 2009). Specifically, DA and serotonin axons and axon terminals are destroyed, and dopamine transporter (DAT), DA D_2 receptor, serotonin transporter, and vesicular monoamine transporter densities are reduced (McCann & Ricaurte, 2004; Zorick et al., 2008). The decrease in monoamine transporter density is widespread, where DAT reductions are particularly prevalent in the orbitofrontal cortex, prefrontal cortex (PFC) and dorsal striatum, whilst the reduction in serotonin transporters is most evident in the midbrain,

dorsal striatum, hypothalamus, thalamus, and the orbitofrontal, temporal and cingulate cortices in METH-dependent users (Krasnova & Cadet, 2009). A decrease in DA metabolism has also been identified in the dorsal and ventral striatum (Zorick et al., 2008).

Morphological studies have identified structural changes to the brain of METH users. A loss of grey matter in the cingulate, limbic and paralimbic cortices has been identified, as well as hippocampal shrinkage (Krasnova & Cadet, 2009). Globally, hyperintensities of white matter, decreases in the neuronal marker n-acetylasparate, reductions in myoinositol, a marker for glial activation, and decreases in creatine, a marker of metabolic integrity, are evident (Krasnova & Cadet, 2009). The damage due to neurotoxicity has been found to persist following abstinence and is associated with cognitive, neurological and behavioural problems (McCann & Ricaurte, 2004). Violent behaviour is one such manifestation of the decline in serotonin transporter density (Zorick et al., 2008). Further, depletions of DA levels have been associated with Parkinson's disease symptoms, due to the common expression of psychomotor dysfunction and damage to DA neurons in the nigrostriatal pathway (Volkow et al., 2001).

1.1.4.3. Physical complications

Methamphetamine use is considered to be cardiotoxic as it places high demands on the cardiovascular system (Darke et al., 2008). As such, a number of cardiovascular problems can result from repeated administration of METH and the subsequent increase in blood pressure and heart rate. Problems include acute coronary syndrome, myocardial ischaemia, and in some cases myocardial infarction, atrial and ventricular dysrhythmias, cardiovascular collapse, cardiomyopathy, pulmonary hypertension, and coronary heart disease (Turnipseed, Richards, Kirk, Diercks, & Amsterdam, 2003). Methamphetamine-dependent individuals are also at a higher risk of experiencing haemorrhagic or ischaemic stroke. In a longitudinal analysis of hospital admissions in Texas, abuse of amphetamines was associated with twice

the risk of having an haemorrhagic stroke than if abusing cocaine, as well as an increased risk of death following stroke (Westover, McBride, & Haley, 2007).

The use of METH during pregnancy is associated with a myriad of difficulties and defects that can become apparent *in utero* and later in life. During early gestation, METH exposure can have substantial teratogenic effects on the foetus (Behnke & Smith, 2013). In addition, METH use in pregnant women has also been associated with loss of the foetus, premature birth, low birth weight, and can also result in neonatal withdrawal syndrome (Linden, Torchalla, & Krausz, 2013). Difficulties can also be evident at an older age, where children can have difficulties with learning and memory, behavioural problems, and be intellectually impaired (Linden et al., 2013).

Dental deterioration often results from chronic METH abuse. Commonly termed "meth mouth", the symptoms include blackened, rotting, or crumbling teeth, as well as xerostomia or dry mouth, bruxism and muscle trismus, or reduced opening of the jaw (Hamamoto & Rhodus, 2008; Shetty et al., 2010). This combination of symptoms typically occurs due to poor oral hygiene, high intake of sugary beverages and refined carbohydrates, increased acidity in the mouth from orally consuming METH, accumulation of chemical residue from smoking METH, as well as vomiting following METH administration (Hamamoto & Rhodus, 2008).

Intravenous injection of METH is associated with users having higher rates of blood-borne viruses. Such diseases include human immunodeficiency virus (HIV) and Hepatitis A, B, and C (Hamamoto & Rhodus, 2008). Endocarditis and pulmonary abscess can also result from poor needle hygiene (Richards et al., 1999). Methamphetamine-dependent individuals, particularly those who intravenously administer the drug, are at a higher risk of having a sexually transmitted disease. This is related to the reduction in inhibition and hypersexuality associated with METH administration, which can result in users engaging in unsafe behaviours including unprotected sex (Hamamoto & Rhodus, 2008; Richards et al., 1999).

Smoking METH regularly is associated with a number of respiratory problems including bronchitis, pulmonary oedema, haemoptysis, and granuloma. Smoking also commonly results in dermatitis around the mouth. Due to the appetite suppressant effects of METH, chronic users are typically malnourished (Hamamoto & Rhodus, 2008). Additional problems relating to repeated use of METH include constipation from dehydration and lack of dietary fibre, muscle cramping also resulting from dehydration and a reduction in electrolytes, as well as renal failure from exposure to toxic fumes during METH production (Rose & Grant, 2008). Chemical burns resulting from contact with precursors, as well as clandestine laboratory accidents and/or explosions are also common (Meredith et al., 2005).

1.1.4.4. Psychiatric symptoms

Psychopathology is typically experienced by both METH-dependent individuals and those withdrawing from METH use. Symptoms of depression are particularly common in this group. In an Australian sample of METH-dependent individuals receiving treatment, 46% had been previously diagnosed with an Axis 1 disorder from the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV), where depression was the diagnosed condition in 35% of the sample (Dyer & Cruickshank, 2007). Depressive symptoms have been reported to be higher during periods of abstinence or withdrawal (Looby & Earleywine, 2007). In keeping with this, high rates of self-harm, suicidal ideation, and suicide attempts are reported in METH users (Dyer & Cruickshank, 2007). In the same aforementioned sample, 20% had been hospitalised for self-harm (Dyer & Cruickshank, 2007). Further, it has been reported that approximately a quarter of METH users will attempt suicide at some point in their lifetime (Zweben et al., 2004).

Consistent with increased reports of depressive symptomatology, high levels of anxiety are typically experienced by METH users. Severe anxiety symptoms are often reported, and approximately 11% of regular METH users have been diagnosed with an

anxiety disorder at some point in their lifetime (Darke et al., 2008). In addition, anxiety - related symptoms are often experienced during periods of abstinence (Rose & Grant, 2008).

It has been well documented that psychostimulant administration can induce psychosis and that this can be experienced whilst regularly using METH or during periods of abstinence. The symptoms experienced are typically indistinguishable from schizophrenia, and include auditory and visual hallucinations, as well as delusions of reference and persecution (Harris & Batki, 2000). Psychosis is usually transiently experienced, where the symptoms abate within a couple of hours (McKetin, McLauren, Lubman, & Hides, 2005). However, in severe cases, psychotic symptoms can persist for a week following abstinence (McKetin et al., 2005). The prevalence of psychosis in METH users is high, with 18% of users in an Australian study reporting psychotic symptoms within the past year (McKetin et al., 2005). Psychotic symptoms can be experienced in METH users who have no family history of a psychotic or schizophrenic illness (McKetin et al., 2005). In those with a family history, METH use can precipitate a schizophrenic episode, or in those who have already been diagnosed with schizophrenia, can exacerbate their symptoms (Harris & Batki, 2000). Further, psychotic symptoms can be triggered by stress in formally psychotic METH users who are now abstinent, which makes correct psychiatric diagnosis substantially more difficult (Harris & Batki, 2000).

The symptom profile associated with the “crash” component of the typical METH administration pattern is characterised more so by psychological difficulties than by physical complaints. Typically, depression with severe dysphoria, irritability, melancholia, anxiety, extreme fatigue, and hypersomnia are experienced, as well as craving for the drug (Meredith et al., 2005). However, these symptoms tend to resolve fairly quickly (Rose & Grant, 2008). Withdrawing from regular METH use is associated with the same psychological symptoms, although they are experienced with increased severity and for a much longer duration, where symptoms may persist for 12 months (Rose & Grant, 2008).

1.1.4.5. Cognitive deficits

In contrast to the improvements in cognitive functioning experienced when administered acutely at low to moderate doses, cognitive deficits have been reported with repeated METH use and following abstinence. Difficulties with learning, memory, sustaining attention, and decision-making have been identified, as well as slowed processing of information (Cruickshank & Dyer, 2009; Dean, Groman, Morales, & London, 2013; Meredith et al., 2005; Ornstein et al., 2000). Such cognitive decline is consistent with damage to the frontostriatal and limbic brain areas (Ornstein et al., 2000). In abstinent users, problems with cognition can persist, with continued difficulties in memory, learning, and executive and motor function (Herbeck & Brecht, 2013; McCann & Ricaurte, 2004). Although the presence and degree of cognitive deficits varies amongst METH users and those who are abstinent, greater difficulties are typically reported by those who had pre-existing cognitive difficulties, are poly-drug users, or are those experiencing psychiatric symptoms (Herbeck & Brecht, 2013; McCann & Ricaurte, 2004).

1.2. Methamphetamine and reward

1.2.1. A short definition of reward

Reward can be broadly defined in terms of the psychological processes and behaviours it generates (Schultz, 2006). Generally, rewards are perceived as pleasurable and desired experiences, which after the initial exposure, promote further engagement in the behaviour or experience that induced such positive emotions (Schultz, 2000). This is attributable to the hedonic function of rewards (Hyman et al., 2006; Schultz, 2006). Learning processes are also involved in experiencing reward, whereby the degree of pleasure produced by the reward and cues that predict its availability are consolidated (Hyman et al., 2006).

Retaining this information influences motivational processes surrounding re-experiencing this particular reward in the future. As such, reward can be perceived as a means of constricting behaviour to increase the likelihood of a particularly pleasurable experience.

1.2.2. Types of reward

Rewarding objects and events can be loosely categorised into natural and artificial rewards. Natural rewards, like food and water are associated with rewarding properties through their importance for survival (Kelley & Berridge, 2002). Social interactions such as sex (Agmo & Gomez, 1993), monogamous pair-bonding (Young & Wang, 2004), and maternal-infant attachment (Insel, 2003) are also considered important evolutionary factors for reproduction, survival, and species fitness, and additionally function as natural rewards.

The administration of pharmacological substances, such as alcohol, nicotine, and various illicit drugs of abuse, including METH, also produce rewarding effects (Kelley & Berridge, 2002). In line with this, the cues and circumstances surrounding METH availability are rapidly consolidated and motivate future behaviour towards obtaining and administering drugs (Hyman et al., 2006). Such cues include; viewing METH and associated drug paraphernalia, being in an environment associated with drug taking, as well as the psychological experiences of craving drug use (Hyman et al., 2006). The hedonic state experienced following METH administration is often valued above that of natural and social rewards, and contributes to the restriction of goals to focus on seeking and administering METH (Hyman et al., 2006).

1.2.3. Neural circuitry of reward

The manner in which all rewarding stimuli are able to elicit positive, pleasurable experiences is through the activation of the mesocorticolimbic DA system (Kelley &

Berridge, 2002; Koob, 1992; Shimosato & Ohkuma, 2000). Originating in the ventral tegmental area, DA neurons project to the nucleus accumbens (NAc), amygdala, hippocampus, PFC, medial dorsal thalamus and ventral pallidum in addition to other forebrain areas (Due, Huettel, Hall, & Rubin, 2002; Koob, 1992). Particular substrates comprising the mesocorticolimbic system are predominantly activated by reward-related cues, namely the NAc, medial PFC, amygdala, and ventral tegmental area (Due et al., 2002; Sparta et al., 2014). In addition to this system, additional substrates are activated and are involved in processing rewarding stimuli. These regions include the dorsal striatum, substantia nigra, subthalamic nucleus (STh) and hypothalamus (Schultz, 2000).

The stimulation of the mesocorticolimbic DA pathway by METH is more extensive and potent than that of natural rewards (Hyman et al., 2006). Moreover, repeated METH use and exposure to METH-related cues are associated with dysregulation of molecular and cellular mechanisms within reward circuits as well as depletion of monoamine systems, particularly DA (Koob, 2009; Nestler, Hope, & Widnell, 1993; Robinson & Berridge, 2003). This includes a reduction of dopamine D2 receptors and decreased dopaminergic activity (Volkow, Fowler, & Wang, 2002; Volkow et al., 1997), which suggests reduced sensitivity of the DA system to rewarding stimuli. However, such changes result in hypersensitive responses to METH administration, which can be explained by the incentive sensitisation theory of addiction (Robinson & Berridge, 2003). This theory stipulates that with repeated drug administration, the salience attributed to the drug and associated cues are intensified whilst the motivation and desire to engage in natural rewards decreases (Kelley & Berridge, 2002; Robinson & Berridge, 2003). This dysregulation of mesocorticolimbic DA neurotransmission and associated shift in motivational salience of the drug are considered critical in the transition to addiction following repeated METH use (Koob, 2009; Robinson & Berridge, 2003).

1.3. Dopamine

1.3.1. Cellular activity and distribution

Dopamine (DA; 3-hydroxytyramine), a metabolite of amino acid tyrosine, is a catecholaminergic neurotransmitter, which constitutes approximately 80% of the catecholamine content in the brain (Beaulieu & Gainetdinov, 2011; Di Chiara, 1995; Vallone, Picetti, & Borrelli, 2000). This neurotransmitter acts as a neuromodulator by slowly mediating the fast neurotransmission of gamma-aminobutyric acid (GABA) and glutamate (Beaulieu & Gainetdinov, 2011). Dopamine cell bodies are located within the substantia nigra pars compacta, ventral tegmental area, and arcuate nuclei of the hypothalamus, from which three principle DA neuronal pathways originate. The nigrostriatal pathway originates in the substantia nigra, projects to the striatum, and is associated with controlling movement (habitual responding) (Baskerville & Douglas, 2010; Civelli, Bunzow, & Grandy, 1993). The tuberoinfundibular pathway originates in the arcuate nuclei of the hypothalamus and projects to the median eminence to regulate hormone release (Baskerville & Douglas, 2010; Civelli et al., 1993). The third pathway is the aforementioned mesocorticolimbic pathway, which originates in the ventral tegmental area and is involved in reward processes (Civelli et al., 1993).

Dopamine acts as a neuromodulator through activating five G-protein coupled receptors that have been divided into two distinct groups (Beaulieu & Gainetdinov, 2011; Vallone et al., 2000). The division of receptors into the D_1-like and D_2-like receptor classification system is based on biochemical, structural and pharmacological factors (Beaulieu & Gainetdinov, 2011; Vallone et al., 2000). The D_1-like receptor category incorporates the D_1 and D_5 receptors. Both receptors have been located post-synaptically and activate adenylyl cyclase to increase cyclic adenosine monophosphate (cAMP) levels (Civelli et al., 1993). The D_1 receptor is the most widespread of the dopamine receptors in the brain, and has been located in the dorsal striatum, NAc, olfactory tubercle, hypothalamus, thalamus,

amygdala, entopeduncular nucleus, globus pallidus, substantia nigra pars reticulata, islands of Calleja, and STh (Jaber, Robinson, Missale, & Caron, 1996; Vallone et al., 2000). The D_5 receptor, conversely, is minimally expressed and restricted to the hippocampus, lateral mammillary nucleus and the parafascicular nucleus of the thalamus (Jaber et al., 1996; Vallone et al., 2000).

The D_2-like receptor subfamily consists of the D_2, D_3, and D_4 receptors. This group inhibits cAMP production and the receptors are located pre-synaptically on dopaminergic neurons as well as post-synaptically on dopamine target neurons (Beaulieu & Gainetdinov, 2011). The D_2 receptor is the most highly expressed following the D_1 receptor, and has been located in the dorsal striatum, NAc, olfactory tubercle, STh, ventral tegmental area, hypothalamus, amygdala, hippocampus and substantia nigra pars compacta (Beaulieu & Gainetdinov, 2011; Johnson, Coirini, Kallstrom, & Wiesel, 1994; Vallone et al., 2000). The D_3 receptor is expressed in various substrates including the NAc shell, dorsal striatum, hippocampus, ventral tegmental area, olfactory tubercle, islands of Calleja, and substantia nigra pars compacta (Beaulieu & Gainetdinov, 2011; Jaber et al., 1996; Vallone et al., 2000). Lastly, the D_4 receptor is the most weakly expressed of the DA receptors, and has been identified in the frontal cortex, amygdala, hypothalamus, medulla, hippocampus, olfactory bulb and globus pallidus (Beaulieu & Gainetdinov, 2011; Jaber et al., 1996; Vallone et al., 2000). Overall, through the widespread distribution of receptors and extensive innervation from dopaminergic neurons throughout the brain, DA neurotransmission plays a significant role in numerous central functions and behaviours.

1.3.2. Behavioural effect

Dopamine is involved in a broad spectrum of central processes, which includes cognition, emotion, arousal, aversion, motivation, movement, perception, and sleep, as well as peripheral effects on the renal and cardiovascular systems (Baskerville & Douglas, 2010;

Kiyatkin, 2002). Disruptions to different aspects of the DA system have also been associated with numerous disorders including Parkinson's Disease, Attention Deficit Hyperactivity Disorder (ADHD), schizophrenia, social anxiety, Major Depressive Disorder and compulsive behaviours (Baskerville & Douglas, 2010; Vallone et al., 2000).

As aforementioned, DA is considered the primary catecholamine to mediate reward processes (Hyman et al., 2006; Koob, 2009; Schultz, 2000). Specifically, DA is critical for reward-related learning and motivating behaviour towards obtaining rewards (Di Chiara, 1995; Hyman et al., 2006; Schultz, 2000). After the presentation of a naturally rewarding stimulus or cues associated with rewarding stimuli, a majority of dopaminergic neurons show short phasic activation (Hyman et al., 2006; Schultz, 1986, 2000). Moreover, this phasic response is strengthened by unexpected reward presentation and is depressed by the omission of an expected or predicted reward. In contrast, minimal dopaminergic neuronal activation occurs when an aversive stimulus is experienced (Hyman et al., 2006; Schultz, Dayan, & Montague, 1997). Together, this demonstrates that phasic DA activation encodes a reward prediction error, rather than coding for the reward itself. These predictions trigger a sensation of 'wanting', which motivates behaviours towards obtaining the reward (Hyman et al., 2006).

In cases of acute METH exposure, the phasic DA reward prediction error appears to be boosted, creating the perception that the reward was better than expected, resulting in a much stronger desire for the drug and much more established reward-related associations (Schultz, 2000). With repeated use, DA activity in the mesocorticolimbic DA pathway decreases, as does subsequent subjective experiences, however, the reward continues to be interpreted as better than expected (Hyman et al., 2006; Koob, 2009). This corresponds with neuroadaptations to the circuit with continual drug exposure and the formation of incentive sensitisation (Hyman et al., 2006; Robinson & Berridge, 1993; Schultz, 2006). The combination of reward-related predictions, alterations to the mesocorticolimbic DA pathway, and increased sensitisation of drug-related cues strengthens the desire for METH and assists with the progression to addiction. However, DA is not solely involved in METH-related

reward and abuse. The neurotransmitters glutamate, GABA, and noradrenaline as well as the neuropeptide corticotropin-releasing-factor (CRF) have been associated with neurobiological changes following chronic METH abuse (Adinoff, 2004; Burrows & Meshul, 1999; Kalivas, 2009; Volkow, Wang, Fowler, Tomasi, & Telang, 2011). In addition, an interaction between DA and the neuropeptide oxytocin has been proposed, which appears to be implicated in the behavioural and neurological responses to METH (Carson, Cornish, Guastella, Hunt, & McGregor, 2010a).

1.4. Oxytocin

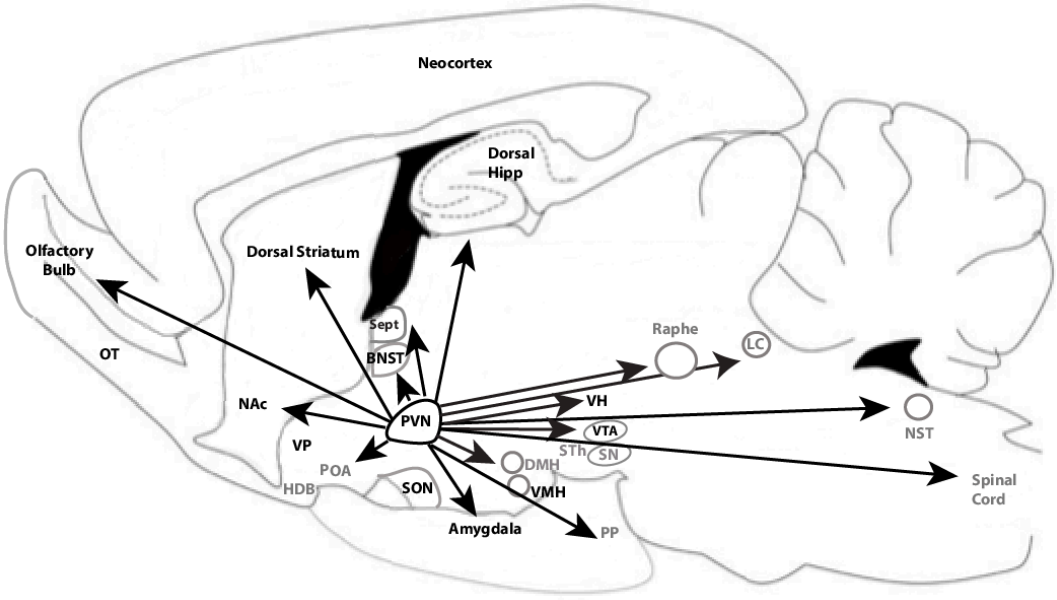
1.4.1. Neurochemistry

Oxytocin is a nine-amino acid neuropeptide (Pow & Morris, 1989). It was discovered by Sir Henry Dale in 1906 who described its functional importance in facilitating uterine contractions, and in the 1950's the chemical structure of oxytocin was described by Vincent Du Vigneaud (Buisman-Pijlman et al., 2014; Carson, Guastella, Taylor, & McGregor, 2013)**.** Oxytocin is primarily synthesised in the central nervous system by the magnocellular neurons located within the paraventricular nucleus (PVN), supraoptic nucleus (SON), and accessory nucleus of the hypothalamus (Pow & Morris, 1989; Swanson & Sawchenko, 1983). The magnocellular neurons project to the posterior pituitary where oxytocin is released into the general blood stream to act as a hormone on peripheral targets (Bargmann & Scharrer, 1951; Brownstein, Russell, & Gainer, 1980). Additionally, oxytocin is released from magnocellular neurons within the SON and PVN to act on oxytocin receptors (OTR) that are distributed widely in a species-specific manner throughout the brain (Landgraf & Neumann, 2004; Ludwig & Leng, 2006; Pow & Morris, 1989). Ascending oxytocinergic pathways largely originate from the PVN to innervate limbic and forebrain regions as well as substrates of the basal ganglia and midbrain (Fuxe et al., 2012; Gimpl & Fahrenholz, 2001). The descending

oxytocin pathway originates in the parvocellular neurons of the PVN, where less oxytocin is synthesised, and projects to the autonomic regions of the lower brain stem, medulla and pons (Fuxe et al., 2012; Lee, Macbeth, Pagani, & Young, 2009; Pinol, Bateman, & Mendelowitz, 2012). The application of optogenetic techniques and retrograde trans-synaptic tracing has resulted in the recent identification of additional oxytocinergic fibres projecting from the mouse PVN to the NAc (Dolen, Darvishzadeh, Huang, & Malenka, 2013) and from the magnocellular neurons of the rat SON to limited forebrain substrates, namely the NAc, central amygdala, lateral septum, CA1 of the ventral hippocampus, and the horizontal limb of the diagonal band of Broca (Knobloch et al., 2012).

In addition to oxytocin acting as a neurotransmitter through fast synaptic signalling along wired axonal pathways, it also acts as a neuromodulator. This is achieved through non-synaptic release from either the dendrites or soma of the neuronal membrane to regulate its own activity (Moos et al., 1984) and target distant brain regions (Landgraf & Neumann, 2004; Ludwig & Leng, 2006; Neumann, 2007). It has long been thought that oxytocin largely released from the SON is diffusely transmitted throughout the brain, and with a half-life of 20 minutes and no spatial restriction to synapses, its release can affect distal brain regions (Landgraf & Neumann, 2004; Ludwig & Leng, 2006; Neumann, 2007). However, recent technological advances have furthered understanding of non-synaptic release, indicating that a combination of focal and non-synaptic release can produce fast behavioural, emotional, and cognitive responses. Knobloch and colleagues (2012) showed through the application of optogenetics, electrophysiology, and retrograde tracers, that oxytocinergic fibres projecting from the dorsolateral accessory nuclei of the hypothalamus to the lateral division of the central amygdala within the rat brain focally release oxytocin through nonsynaptic means. This focal release was through diffuse transmission within a short 100-micrometre distance. At present, it appears that oxytocin can communicate in a fast point-to-point manner through synaptic and non-synaptic signalling, as well as through slow and global release as a neuromodulator to have longer lasting effects.

a.



b.

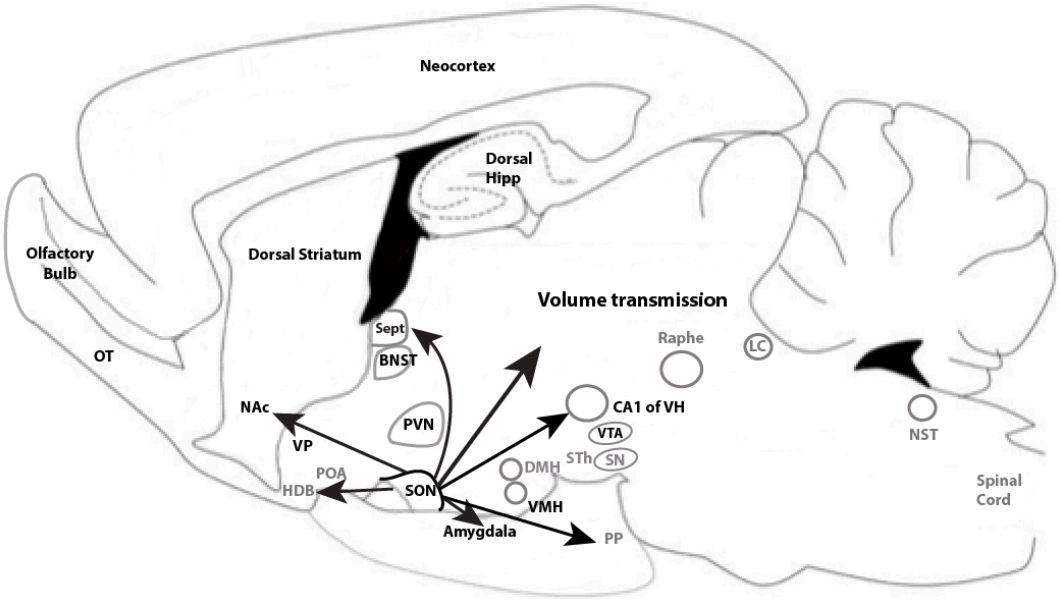


Figure 1. Oxytocin receptor localisation and a) projections from the PVN and b) SON in the rat brain. PVN, paraventricular nucleus; SON, supraoptic nucleus; NAc, nucleus accumbens; sept, lateral septum; BNST, bed nucleus of the stria terminalis; POA, preoptic area; PP, posterior pituitary; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus, Dorsal Hipp, dorsal hippocampus; VH, ventral hippocampus; VTA, ventral tegmental area; SN, substantia nigra; STh, subthalamic nucleus; VP, ventral pallidum; OT, olfactory tubercle; HDB, horizontal limb of the diagonal band of Broca; LC, locus coeruleus; NST, nucleus of solitary tract. Brain regions in black indicate oxytocin receptor localisation, brain regions in grey indicate that the oxytocin receptor has not been located in this substrate. Diagram adapted from McGregor et al. (2008).

At the present time, one OTR has been identified, which is a member of the rhodopsin-type class I G-protein coupled receptor family (Gimpl & Fahrenholz, 2001). Oxytocin receptors are functionally coupled to G_q, G_s, and G_i proteins to activate various intracellular signalling pathways. Coupling with the G_q protein activates phospholipase C, which subsequently increases intracellular Ca^{2+} ions and protein kinases type C, G_s coupling activates adenylyl cyclase and stimulates cAMP production, and coupling with G_i inhibits cAMP production (Gimpl & Fahrenholz, 2001; Strunecka, Hynie, & Klenerova, 2009; Viero et al., 2010). As such, various intracellular signalling pathways can be activated to produce different behavioural outcomes. This is dependent on the combination of different G proteins that are coupled to OTRs within that particular brain region and whether a single or multiple G proteins are simultaneously activated (Viero et al., 2010).

The OTR is extensively distributed throughout the brain in a species dependent manner. In the rat, the OTR has been abundantly located in the basal ganglia, limbic system, peduncular cortex, thalamus, hypothalamus, olfactory system, brain stem and spinal cord (Adan et al., 1995; Gimpl & Fahrenholz, 2001). Additionally, due to their structural similarity, oxytocin binds to the receptors of the neuropeptide arginine vasopressin (AVP) at a slightly lower affinity than the OTR (Chini & Manning, 2007; Tribollet, Barberis, Jard, Dubois-Bauphin, & Dreifuss, 1988). This extends the effect oxytocin can have within the brain, as receptors for the neuropeptide AVP are largely distributed in different brain regions to the OTR (Gimpl & Fahrenholz, 2001). Altogether, the widespread localisation of oxytocin and AVP receptors, both the focal and global transmission of oxytocin, and the diverse intracellular signalling pathways, which can be activated highlight that oxytocin can modulate a wide range of behaviours.

1.4.2. Effect on behaviour

Commonly termed the social neuropeptide, oxytocin is involved in the modulation of numerous social, emotional, and sexual behaviours, in addition to its classical peripheral effects on uterine contractions during parturition and milk letdown during lactation (Dawood, Khan-Dawood, Wahi, & Fuchs, 1981). Oxytocin promotes maternal behaviour, sexual arousal, erectile function and ejaculation, social interaction, the formation of mother-infant and monogamous pair bonds, and can enhance trust in humans (Hollander et al., 2007; Lee et al., 2009; McGregor, Callaghan, & Hunt, 2008; Young, Lim, Gingrich, & Insel, 2001). Further, oxytocin has an anxiolytic and antidepressant effect, and reduces stress responses by attenuating the hypothalamic-pituitary-adrenal axis through the inhibition of CRF expression in the hypothalamus (Bülbül et al., 2011; Neumann & Landgraf, 2012).

Drug taking is strongly associated with social context, where the initiation of drug use can be influenced by social groups or settings, and can result in numerous social consequences when taken acutely or chronically. In keeping with this, the regulation of the endogenous oxytocin system has been associated with resilience to addiction in humans (Buisman-Pijlman et al., 2014) and exogenously administered oxytocin has been associated with modulating licit and illicit drug-related behaviours and experiences in animals (Sarnyai & Kovacs, 2014; see Table 1). Rodent studies have demonstrated that systemic or central injections of oxytocin decreased the self-administration of heroin in heroin-tolerant rats (Kovacs, Borthaiser, & Telegdy, 1985), reduced the development of tolerance to, and physical dependence on morphine (Kovacs, Horvath, Sarnyai, Faludi, & Telegdy, 1985), and that central oxytocin administration decreased cannabinoid withdrawal symptoms (Cui et al., 2001). Systemic oxytocin administration has also been shown to attenuate the self-administration of cocaine (Sarnyai & Kovacs, 1994), as well as cocaine-induced locomotion (Kovacs, Sarnyai, Babarczi, Szabo, & Telegdy, 1990), stereotypy (Sarnyai et al., 1991), and tolerance (Sarnyai et al., 1992a). Both peripheral and central administration of oxytocin

Table 1: Behavioural studies examining the effect of oxytocin administration on drug-related behaviour

Drug Treatment	Species	route of oxytocin administration	Effect of oxytocin	Reference
Heroin	rat	Subcutaneous	Decreased self-administration	Kovacs et al., 1985a
Heroin	rat	Intracranial	Decreased self-administration	Ibragimov et al., 1987
Cocaine	rat	Intracranial	Decreased sniffing	Sarnyai et al., 1991
Morphine	mice	Subcutaneous	Decreased development of tolerance to morphine	Kovacs et al., 1986
Morphine	mice	Subcutaneous	Decreased development of tolerance and physical dependence	Kovacs et al., 1985b
Cannabinoid agonist	rat	Subcutaneous	Decreased cannabinoid withdrawal symptoms	Cui et al., 2001
Marijuana	human	Intranasal	Decreased craving following psychosocial stressor	McRae-Clark et al., 2013
Cocaine	rat	Subcutaneous	Decreased self-administration	Sarnyai & Kovacs 1994
Cocaine	rat	Subcutaneous	Decreased locomotion	Kovacs et al., 1990
Cocaine	rat	Subcutaneous	Decreased stereotypy	Sarnyai et al., 1991
Cocaine	rat	Subcutaneous	Decreased tolerance	Sarnyai et al., 1992a
Cocaine	human	Intranasal	Reduced the association between state anger and cocaine-related cues	Lee et al., 2014
Alcohol	rat	ip	Decreased preference and consumption	McGregor & Bowen, 2012
Alcohol	mice	icv	Decreased tolerance	Szabo et al., 1989
Alcohol	human	Intranasal	Decreased alcohol withdrawal	Pedersen et al., 2012
Methamphetamine	mice	icv	Decreased acquisition of CPP	Qi et al., 2009
Methamphetamine	mice	icv	Decreased locomotor	Qi et al., 2008
Methamphetamine	rat	ip	Decreased self-administration and drug-induced reinstatement	Carson et al., 2010a
Methamphetamine	rat	ip	Decreased cue-, drug-, and stress-induced reinstatement	Cox et al., 2013
Methamphetamine	rat	Intracranial	Decreased acquisition of CPP	Baracz et al., 2012
Methamphetamine	rat	ip	Decreased motivation to self-administer Decreased drug-primed reinstatement	Hicks et al., in press

reduced preference and consumption of alcohol in alcohol preferring rats (McGregor & Bowen, 2012) and reduced tolerance to alcohol in mice (Szabo, Kovacs, & Telegdy, 1989). The oxytocin analogue carbetocin has also been used to examine its effect on morphine withdrawal. The sole peripheral administration of carbetocin following seven days of withdrawal from chronic morphine administration restored social behaviour, reduced anxiety and depressive-like symptoms, and attenuated reinstatement to morphine-seeking behaviour in mice (Zanos et al., 2014). Further, preliminary clinical findings examining alcohol, marijuana, and cocaine-dependent humans suggest that intranasal administration of oxytocin reduced alcohol withdrawal symptoms (Pedersen et al., 2012), marijuana craving following exposure to a psychosocial stressor (McRae-Clark, Baker, Maria, & Brady, 2013), and the association between state anger, a factor that can impact drug taking behaviour, and cocaine-related cues (Lee et al., 2014).

An examination of the ability for oxytocin administration to modulate behaviour following METH administration was initiated much more recently. Acute intracerebroventricular (icv) oxytocin administration was shown to reduce METH-induced hyperactivity in mice (Qi et al., 2008). Oxytocin administration also modulates reward processes (although see Subiah, Mabandla, Phulukdaree, Chuturgoon, & Daniels, 2012), whereby icv oxytocin injections in mice attenuated the acquisition of a conditioned place preference for METH and blocked stress induced relapse to METH-seeking behaviour (Qi et al., 2009). Further, systemic injections of oxytocin have been shown to reduce the self-administration of METH in rodents, as well as reinstatement to METH-seeking behaviour when exposed to a drug prime (Carson et al., 2010a). This effect has also been shown in female rats, where regardless of the stage of the estrous cycle, oxytocin reduced the self-administration of METH, as well as cue-, drug-, and stress-induced reinstatement (Cox, Young, See, & Reichel, 2013). The impact of oxytocin administration during adolescence on

addiction-related behaviours in adulthood has also been examined in female rats (Hicks, Cornish, Baracz, Suraev, & McGregor, in press). Pretreatment with oxytocin reduced lever press responding for METH under a progressive ratio schedule of reinforcement as well as during METH-primed reinstatement. Altogether, this suggests that oxytocin administration can affect addiction-related behaviours when injected prior to, as well as following acute and chronic drug administration.

1.5. Oxytocin and dopamine interactions

At a neurochemical level, one of the ways in which oxytocin modulates socio-affiliative behaviours and drug reward within the brain is through an interaction with DA. Oxytocin and DA have been shown to influence the central release of each other. Dopamine administration using *in vivo* and *in vitro* techniques increases oxytocin synthesis and secretion, whilst the administration of DA antagonists to cell culture media blocks oxytocin production (Galfi et al., 2001; Mason, 1983). Similarly, central oxytocin administration has been shown to increase DA release (Young & Wang, 2004).

Anatomical and immunocytochemical studies have shown that oxytocin and DA receptors and neuronal fibres are largely located within the same brain regions and are in close apposition. Dopamine D_2 and D_3 receptors have been identified on the cell bodies and dendrites of magnocellular and parvocellular oxytocin neurons of the hypothalamus, and D_4 receptors have been located exclusively on magnocellular oxytocin neurons in the PVN (Baskerville, Allard, Wayman, & Douglas, 2009). In addition to the expression of DA receptors on oxytocinergic neurons, an overlap in oxytocin and D_2 receptor distribution is evident in the dorsal and ventral striatum (Fuxe et al., 2012) as are D_2 receptor– OTR heteromers (Romero-Fernandez, Borroto-Esuela, Agnati, & Fuxe, 2013). In terms of fibre innervation, dopaminergic fibres from the incertohypothalamic system extend to oxytocin nuclei in the hypothalamus, and from the ventral tegmental area to the hippocampus and

amygdala (Baskerville & Douglas, 2010). Oxytocinergic fibres also innervate mesocorticolimbic DA cell bodies in the ventral tegmental area that then terminate in the NAc (Baskerville & Douglas, 2010). Together, this highlights the potential circuitry through which oxytocin and DA could interact.

The manner in which oxytocin and DA interact to influence behavioural outcomes is quite complex, is associated with a wider neural network, and appears to be dependent on both the behaviour (Baskerville & Douglas, 2010) and the brain region involved (Kovacs et al., 1990). Pair bond formation in voles has been shown to require the concurrent activation of both oxytocin and D_2 receptors at the level of the NAc (Liu & Wang, 2003; Young et al., 2001). Liu and Wang (2003) microinjected oxytocin or the D_2 receptor agonist quinpirole into the NAc of female prairie voles and found that both induced partner preference formation, and that this was blocked by the co-administration of either agonist (oxytocin or quinpirole) with the D_2 receptor antagonist eticlopride or the OTR antagonist $d(CH_2)_5[Tyr(Me)^2, Thr^4, Tyr\text{-}NH_2^9]$-OVT.

In contrast, the initiation of maternal behaviour appears to be oxytocin driven, whereby microinjections of an OTR antagonist into either the medial preoptic area or ventral tegmental area interferes with DA release within the mesocorticolimbic system, which in turn disrupts the onset of such behaviour in parturient female rats (Numan & Stolzenberg, 2009). The maintenance of maternal behaviour is also oxytocin driven, although oxytocin seems to exert a modulatory role, rather than control, over engagement in maternal behaviours. This has been demonstrated through direct oxytocin administration into the ventral tegmental area, which increased DA signalling in the NAc, resulting in heightened grooming and licking behaviour, whereas this behaviour was decreased when an OTR antagonist was microinjected into the ventral tegmental area (Shahrokh, Zhang, Diorio, Gratton, & Meaney, 2010).

Sexual behaviour in male rats, alternatively, appears to be driven by DA (Baskerville et al., 2009; Baskerville & Douglas, 2010). Further, DA appears to activate select oxytocin neurons depending on the context in which penile erection occurs. Baskerville and colleagues

(2009) demonstrated that the icv administration of a D_4 receptor antagonist prior to placement of the male in the same chamber as a receptive female attenuated both intromission and activation of oxytocin magnocellular neurons in the SON. In the absence of a female rat, penile erection was induced by the central administration of the D_2/D_3 agonist quinelorane, which also activated oxytocin neurons in the PVN (Baskerville et al., 2009).

Unlike investigations into the oxytocin-DA interaction in socio-affiliative behaviours, behavioural pharmacology studies examining this interaction in drug reward and abuse are limited. It has been postulated that oxytocin attenuates the increased DA neurotransmission typically experienced following psychostimulant administration within the mesocorticolimbic circuit (McGregor et al., 2008; Qi et al., 2009; Qi et al., 2008). Central or peripheral administration of oxytocin has been shown to decrease the increased utilisation of DA in the NAc following cocaine administration (Kovacs et al., 1990) and in both the dorsal and ventral striatum following METH administration (Qi et al., 2008). In a recent study, oxytocin microinjected into the prelimbic cortex reduced the increase in NAc DA levels evident following repeat amphetamine injections which rescued amphetamine-induced impairments in the formation of pair bonds in female prairie voles (Young, Liu, Gobrogge, Wang, & Wang, 2014). As oxytocin neuronal fibres have been located in apposition to DA cell bodies in the ventral tegmental area and innervate the PFC, which also receives dopaminergic input, this provides an anatomical foundation for hypothalamic oxytocin input into the mesocorticolimbic DA system (Baskerville & Douglas, 2010). Although the exact mechanisms by which oxytocin modulates DA neurotransmission in drug reward and abuse remain unclear.

1.6. Brain regions involved in oxytocin and methamphetamine interactions

Recent investigations have furthered our understanding of the neurobiological substrates involved in oxytocin modulation of acute METH exposure. Carson and colleagues (2010b) demonstrated that peripherally administered oxytocin prior to an acute METH injection reduced METH-induced c-Fos expression in the NAc core and the STh in addition to METH hyperactivity. Such findings indicate that both regions are critically involved in oxytocin reduction of acute METH-related effects. To explore this further, a study conducted prior to this thesis (Baracz et al., 2012) examined the involvement of both the NAc core and STh in the ability of oxytocin to reduce METH reward. Oxytocin microinjected into either the NAc core or STh attenuated the formation of a place preference to the METH-associated context using a single conditioning session. This demonstrates that oxytocin is acting in either region to reduce the rewarding effects of acutely administered METH. Thus, the studies of Carson et al. (2010b) and Baracz et al. (2012) highlight that the NAc core and STh both play an important role in oxytocin modulation of acute METH exposure.

1.6.1. Nucleus accumbens core

The NAc is considered a key neural substrate involved in reward and motivation (Ito, Robbins, & Everitt, 2004; Sellings & Clarke, 2003; Wise & Bozarth, 1985). This brain region makes up the ventral component of the striatum and is the terminal region of the mesocorticolimbic DA pathway (Carlezon & Thomas, 2009). The NAc receives glutamatergic input from the PFC, the hippocampus, and amygdala, and dopaminergic input from the ventral tegmental area (Joffe, Grueter, & Grueter, 2014; Koch, Schmid, & Schnitzler, 2000). In terms of output, the NAc is divided into two subregions, the medioventral shell and the dorsolateral core, both of which project to independent brain regions (Usada, Tanaka, & Chiba, 1998). The medioventral shell projects to the medial ventral pallidum, lateral

hypothalamus, ventral tegmental area, substantia nigra pars reticulata, and the extended amygdala (Joffe et al., 2014; Tripathi, Prensa, Cebrian, & Mengual, 2010; Usada et al., 1998). Projections from the core subregion extend to the dorsal portion of the ventral pallidum, medial globus pallidus, substantia nigra pars compacta and the STh (Joffe et al., 2014; Tripathi et al., 2010; Usada et al., 1998).

The NAc is largely involved in the initial stages of drug addiction (Wise & Bozarth, 1985). Following acute psychostimulant administration, increased DA is released in this brain region, which instigates the behavioural expression and pleasure associated with reward (Broom & Yamamoto, 2005; Di Chiara, 2002; Sellings & Clarke, 2003). Behaviourally, this has been shown by the direct self-administration of amphetamine into the NAc (Carlezon & Thomas, 2009). In addition, DA release in the NAc is associated with exposure to environments and cues which are related to drug administration (Ito, Dalley, Howes, Robbins, & Everitt, 2000).

As aforementioned, the NAc is divided into two anatomically and functionally distinct subregions, both of which are involved in aspects of drug-related reward (Di Chiara, 2002; Ito et al., 2004). The shell subregion has been associated with the acute rewarding effects of psychostimulants (Pontieri, Tanda, & Di Chiara, 1995; Sellings & Clarke, 2003). Through the application of microdialysis, it has been shown that METH administration increases DA release specifically within the NAc shell (Broom & Yamamoto, 2005). Further, lesions to the shell subregion attenuate the psychostimulant effects of cocaine (Ito et al., 2004). The core, alternatively, is associated with behavioural responses to environmental cues and stimuli that have motivationally significant outcomes (Di Chiara, 2002; Ito et al., 2004). Bilateral lesions to the core have been shown to disrupt conditioned reinforcement between cues and the drug, such that cocaine-seeking behaviour decreased in core-lesioned rats (Ito et al., 2004). Moreover, direct application of the DA receptor antagonist flupenthixol attenuated cue-evoked cocaine seeking in rats experienced at cocaine self-administration, whilst a microinjection of amphetamine facilitated cocaine-seeking behaviour (Saunders, Yager, &

Robinson, 2013). This highlights the influence of DA and the core subregion on behavioural outcomes to motivationally salient stimuli.

Oxytocin modulation of drug-related reward and associated behaviours within the NAc has been minimally examined. Seminal research conducted by Kovacs laboratory found that oxytocin microinjected into the NAc reduced intravenous self-administration of heroin in heroin-tolerant rats (Ibragimov, Kovacs, Szabo, & Telegdy, 1987) and reduced cocaine-induced stereotyped sniffing behaviour (Sarnyai et al., 1991). Since then, only one published study (Baracz et al., 2012) has examined oxytocin modulation of acute drug-related reward within this region and is also the only study thus far to specifically focus on the core subregion. Further, less is known about whether oxytocin modulates chronic exposure to METH within the NAc or within one of its subregions.

1.6.2. Subthalamic nucleus

The STh has traditionally been considered a key substrate involved in modulating motor behaviour and its dysfunction. Relatively recent research has identified that it also plays a crucial role in motivational aspects of both natural and drug reward. The STh is a small nucleus located within the basal ganglia (Parent & Hazrati, 1995). It is ventral to the zona incerta and dorsal to the cerebral peduncle (Nambu, Tokuno, & Takada, 2002). The STh is an input structure, which uniquely is the sole basal ganglia nucleus to have glutamatergic neurons and an excitatory input on its target brain structures (Parent & Hazrati, 1995; Wilson & Bevan, 2011). The STh receives glutamatergic input from the cortex, GABAergic projections from the globus pallidus and ventral pallidum, and dopaminergic input from the midbrain (Bell, Churchill, & Kalivas, 1995; Hamani, Saint-Cyr, Fraser, Kaplitt, & Lozano, 2003; Hassani, Francois, Yelnik, & Feger, 1997; Moriizumi, Nakamura, Kitao, & Kudo, 1987; Mouroux, Hassani, & Féger, 1995). It largely projects to the globus pallidus and substantia nigra pars reticulata; the output nuclei of the basal ganglia (Lintas, Silkis, Alberi, &

Villa, 2012). To a lesser extent, STh neurons also project to the amygdala, striatum and substantia nigra pars compacta (Hassani et al., 1997).

The STh is influential in regulating behaviour associated with higher order cognitive processes. This is related to a direct connection with the medial PFC, whereby disconnection of the cortico-subthalamic pathway in rats has resulted in impairments in attention, perseverative responding and longer response latencies (Chudasama, Baunez, & Robbins, 2003). Bilateral lesions to the STh have also been shown to increase impulsive action and decrease impulsive choice, suggesting that lesions to the STh result in alterations to the attribution of incentive salience (Uslaner & Robinson, 2006; Winstanley, Baunez, Theobald, & Robbins, 2005). Inactivation of the STh reduced the pleasant reinforcing effects of a sweet solution and the negative reinforcement of foot shocks and lithium-chloride induced sickness, demonstrating the involvement of this region in emotional processing (Pelloux, Meffre, Giorla, & Baunez, 2014). High frequency stimulation and pharmacological inactivation of the region have also been shown to block motor stereotypies in rodents sensitised to cocaine (Aliane, Perez, Deniau, & Kemel, 2012).

Beyond the modulation of motor behaviour, the inactivation of the STh differentially modulates motivation for natural and drug reward. Bilateral lesions to the STh or high frequency stimulation increased motivation for food, whilst decreasing motivation for intravenous infusions of cocaine (Baunez, Dias, Cador, & Amalric, 2005; Rouand et al., 2010). The role of the STh on motivation was found to be even more complex when Lardeux and Baunez (2008) identified that preference for alcohol differentially affected motivation following bilateral lesions to the STh. Specifically, motivation for alcohol increased in rats who preferred alcohol, and decreased in rats exhibiting a low preference. Contradictory results have also been reported where STh lesions increased hyperactivity induced by cocaine and assisted in the initiation of cocaine self-administration (Uslaner, Yang, & Robinson, 2005). However, the basal intake of cocaine in this study was particularly low, making it more likely that the increased intake in cocaine self-administration was associated with

acquisition of intake, rather than motivation for cocaine. Stimulation of the STh in humans using deep brain stimulation (DBS) has also shown reductions in pathological gambling in individuals with Parkinson's disease (Ardouin et al., 2006; Bandini, Primavera, Pizzorno, & Cocito, 2007) and addiction to their L-dopa medication (Witjas et al., 2005) and has been proposed as a potential treatment for addiction (Pelloux & Baunez, 2013).

The STh is also associated with cues predicting rewarding outcomes. Specifically, the STh is involved in coding reward-related predictions and reward magnitude (Darbaky, Baunez, Arecchi, Legallet, & Apicella, 2005; Lardeux, Pernaud, Paleressompoulle, & Baunez, 2009). Further, independent neuronal populations in the STh code for different concentrations of sucrose (Lardeux et al., 2009) as well as encode reward value for sucrose or cocaine (Lardeux, Paleressompoulle, Pernaud, Cardor, & Baunez, 2013). Considering that DA is associated with coding reward-related properties, it seems likely that STh neurons associated with reward value and magnitude are modulated by midbrain dopaminergic projections (Hassani et al., 1997; Lardeux et al., 2009). Conversely, little research has examined oxytocin activity within the STh. Indeed, only OTR mRNA expression has been reported in this brain region (Vaccari, Lolait, & Ostrowski, 1998), which does not indicate a functional receptor. Considering this, Carson et al's (2010b) study was not surprisingly, the first to identify that oxytocin modulates activity within this region.

1.7. Oxytocin as a pharmacotherapy

The ability of oxytocin administration to attenuate drug reward, craving, and symptoms of withdrawal highlight its applicability as a pharmacological treatment for drug dependency. This potential is strengthened by studies demonstrating that oxytocin itself does not elicit rewarding effects when centrally administered in rodents (Baracz et al., 2012; Qi et al., 2009) and does not reduce motivational behaviours (Gordon, Martin, Feldman, & Leckman, 2011; Velazquez-Sanchez, Ferragud, Renau-Piqueras, & Canales, 2011). In a

human population, nasal delivery of oxytocin appears to be the most effective (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013) and non-invasive means of administration as systemically administered oxytocin has poor penetration of the blood brain barrier (MacDonald & MacDonald, 2010). Indeed, intranasal administration of oxytocin has been applied in human studies to examine its involvement in evaluating socially salient information (Domes, Heinrichs, Michel, Berger, & Herpertz, 2006; Guastella, Mitchell, & Dadd, 2007), in social interactions (Ditzen et al., 2013; Ditzen et al., 2009; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003), increasing trust (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), and memory and cognition (Bruins, Hijman, & Van Ree, 1992; Fehm-Wolfsdorf, Born, Voigt, & Fehm, 1984; Greenen, Adam, & Baro, 1988) in nonclinical subjects.

Nasal delivery has also been used in psychiatric populations to evaluate the effectiveness of oxytocin in treating the presenting symptoms and associated social and emotional dysfunction. Specifically, the effect of oxytocin intranasal administration in patients with autism spectrum disorder (Anagnostou et al., 2012; Guastella et al., 2010; Hollander et al., 2007; Hollander, Novotny, & Hanratty, 2003), schizophrenia (Bujanow, 1972, 1974; Davis et al., 2014; Gibson et al., 2014; Woolley et al., 2014), obsessive-compulsive disorder (Epperson, McDougle, & Price, 1996), post-traumatic stress disorder (Pitman, Orr, & Lasko, 1993), and social anxiety (Guastella, Howard, Dadds, Mitchell, & Carson, 2009; Labuschagne et al., 2010) have been reported with largely positive findings. Considering that oxytocin has mostly been used to improve social deficits and regulate emotion in a variety of disorders, it is likely that oxytocin administration could also improve psychosocial deficits typically apparent in METH abuse and addiction. However, the limited understanding of the mechanisms of oxytocin action restricts its clinical utility. As aforementioned, it is also unclear how oxytocin is interacting with DA to modulate METH-related reward and METH-seeking behaviour. As such, further investigation into oxytocin action is required. In relation to METH abuse, a means of examining the mechanisms by

which oxytocin is acting is through the utilisation of animal models of reward and addiction, whereby the effect of central oxytocin administration can be investigated in animals that are acutely or chronically exposed to METH.

1.8. Behavioural paradigms utilised for measuring methamphetamine reward and abuse

1.8.1. Conditioned place preference paradigm

The conditioned place preference (CPP) paradigm is a widely used method for examining reward in the laboratory setting. This paradigm employs classical conditioning principles where a distinct set of neutral contextual stimuli are paired with a primary reinforcer to subsequently acquire secondary reinforcing properties (Bardo, Rowlett, & Harris, 1995; Tzschentke, 1998). As such, when exposed to the contextual stimuli in a drug free state, an operant approach response or preference becomes evident (Bardo et al., 1995; Tzschentke, 1998). In humans, a crucial component of the drug taking experience is the environment and cues associated with drug use, as approaching or seeking such stimuli is strongly associated with acquiring and maintaining drug taking behaviour (Bardo & Bevins, 2000). Thus, CPP provides a valid method for examining drug reward and motivation to seek out the drug following cue exposure.

Typically, the conditioning stage of CPP involves pairing a distinct context with a stimulus of interest, and pairing a second, different environment with a neutral stimulus such as saline. The context of each chamber can differ by size, shape, flooring, wall colour, patterns, olfactory cues or a combination (Bardo & Bevins, 2000). Following the conditioning stage, animals are allowed to freely explore both compartments in a drug free state to determine which context they spend more time in. A preference for the context paired with reinforcing stimuli has been established with natural rewards such as food, water, and sex, as

well as with drugs of abuse such as opiates, alcohol, and psychostimulants (Bardo et al., 1995; Mucha, Van Der Kooy, O'Shaughnessy, & Bucenieks, 1982). Generally, four conditioning sessions with each stimuli and compartment is required for the formation of a place preference (Bardo, Gehrke, Shortridge, & Rauhut, 2003). However, a single conditioning session has been shown to be sufficient for the establishment of a place preference to drugs with high reward value, namely morphine (Bardo et al., 2003; Bardo & Neisewander, 1986; Mucha et al., 1982), cocaine (Bardo & Neisewander, 1986), amphetamine (Bardo, Valone, & Bevins, 1999), and methamphetamine (Baracz et al., 2012; Herrold et al., 2009).

There are three approaches that can be applied to quantify the existence and magnitude of a preference for a particular context. The biased approach involves animals being pretested for their compartment or context preference. After identifying the preferred context, this is paired with saline, and the non-preferred compartment is paired with the drug during conditioning. CPP is then determined by the change in time spent in the non-preferred compartment from pre to post conditioning. The unbiased approach does not incorporate pretesting for compartment preference, but rather equally distributes the pairing of each compartment to the drug or vehicle stimuli. Using this approach, CPP is calculated by the difference in time spent on the test day in the drug-paired and vehicle-paired compartments. In the third approach, one group of animals receives the drug and vehicle treatment and another group of animals receive vehicle in both compartments. CPP is then determined by the difference in time spent in the drug-paired context in the drug treated animals and the vehicle treated animals.

The CPP paradigm is an effective means for measuring reward. The capability to conduct a single conditioning session with positive results is of great benefit. This allows for an examination of the neuropharmacological mechanisms involved in acute drug reward in the absence of tolerance or sensitisation (Bardo & Bevins, 2000; Bardo et al., 1999). In addition, as animals are tested in a drug free state, this allows for a measurement of reward, which is not biased or influenced by the sensory and locomotor changes experienced

following drug administration (Mucha et al., 1982). Further, a variety of injection techniques have been utilised to administer treatments during conditioning sessions with a successful outcome, including subcutaneous, intraperitoneal, icv, or intravenous administration (Mucha et al., 1982). As such, this provides choice in route of administration that is not restricted to requiring a surgical procedure. Although, if intracranial surgery is possible, target substrates can be examined for their role in reward processes for the drug under examination, which could provide a greater understanding of reward circuitry. Overall, CPP is a sensitive and reliable procedure for measuring the rewarding properties of a variety of natural and artificial stimuli.

1.8.2. Intravenous drug self-administration procedure paradigm

The self-administration procedure is a common behavioural paradigm utilised within addiction research to model and investigate human drug abuse. In this approach, an animal engages in a particular behaviour, which most commonly is lever pressing, that results in delivery of the stimulus most often through an intravenous catheter. The association between the behavioural response and stimulus exposure is learnt and maintained through both operant and classical conditioning. In regards to operant conditioning, the stimulus acts as the reinforcer, as exposure to the rewarding stimulus increases the likelihood that the behaviour to gain the stimulus will be engaged in in the future (Panlilio & Goldberg, 2007). In addition, classical conditioning strengthens the reoccurrence of this behaviour through an association forming between the reinforcing stimulus and cues in the environment that predict the availability of the stimulus and the onset of its effects (Panlilio & Goldberg, 2007). This procedure has been shown to be effective for examining the reinforcing properties of numerous stimuli including sucrose (Figlewicz, Bennett-Jay, Kittleson, Sipols, & Zavosh, 2011), alcohol (Mello, 1976), nicotine (Corrigall, 1992), cocaine (Ross, Laska, & Fennessy, 1978), amphetamine (Yokel & Pickens, 1974), methamphetamine (Munzar, Baumann,

Shoaib, & Goldberg, 1999), ecstasy (Cornish et al., 2003), heroin (Babor, Meyer, Mirin, McNamee, & Davies, 1976), and mephedrone (Motbey et al., 2013).

The reinforcement of behavioural responses with drug exposure is often based on two forms of scheduling; the number of responses required to receive drug administration or the lapsing of a set amount of time before a response will result in drug delivery. Ratio schedules are more commonly utilised, where the number of responses required for the drug is either fixed or varied (Richardson & Roberts, 1996). The fixed ratio schedule is extensively used as it provides a good indication of whether the stimulus is reinforcing and allows for a direct examination of the relationship between the behaviour and drug intake (Richardson & Roberts, 1996; Sanchis-Segura & Spanagel, 2006). Progressive ratio schedules are typically used to examine the reinforcing efficacy of the drug through examining the motivation of the animal to continue engaging in the response as the ratio of responses required for drug delivery increases (Panlilio & Goldberg, 2007; Richardson & Roberts, 1996). The application of either fixed or progressive ratio schedules were traditionally the only methods used for examining drug-taking behaviour within this paradigm. Modifications to the self-administration procedure have developed to examine different factors and neurobiological changes associated with drug abuse. This includes examining the effect of increased access to the drug, (Ahmed & Koob, 1998; Cornett & Goeders, 2013), the difference between active and passive drug administration (Galici, Pechnick, Poland, & France, 2000), the ability to discriminate between different drugs (Goodwin & Baker, 2000) and the impact of choice on drug administration (Ahmed, 2010).

The self-administration paradigm has additionally been utilised to examine relapse to drug taking following a period of abstinence. This procedure initially involves training the animals to self-administer the drug, then discontinuing drug infusions until the behavioural response associated with drug delivery is reduced to a specified criterion, and lastly, non-contingent exposure to a drug or non-drug trigger (Shaham, Shalev, Lu, De Wit, & Stewart, 2003). Reinstatement to drug-seeking behaviour following a trigger or drug prime is

determined by significantly higher engagement in the behaviour associated with drug delivery in comparison to a control treatment or prior extinction session (Panlilio & Goldberg, 2007; Shaham et al., 2003). Three types of triggers have been found to reinstate previously learnt behaviour, namely exposure to a drug, environmental cue, or stressor (Bossert, Marchant, Calu, & Shaham, 2013). In terms of drug exposure, a priming injection of either the self-administered drug or another reinforcing drug is administered (Sanchis-Segura & Spanagel, 2006). Stress-induced reinstatement typically incorporates exposure to various stressors such as foot shocks, pharmacological stressors, loud noise or bright lights (Bossert et al., 2013; Shaham et al., 2003). Exposure to cues during reinstatement are either those that were present contingently during drug delivery or are new, previously unexposed cues (Sanchis-Segura & Spanagel, 2006). A combination of different triggers can be investigated on separate reinstatement testing sessions to determine each triggers ability to elicit relapse and can provide a greater understanding of relapse in humans.

The self-administration procedure provides an excellent means for investigating human drug abuse and relapse, and for developing and testing interventions for drug-addicted humans. Indeed, the utilisation of this paradigm has been instrumental in the development of effective treatments, including methadone, naltrexone and buprenorphine (Panlilio & Goldberg, 2007). This is largely associated with the high face validity and reliability of the paradigm as the findings produced using this model with animals are similar to those from the human reinstatement model and to patterns of human drug abuse and relapse (Shaham et al., 2003). Further, the self-administration paradigm can be combined with numerous techniques to gain a greater understanding of the neurobiological and neurochemical processes involved in drug abuse and relapse. Such techniques include neuroimaging, microdialysis, microinjecting into specific brain regions, and temporary lesioning of specific brain areas or circuits (Panlilio & Goldberg, 2007). Altogether, the self-administration paradigm can provide valuable insight into neural mechanisms and the development of treatment strategies for drug-addicted humans.

1.9. Aims

The limited research examining the effects of oxytocin on METH reward and abuse emphasises the current limited understanding of oxytocin action in the brain and restricts a thorough understanding of the implications of using oxytocin as a pharmacotherapy for METH dependence. Considering this, the current thesis will examine: i) the mechanisms by which oxytocin is interacting with DA to reduce METH-related reward; ii) the involvement of the STh and NAc core in oxytocin modulation of chronic METH use and iii) the neural changes of the oxytocin system at a cellular level in the NAc core and STh following chronic METH use. This will be primarily investigated through animal models of reward and addiction and incorporate pharmacological, cellular and biochemical techniques.

1.10. References

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Oxytocin modulates dopamine-mediated reward in the rat subthalamic nucleus

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ABSTRACT

The subthalamic nucleus (STh) is increasingly recognized as an important region involved in the motivation for drug reward. It is not yet known if dopamine, the neurotransmitter primarily responsible for reward signaling, is also involved in mediating reward-related activity in the STh. The neuropeptide oxytocin acts within the STh to reduce the rewarding effects of the psychostimulant methamphetamine, through a proposed interaction with dopamine. However, the mechanisms of this interaction are unclear. The current study aimed to determine whether (i) dopamine microinjected into the STh would result in a significant place preference following a single-trial conditioning session, (ii) co-administered dopamine receptor antagonist would block the formation of a conditioned place preference (CPP) for dopamine, (iii) co-administered oxytocin would prevent CPP for dopamine and (iv) whether the selective oxytocin antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT, when co-administered with oxytocin and dopamine, would reverse the effects of oxytocin and result in a CPP for dopamine. Results showed that male Sprague Dawley rats i) formed a preference for the context paired with dopamine (100 nmol/side) administration into the STh, which was prevented by co-administration of ii) the mixed dopamine receptor antagonist fluphenazine (10 nmol/side) or iii) oxytocin (0.6 nmol/side), with the oxytocin effect on dopamine CPP reversed by the co-administration of the oxytocin receptor antagonist (3 nmol/side). These data suggest that dopamine neurotransmission in the STh produces rewarding effects that can be reduced by activation of local oxytocin receptors.

Introduction

The subthalamic nucleus (STh) has traditionally been considered a key brain region modulating basal ganglia motor circuitry (Baunez and Lardeux, 2011; Janssen et al., 2012; Tofighy et al., 2003). More recently, this region has been recognized for its involvement with motivational aspects of natural and drug reward (Baunez and Lardeux, 2011). Both bilateral lesions and deep brain stimulation of the STh reduce the reinforcing effects of ethanol and cocaine and increase the motivation for food as measured through the conditioned place preference (CPP) and self-administration paradigms (Baunez et al., 2005; Lardeux and Baunez, 2008; Rouand et al., 2010).

The role of dopamine neurotransmission in reward has long been implicated and demonstrated by the self-administration of D_1- and D_2-like receptor agonists (Self and Stein, 1992; Sinnott et al., 1999; Woolvertonet al., 1984), the formation of a CPP to systemically administered D_1-like receptor agonists (Abrahams et al., 1998) and the prevention of a place preference for amphetamine through pre-treatments of D_1- and D_2-like receptor antagonists (Bardo et al., 1999). In addition, dopaminergic neurons encode reward-related information (Di Chiara, 1995). As STh neurons are also involved in coding reward magnitude and reward-related predictions (Darbaky et al., 2005; Lardeux et al., 2009), and are innervated by midbrain dopamine neurons, it seems likely that STh neuronal activity is modulated by dopamine signalling (Di Chiara, 1995; Lardeux et al., 2009). However a direct effect of dopamine neurotransmission in the STh on the modulation of reward behaviour has yet to be shown.

The neuropeptide oxytocin has been suggested as a potential novel pharmacotherapy for drug dependence. Oxytocin administration modulates the rewarding effects and abuse potential various illicit drugs, one of which being methamphetamine (METH) (Baracz et al., 2012; Carson et al., 2010a,b; Cui et al., 2001; Kovacs et al., 1985a, 1985b, 1990; Qi et al., 2008, 2009; Sarnyai et al., 1991). We have also recently shown that systematically administered oxytocin reduced METH-induced Fos expression in the STh (Carson et al., 2010b) and oxytocin microinjected into this region reduced the formation of a conditioned place preference (CPP) for METH (Baracz et al., 2012). These studies highlight the involvement of oxytocin in reducing drug reward and the involvement of the STh in this process.

The ability of oxytocin to attenuate drug-related reward is thought to be through the modulation of dopamine neurotransmission (McGregor and Bowen, 2012; McGregor et al., 2008; Qu et al., 2008, 2009; Yang et al., 2010). Oxytocin and dopamine interact to regulate a number of socio-affiliative behaviors in addition to drug reward. Such behaviors include pair bonding (Liu and Wang, 2003), maternal behavior (Shahrokh et al., 2010), social memory (Ferguson et al., 2000), and sexual behavior (Baskerville et al., 2009; Succu et al., 2007). This

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interaction between oxytocin and dopamine can either be facilitatory or inhibitory, depending on the behaviour (Baskerville and Douglas, 2010) and the brain regions involved (Kovacs et al., 1990).

The exact mechanisms by which oxytocin and dopamine interact to reduce drug reward are not well understood. In the STh in particular, very limited research into the function of dopamine and oxytocin has been conducted. Dopamine terminals, as well as D_1 and D_2 receptors have been located within this region (Boyson et al., 1986; Hassani et al., 1997; Johnson et al., 1994), although, dopamine has not been independently investigated for its rewarding effects in the STh. Oxytocin containing cells of the supraoptic nucleus (SON) and paraventricular hypothalamic nucleus (PVN) are known to release oxytocin by volume transmission, affecting diverse midbrain and forebrain areas (McGregor et al., 2008). In addition, the PVN provides classical synaptic transmission to forebrain areas (McGregor et al., 2008), however it is not known if these cells project to the STh. It is known that oxytocin receptor mRNA is expressed in STh neurons (Vaccari et al., 1998), yet the lack of visualization of oxytocin receptors in this area limits our understanding of their location on STh neurons, and how these may interact with dopamine neurotransmission.

The purpose of the present study was to investigate the possible interaction between dopamine and oxytocin in mediating reward behavior in the STh using a single-trial CPP paradigm. Firstly, we examined whether dopamine microinjected into the STh would result in a significant place preference and if this effect was specific for dopamine receptors in the STh. We then examined if co-administered oxytocin would block the formation of a CPP for dopamine administered into the STh. The specificity of oxytocin to block dopamine CPP was then examined by the concomitant antagonism of oxytocin receptors in the STh.

Materials and methods

Animals

One hundred and five male Sprague Dawley rats (weighing 200–250 g) were obtained from the Animal Research Centre (Perth, WA, Australia). Rats were housed in pairs (cage size: 40×27×16 cm) with the exception of a two-day postoperative period of individual housing. Food and water were available ad libitum in the home cages and not during experimental procedures. Lighting was kept on a 12-hour light/dark cycle (lights on 06:00), with all experiments conducted during the light cycle. Housing room temperature was maintained at 21 °C (±1 °C). Prior to the start of experimentation, rats were acclimatized to the facility for seven days and were handled daily for a further seven days. All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004) and were approved by the Macquarie University Animal Ethics Committee.

Drugs

Dopamine hydrochloride (DA) and fluphenazine dihydrochloride were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Oxytocin (OXY) was synthesized by AusPep Ltd (Parkville, VIC, Australia). 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). All drugs were dissolved in saline (0.9%) for injection purposes with the OXY and cocktail solutions freshly prepared for each conditioning day. Vehicle (VEH) administration was a 0.9% saline solution.

Apparatus

The CPP apparatus used were as described previously (Baracz et al., 2012). The three chambers consisted of three compartments separated by two removable guillotine doors. The two side compartments were distinguished by distinct odour cues, these being drops of essential oils (Sunspirit aromatherapy oils) in small caps placed on a tray under the opposite far corner of each compartment. Frankincense oil was placed under one compartment and rosewood oil under the other compartment. Infrared cameras were positioned above each compartment and were used to film activity. Locomotor activity and the time spent in each compartment were recorded by automated video tracking software (Motion Mensura, Cooks Hill, NSW, Australia).

Surgery

Rats were anesthetized with isoflurane gas (3% in oxygen 2 l/min) and placed in a stereotaxic apparatus for bilateral implantation of guide cannulae (26 gauge; 15 mm) to 1 mm above the STh (with nosebar = −3.3 mm, measured from bregma: anterior/posterior, − 3.8 mm; lateral, + 2.5 mm; dorsal/ventral, − 7.0 mm) as previously described (Baracz et al., 2012). Co-ordinates were adapted from the rat brain atlas of Paxinos and Watson (1997). For analgesia, rats were administered with Carpofen (5 mg/kg) subcutaneously (s.c.) at the time of surgery and daily for the following two days. Rats were allowed 5–7 days to recover before experimentation began.

Microinjection procedure

Rats were randomly allocated to one of seven treatment groups (n=15 per group): 1) dopamine (DA; 100 nmol/side), 2) co-administered dopamine and fluphenazine (DA+DA ANT; 10 nmol/side), 3) co-administered dopamine and oxytocin (DA+OXY; 0.6 nmol/side), 4) the low oxytocin receptor antagonist dose (LOW OXY ANT; 1 nmol/side), 5) the high oxytocin receptor antagonist dose (HIGH OXY ANT; 3 nmol/side), and co-administered dopamine and oxytocin with the addition of 6) the low oxytocin receptor antagonist dose (COCKTAIL1) or 7) the high oxytocin receptor antagonist dose (COCKTAIL 2).

The dose of dopamine and fluphenazine used in this study was extrapolated from a known pharmacologically effective dose in the nucleus accumbens (Cornish and Kalivas, 2000). The oxytocin dose was based on our previous study examining oxytocin in the STh (Baracz et al., 2012). As the oxytocin receptor antagonist has not, to our knowledge, been examined in the STh, the low dose was determined from published studies that microinjected the antagonist into other brain regions (Yang et al., 2011) and a logarithmic scale was used to determine the high dose. Rats received a bilateral infusion of treatment or VEH into the STh at a volume of 200 nl/side. Both microinjectors (33 gauge; 16 mm) were attached by polyethylene tubing to a 1 µl Hamilton syringe with infusions being driven by a microinjection pump (Harvard Apparatus, USA). The microinjectors remained in position 30 s after the completion of the microinjection to ensure that the entire dose had infused into the brain region.

Conditioned place preference (CPP) procedure

The CPP procedure (as previously described in Baracz et al. (2012)), consisted of a pre-test, conditioning and post-test.

Pre-test

Rats were placed in the central compartment and were able to freely explore the entire apparatus for 15 min. The odour cues were counterbalanced across side compartments for all treatment groups. Time (s) spent in each compartment was recorded and if, after testing, a large preference was apparent for one compartment, rats were retested up to a maximum of three times until a lesser preference was evident. Thus, rats were retested if their bias was greater than 120 s or if the time spent in the central compartment was more than double the time spent in the two side compartments combined. Typically, most rats reached criterion following the first pre-test session with 25% of the rats requiring testing on the second pre-test and 15% on the

third pre-test. These rats were evenly distributed across treatment groups.

Conditioning

Conditioning sessions began 48 h following the last pre-test day. Two conditioning sessions were conducted (once daily for two consecutive days). On each conditioning day, rats received either a VEH or treatment microinjection with this order counterbalanced such that half of the animals received conditioning with one of the treatments and half received VEH on each conditioning day. To prevent any association between the conditioning compartment and the microinjection procedure, and to capture the peak effective period of the treatments administered, the microinjection was administered 5 min prior to being placed in a conditioning compartment. After this time, rats were confined to the designated conditioning compartment (VEH or treatment) for 30 min.

Post-test

Forty-eight h following the last conditioning session for each experimental condition, the post-test was conducted. Rats, in a drug-free state, were placed in the central compartment and were given free access to the CPP apparatus for 15 min. Time (s) spent in each compartment was recorded.

Histology

Following the completion of the experiments, rats were deeply anesthetized with sodium pentobarbitone (135 mg in 1 ml, i.p.) and underwent intracardiac perfusion with 50 ml of 0.9% saline followed by 50 ml of 10% formalin. Brains were extracted, post-fixed in a 10% formalin solution for seven days, and sliced into 60 µm thick coronal sections using a cryostat. Sections were mounted on gel slides. The rat brain atlas of Paxinos and Watson (1997) was used to verify cannulae placement. Only data from rats with correct cannulae placements were analyzed.

Statistical analysis

Data are presented as the mean ± the standard error of the mean (SEM). CPP was assessed as the difference in time (s) spent in the treatment-paired compartment from pre to post-test in comparison to the difference in time spent in the VEH-paired compartment from pre to post-test. This was analyzed using a two-tailed paired samples t-test (Baracz et al., 2012; Herzig and Schmidt, 2004; Tzschentke, 2004). Therefore a significant difference between the shift in time spent in each side compartment, where more time was spent in the treatment-paired compartment post conditioning, indicated that treatment was rewarding and that a CPP had developed.

Locomotor activity during conditioning sessions in each experiment was analyzed using a mixed analysis of variance (ANOVA) model, with treatment as the between-subjects factor and conditioning days as the within-subjects factor. Any required post-hoc pairwise comparisons were corrected using the Bonferroni rule. Statistical analysis was undertaken using SPSS 19 Graduate Student Version for Mac. Statistical significance was set at $P < 0.05$ for all statistical tests, and for post hoc tests, statistical significance was set in accordance to the Bonferroni decision rule at $P < 0.0071$.

Results

Locomotor activity

As shown in Fig. 1, locomotor activity was not altered by the administration of the treatment or vehicle microinjection across all of the treatment groups during the conditioning sessions. Analysis of locomotor activity on the conditioning days showed no significant difference between treatment and vehicle conditioning days ($F(1,48)$ 2.529, $p = 0.118$), no significant group effect ($F(4,48)$ 1.651, $p = 0.177$) and no significant conditioning day × treatment interaction ($F(4,48)$ 0.467, $p = 0.759$). A paired samples t-test indicated no significant

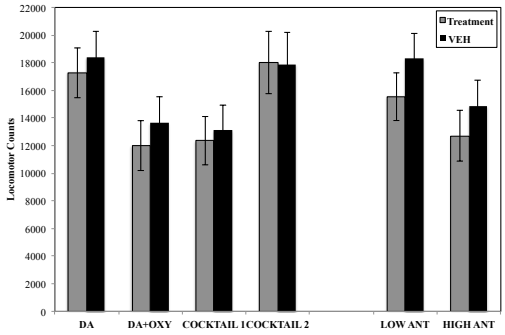

Fig. 1. Effect of single-trial treatment conditioning on locomotor activity. (DA, n=11; DA+DA ANT, n=12; DA+OXY, n=11; COCKTAIL 1, n=12; COCKTAIL 2, n=7; LOW OXY ANT, n=12; HIGH OXY ANT, n=11). Locomotor activity was recorded during the 30 minute conditioning sessions and is expressed as the mean±SEM. There was no significant effect of treatment on locomotor activity compared to VEH.

difference in locomotor activity for the rats in the LOW OXY ANT and the HIGH OXY ANT groups ($t(11) = -1.921$, $p = 0.081$; $t(10) = -1.765$, $p = 0.108$, respectively).

Conditioned place preference

The DA group exhibited a significant preference for the treatment paired compartment in relation to the vehicle-paired compartment from pre to post-test ($t(10) = 3.201$, $p = 0.009$; Fig. 2). A preference for the treatment-paired compartment was not evident in the DA+DA ANT ($t(11) = 1.378$, $p = 0.195$) or the DA+OXY groups ($t(10) = 0.951$, $p = 0.364$).

The COCKTAIL 1 group did not exhibit a significant preference for the treatment-paired compartment relative to the vehicle-paired compartment from pre to post-test ($t(11) = 0.492$, $p = 0.632$). The COCKTAIL 2 group did display a significant preference for the treatment-paired compartment relative to the vehicle-paired compartment from pre to post-test ($t(6) = 2.898$, $p = 0.027$).

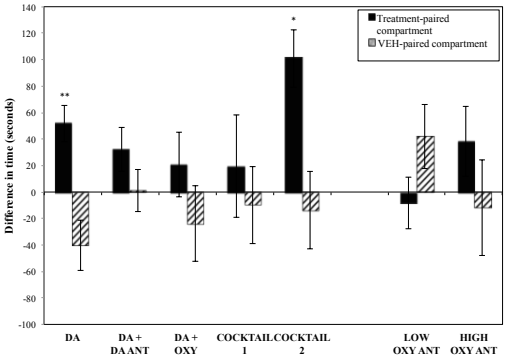

Fig. 2. Effect of single-trial treatment conditioning on CPP for dopamine (DA, n=11;DA+DA ANT, n=12; DA+OXY, n=11; COCKTAIL 1, n=12; COCKTAIL 2, n=7; LOWOXY ANT, n=12; HIGH OXY ANT, n=11). Place preference was determined as the difference in time (seconds) spent in the treatment and VEH-paired compartments from pre to post-test (mean ± SEM). Time spent in the middle compartment has been omitted, as there was no significant difference between testing days. *Pb0.05, **Pb.005, treatment paired compartment vs. VEH-paired compartment

Both the low and the high oxytocin antagonist groups did not display a significant place preference for the treatment-paired compartment relative to the vehicle-paired compartment from pre to post-test ($t(11) = -1.527$, $p = 0.155$; $t(10) = 0.968$, $p = 0.356$, respectively). The data trends towards a dose-dependent effect of producing a reward association to antagonist treatment, with the low dose unable to shift the bias of the preference test and the high dose tending to encourage more time spent in the treatment-paired compartment ($M = 38.2$, $SE = 26.5$) than in the vehicle-paired compartment ($M = -11.7$, $SE = 36.2$) from pre to post-test.

Fig. 3 illustrates the correctly located cannulae in the STh. Rats were removed if the cannulae were not bilaterally located within this brain region. Due to the small brain area this resulted in a total of 29 excluded rats, producing sample sizes of DA = 11, DA + DA ANT = 12, DA + OXY = 11, COCKTAIL 1 = 12, COCKTAIL 2 = 7, LOW OXY ANTAG = 12 and HIGH OXY ANTAG = 11. There were 7 animals that received dopamine microinjections dorsal to the STh into the zona incerta. Dopamine administration dorsal to the STh did not result in a CPP ($t(6) = -0.642$, $p = 0.544$; treatment-paired $M = -19.9$, $SE = 26.9$, saline-paired $M = 12.0$, $SE = 47.1$).

Discussion

The aim of the present study was to investigate: (i) the effect of a dopamine microinjection in the STh on the formation of a CPP using a single-trial conditioning procedure, (ii) the specificity of this effect at dopamine receptors in the STh, (iii) the effect of co-administered oxytocin on the acquisition of a dopamine-induced CPP, and (iv) the effect of microinjecting a cocktail of the oxytocin receptor antagonist, oxytocin and dopamine on the formation of a CPP to dopamine administration. We determined that using a single-trial conditioning session, a dopamine microinjection in the STh resulted in the formation of a place preference that was prevented by the co-administration of a mixed dopamine D_1/D_2 receptor antagonist.

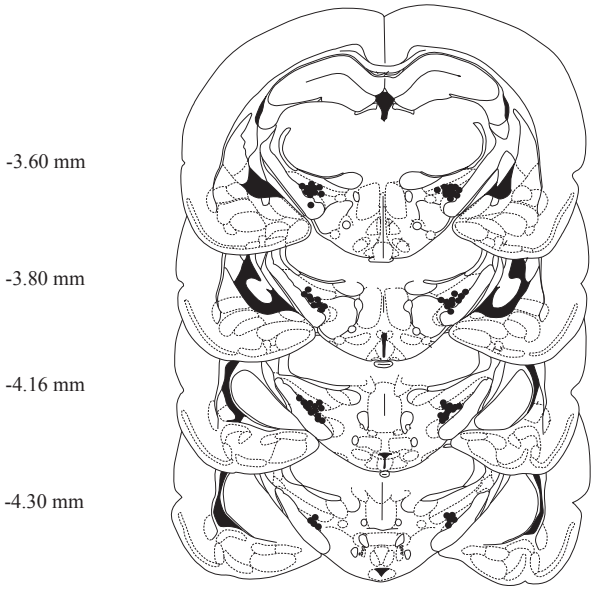

Fig 3. Anatomical coronal diagrams depicting the microinjection sites in the STh. Sites for each treatment group were equally distributed across the AP asix. The numbers to the left of the image depict the distance in mm from bregma.

We also identified that the co-administration of oxytocin and dopamine in the STh prevented the formation of a place preference to dopamine. In addition, we showed that there was a dose-dependent effect of the oxytocin receptor antagonist on the formation of a CPP when co-administered with dopamine and oxytocin. The low dose of the oxytocin receptor antagonist did not alter the attenuating effects of oxytocin on dopamine-related reward, resulting in the prevention of a place preference. In contrast, the high dose appeared to reduce the effect of oxytocin and permitted the formation of a CPP to dopamine. It is also of interest that locomotor activity was not affected by any of the treatment conditions.

Dopamine is well described for regulating motor control (Ikemoto, 2010). As D_1 and D_2 receptors have been identified in the STh (Boyson et al., 1986; Johnson et al., 1994) and this nucleus plays a crucial role in movement disorders such as Parkinson's disease (Coyle and Snyder, 1969; Greer and Williams, 1963), it would seem likely that local administration of dopamine into this nucleus would alter locomotor activity in some way. However, we found no difference in locomotor activity following dopamine administration into the STh when compared to vehicle controls.

The literature on dopamine activity in the STh is sparse, and largely consists of electrophysiological studies. The local application of dopamine agonists or iontophoretic stimulation of D_2 receptors in the STh has produced inconsistent findings, where either firing patterns did not change (Kreiss et al., 1997), were inhibited (Hassani and Feger, 1999), or increased in intact rats (Mintz et al., 1986). As the association between neuronal activity and motor responses is not as strong as previously thought (Wilson and Bevan, 2011), and none of the aforementioned studies examined behavioral responsivity, it is difficult to discern what changes in locomotor activity, if any, would have occurred. Research involving lesions of regions connected to the STh however, have determined that ablations to the striatum or substantia nigra impact on dopamine neuronal firing in the STh in addition to other basal ganglia regions and the motor system, which together alters motor output (Hassani and Feger, 1999; Janssen et al., 2012; Lintas et al., 2012). Together this suggests that the STh is not solely involved in motor co-ordination, but interacts with other regions to produce a behavioral outcome.

Dopamine is strongly associated with reward-related learning (Hyman et al., 2006; Ikemoto, 2010; Koob, 2009; Schultz, 2000) and is primarily involved with the rewarding effects of psychostimulants such as METH (Cruickshank and Dyer, 2009; Elkashef et al., 2008). Again, the effect of dopamine on reward has not been previously examined within the STh. Our study showed that a CPP formed following a microinjection of dopamine into the STh, and that the dopamine receptor antagonist fluphenazine, when microinjected with dopamine, prevented the formation of a place preference. This suggests that dopamine neurotransmission in the STh modulates reward behavior. In addition, our study demonstrated that the co-administration of oxytocin prevented the formation of a CPP for dopamine. Using the same procedures, we have previously determined that an independent microinjection of oxytocin did not alter baseline preferences (Baracz et al., 2012). This suggests that oxytocin is not producing an aversive outcome to negate dopamine reward, but rather is inhibiting the rewarding effects elicited by dopamine administration. A possible dopamine/oxytocin interaction supports previous postulations that oxytocin modulates METH-related reward through reductions in dopamine neurotransmission (McGregor and Bowen, 2012; McGregor et al., 2008; Qi et al., 2008; Yang et al., 2010). It is also known that oxytocin may independently increase reward associations, however this effect appears to be regionally specific (Kovacs et al., 1990), with systemic administration enhancing reward (Baracz et al., 2012), and intracerebroventricular or local microinjection of oxytocin into the STh or nucleus accumbens having no effect in the place preference paradigm (Baracz et al., 2012; Qi et al., 2009).

To gain a greater understanding of a possible interaction between oxytocin and dopamine in modulating reward in the STh, we co-administered the selective oxytocin receptor antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT. This oxytocin receptor antagonist is 95 times more selective for the oxytocin receptor than for the vasopressin V1a receptor (Manning et al., 2008). For this reason, it has been used to examine the role of the oxytocin receptor in numerous behaviors such as pain modulation (Yang et al., 2011) and anxiety (Figueira et al., 2008). However, the oxytocin receptor antagonist has not been previously studied in relation to drug reward and abuse.

The inclusion of the oxytocin receptor antagonist in our study produced an interesting outcome when co-administered with dopamine and oxytocin. The low oxytocin receptor antagonist dose in COCKTAIL 1 was not sufficient to alter the attenuating effect of oxytocin on dopamine. The higher dose in COCKTAIL 2, however, blocked oxytocin activity, resulting in a preference for dopamine that appeared greater than when dopamine was solely administered. It is possible that a tonic level of endogenous oxytocin is present in the STh, which could have

reduced the rewarding effect of independently administered dopamine. A tonic level of oxytocin would also contribute to the ineffectiveness of the low oxytocin receptor antagonist dose on combined dopamine and oxytocin administration. Furthermore, the robust rewarding effect of COCKTAIL 2 may be the result of enhancing the effect of administered dopamine, through high dose antagonism of both exogenous and endogenous oxytocin. This possibility is also consistent with the effect of oxytocin antagonist administration alone, as there was a trend towards a significant place preference when the high dose was administered, yet not with low dose antagonist administration. However, in order to accurately interpret the current data, more research is needed to investigate the presence and role of oxytocin and oxytocin receptors in the STh.

The oxytocin receptor has been identified in numerous brain regions, however, only oxytocin receptor mRNA expression has been reported in the STh (Vaccari et al., 1998), which does not assure that functioning oxytocin receptors are situated within this brain region. If the oxytocin receptor is acting within this region, it may be of low affinity and has not yet been detected by traditional methods such as autoradiography (Freund-Mercier et al., 1988). Indeed, our results demonstrate that within the STh, the high dose of a highly selective oxytocin receptor antagonist blocked oxytocin activity, suggesting that oxytocin receptor activation reduces the CPP to dopamine administration in this region. As the oxytocin receptor has not been visualized on STh neurons, it is unclear if they are located pre- or post-synaptically, to inform how oxytocin modulates this interaction with dopamine administration. In addition to the oxytocin OT receptor, it has been proposed that a further oxytocin receptor subtype exists, and it is not yet known if either subtype is present in the STh (Adan et al., 1995; Chan et al., 2003). Future studies will importantly characterize oxytocin neurotransmission in the STh, and how this may interact with dopamine receptors, including the use of specific receptor ligands across a range of doses.

The ability for oxytocin to reduce METH-related reward, METH induced hyperactivity and relapse to METH-seeking behavior highlights its potential as a pharmacological treatment for METH abuse. The ability for oxytocin to modulate dopamine reward in the absence of reducing motivational behaviors (Gordon et al., 2010, 2011; Melis and Argiolas, 2011) further highlights this peptide as a pharmacological treatment for the effective treatment of drug abuse (Izzo et al., 2001; Kovacs et al., 1990; Velazquez-Sanchez et al., 2011). As oxytocin also reduces the behavioural effects of other psychostimulants such as cocaine (Kovacs et al., 1990; Sarnyai et al., 1991), reduces cannabis withdrawal symptoms (Cui et al., 2001) and physical tolerance and dependence on morphine in rodents (Kovacs et al., 1985b), its applicability as a pharmacotherapy extends beyond METH to include other drugs of abuse. Additionally, we (Baracz et al., 2012) and others (Qi et al., 2009) have previously shown that oxytocin, when administered alone via a central route, does not produce a rewarding effect, further emphasizing the potential of oxytocin as an intranasally administered pharmacotherapy. Oxytocin has already been used pre-clinically as an effective intranasal treatment in human populations, largely examining the involvement of oxytocin in stress responses of drug dependent individuals. A number of clinical trials are listed on the National Institute of Health Clinical Trials registry (USA) and the Australian New Zealand Clinical Trials registry.

In addition to an examination of the applicability of oxytocin as a pharmacotherapy, the involvement of the STh in the effect of oxytocin on METH-related reward, and addiction in general, should be investigated further. This region has recently been considered an input structure of the basal ganglia due to the direct projections it receives from a number of substrates (Baunez and Lardeux, 2011). Additionally, some of the connections are to regions associated with reward, including the prefrontal cortex, ventral pallidum, nucleus accumbens and midbrain dopamine nuclei (Lardeux et al., 2009). It is thought that the STh integrates information received from these regions, and modulates the output of the reward system depending on the salience and nature of the reward (Baunez and Lardeux, 2011). This highlights the involvement of the STh in motivational processes, which is typically considered a frontal function, and its critical role in complex output modulation. Thus a potential central role for the STh in reward and addiction needs to be further examined.

In conclusion, the present study demonstrates that dopamine neurotransmission in the STh elicits a place preference that is blocked by co-administered oxytocin, acting at local oxytocin receptors. This suggests that the oxytocin receptor is present in the STh and also supports important roles for dopamine, oxytocin and the STh in the modulation of drug-related reward.

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Addendum

Page 59 (abstract) and page 60 (right column, paragraph 3):

Where it states that the dose of oxytocin is 0.6 nmol/side, it should read 0.6 pmol/side.

Chapter 3: Oxytocin in the nucleus accumbens core reduces reinstatement of methamphetamine-seeking behaviour in rats

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Cornish, J.L.

Contributed to research design, provided technical assistance and manuscript editing 6%

Everett, N.A.

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Oxytocin in the nucleus accumbens core reduces reinstatement of methamphetamine-seeking behaviour in rats

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ABSTRACT

The psychostimulant methamphetamine (METH) is an addictive illicit drug. Systemic administration of the neuropeptide oxytocin modulates METH-related reward and METH-seeking behaviour. Recent findings demonstrated a reduction in METH-induced reward by oxytocin administration into the nucleus accumbens (NAc) core. It is not known, however, if oxytocin acts in this region to reduce relapse to METH-seeking behaviour. Using the drug reinstatement paradigm in rats experienced at METH self-administration, we aimed to determine whether oxytocin pre-treatment within the NAc core would reduce relapse to METH use and if this could be reversed by the co-administration of the oxytocin receptor (OTR) antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT. Male Sprague-Dawley rats underwent surgery to implant an intravenous jugular vein catheter and bilateral microinjection cannulae in the NAc core. Rats were then trained to self-administer intravenous METH (0.1 mg/kg/infusion) by lever press during 2-hour fixed ratio 1 scheduled sessions for 20 days. Following extinction of lever press activity, the effect of microinjecting saline, oxytocin (0.5 pmol, 1.5 pmol, 4.5 pmol) or co-administration of oxytocin (1.5 pmol) and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (1 nmol, 3 nmol) in the NAc core (500 nl/side) was examined on METH-primed (1 mg/kg, i.p.) reinstatement of drug-seeking behaviour. Our results showed oxytocin directly administered into the NAc core decreased METH-primed reinstatement in a dose-dependent manner. Co-administration of the selective OTR antagonist did not specifically reverse the inhibitory effects of oxytocin on METH priming, suggesting mediation by receptors other than the OTR. These findings highlight an important modulatory effect of oxytocin in the NAc core on relapse to METH seeking.

Keywords Addiction, methamphetamine, nucleus accumbens core, oxytocin, self-administration.

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INTRODUCTION

The psychostimulant methamphetamine (METH) is a potent and addictive illicit drug that is abused frequently worldwide (United Nations Office on Drugs and Crime 2010). Chronic METH use can result in serious and pronounced cognitive (Ornstein *et al.* 2000), neurological (Volkow *et al.* 2001) and psychiatric dysfunction (Dyer & Cruickshank 2007) in addition to physical health problems (Turnipseed *et al.* 2003; Westover, McBride & Haley 2007). The reinforcing properties of METH are associated with prolonged and enhanced functionality of the monoamine neurotransmitter dopamine within the mesocorticolimbic circuit (Koob 1992; Rose & Grant 2008). Currently, pharmacotherapies for METH addiction, including monoamine agonists, mixed monoamine agonists and monoamine antagonists have limited efficacy (Ciketic *et al.* 2012).

The neuropeptide oxytocin has been proposed as a potential pharmacotherapy for drug dependence. Oxytocin administration can reduce the rewarding effects and addictive potential of various illicit drugs, including METH (Qi *et al.* 2008, 2009; Carson *et al.* 2010a,b; Baracz *et al.* 2012; Baracz & Cornish 2013; Cox *et al.* 2013). In particular, intracerebroventricular (icv) administration of oxytocin prevented the acquisition of a place preference for METH and blunted METH-induced hyperactivity (Qi *et al.* 2009). In addition, Carson *et al.* (2010a) showed that intraperitoneal (i.p.) injections of oxytocin reduced METH self-administration, METH- primed reinstatement of METH-seeking behaviour and METH-induced hyperactivity.

Recently, we have reported that the core region of the nucleus accumbens (NAc) is involved in oxytocin modulation of acute METH effects. Specifically, peripherally administered oxytocin reduced acute METH-induced c-Fos expression in the accumbens core (Carson *et al*. 2010b), and a microinjection of oxytocin into this region attenuated the formation of a place preference to METH following a single conditioning session (Baracz *et al*. 2012.

Considerable evidence has identified the NAc as an important brain region for reward and addiction-related mechanisms (Sellings & Clarke 2003; Ito, Robbins & Everitt 2004). The increase in dopamine neurotransmission within this region following psychostimulant administration drives reward processes (Sellings & Clarke 2003). Rats will also self-administer amphetamine directly into the NAc, highlighting the involvement of this region in the reinforcing effects of psychostimulants (Hoebel *et al*. 1983). In addition, exposure to cues associated with drug-taking behaviour increases dopamine activity and release in the NAc, contributing to the motivation to instigate drug-seeking behaviour (Ito *et al*. 2000). The core region, more specifically, is crucial for behavioural responses to stimuli associated with motivationally significant outcomes (Di Chiara 2002; Ito *et al*. 2004), which includes cue-evoked cravings for cocaine in drug-seeking rodents (Saunders, Yager & Robinson 2013). Currently, minimal literature has examined the involvement of the NAc in oxytocin modulation of acute METH-related behaviours (Ibragimov *et al*. 1987; Sarnyai *et al*. 1991). Furthermore, no published studies have investigated whether oxytocin can modulate the effects of chronic exposure to METH when injected into this brain region.

The purpose of the present study was to investigate the ability of oxytocin in the NAc core to modulate reinstatement to METH-seeking behaviour using the drug-induced reinstatement model of intravenous METH self-administration. Firstly, we examined whether oxytocin microinjected into the NAc core would reduce lever pressing associated with METH self-administration when exposed to a METH priming injection after a period of extinction. Secondly, by using a highly selective oxytocin receptor (OTR) antagonist (Manning *et al*. 2008), we examined if oxytocin modulation of METH lever pressing activity was occurring through an action on the OTR in the NAc core.

MATERIALS AND METHODS

Animals

Thirty male Sprague Dawley rats (weighing 200–250 g) were obtained from the Animal Research Centre (Perth, WA, Australia). Rats were housed in pairs (cage size: 40 x 27 x16 cm until week 6 when they were relocated to larger cages: 64 x 20 x 40 cm) with the exception of a 2-day post-operative period of individual housing. Food and water were available *ad libitum* in the home cages and not during experimental procedures. Lighting was kept on a 12-hour light/dark cycle (lights on 6:00 AM), with all experiments conducted during the light cycle to be consistent with our previous research on oxytocin modulation of METH self-administration (Carson *et al*. 2010a). Housing room temperature was maintained at 21°C (± 1°C). Prior to the start of experimentation, rats were acclimatized to the facility for 7 days and were handled daily for a further 7 days. All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004) and were approved by the Macquarie University Animal Ethics Committee.

Drugs

Methamphetamine hydrochloride (METH) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). Oxytocin was synthesized by AusPep Ltd (Parkville, VIC, Australia). The selective OTR 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). All drugs were dissolved in saline (0.9%) for injection purposes with the oxytocin and OTR antagonist cocktail solutions freshly prepared for each reinstatement session. Vehicle administration was a 0.9% saline solution.

Apparatus

Testing was conducted in 16 standard operant response chambers (32 x 25 x 34 cm; Med Associates, St Albans, VT, USA), which were housed in sound-attenuating boxes (41 x 56 x 56 cm) equipped with a fan for masking noise and to provide ventilation. Each chamber was equipped with two retractable levers (one active, one inactive) and a house light. The chambers also contained a metal arm with an adjustable weight and a spring connector, which were attached to a swivel. Polyethylene tubing threaded through the spring connector was attached to a 10 ml syringe driven by an infusion pump (Med Associates) located outside of the sound-attenuating chamber. The tubing exiting from the base of the spring connector was connected to the back mount of the intravenous catheter.

Four infrared photobeam detectors were also positioned on the sidewall of each operant chamber to measure locomotor activity. Active and inactive lever presses, number of infusions and locomotor activity was collected and recorded using MED-PC software (Med Associates).

Surgery

Rats were firstly implanted with a chronic indwelling catheter in the right jugular vein, followed by insertion of bilateral intracranial cannulae to 1 mm above the NAc core. To achieve this, rats were anaesthetized with isoflurane gas (3% in oxygen 2 l/min) and aseptic surgical techniques were used. Catheter implantation, as well as catheter construction, is as previously described (Motbey *et al*. 2013). Subsequently, rats were placed in a stereotaxic apparatus for bilateral implantation of guide cannulae (26 gauge; 14 mm) to 1 mm above the NAc core (with nosebar = −3.3 mm, measured from bregma: anterior/posterior, +1.3 mm; lateral, +1.5 mm; dorsal/ventral, −6.5 mm) as previously described (Baracz *et al*. 2012). Coordinates were adapted from the rat brain atlas of Paxinos & Watson (1997). Rats were treated with 0.2 ml of the antibiotic cephazolin sodium (100 mg/ml) intravenously and the analgesic carpofen (5 mg/kg) subcutaneously at the time of surgery and daily for the following 2 days. Following this, catheter patency was maintained by a daily intravenous flush of 0.2 ml of cephazolin sodium in heparinized saline (300 IU/ml). Rats were allowed 5–7 days to recover before experimentation began.

Acquisition and maintenance of METH self-administration

Rats were allowed to acquire self-administration of METH during 2-hour fixed ratio 1 schedule sessions conducted 5 days a week. At the beginning of each session, catheters were flushed with 0.1 ml heparinized saline (10 IU/ml) and were connected to the infusion line. Lever extension and house light illumination indicated the initiation of the session. Levers were allocated as active or inactive, where the location of the active lever was counterbalanced across chambers. Depression of the active lever delivered a 3-second infusion of METH (0.1 mg/kg/infusion, 0.05 ml), immediately followed by the house light extinguishing and a 20-second time out period, during which depression of the active lever was recorded, yet had no consequences. Depression of the inactive lever had no programmed consequences at any time. To avoid overdose, each rat was limited to a maximum of 60 infusions per session. The session ended when either 2 hours had elapsed or the rat had received 60 infusions of METH, and was indicated by lever retraction and the house light turning off. At the end of each session, the infusion line was disconnected and catheters were flushed with 0.2 ml of cephazolin sodium in heparinized saline solution. Self-administration sessions were undertaken for 20 days.

Extinction

Following the last day of METH self-administration, rats were exposed to daily 2-hour extinction sessions. Depression of the active lever resulted in a saline infusion. Otherwise, the sessions were identical to self-administration sessions. Rats continued under extinction conditions for a minimum of 10 days and until < 25 active lever presses were made per session for 2 consecutive days. During the second week of extinction, rats were given one sham saline microinjection into the NAc core and one i.p. (1 ml/kg saline) injection before the 2-hour session.

Reinstatement and microinjection procedure

Once extinction criteria were met, rats received five reinstatement tests, with three extinction days between each test session. Prior to the reinstatement test session, rats received a bilateral infusion of treatment or vehicle into the NAc core (500 nl/side delivered over 1 minute). Both microinjectors (33 gauge; 15 mm) were attached by polyethylene tubing to a 1µl Hamilton syringe with infusions being driven by a microinjection pump (Harvard Apparatus, Holliston, MA, USA). The microinjectors remained in position for 30 seconds after the completion of the microinjection to ensure the entire dose had infused into the brain region. Five minutes following, rats received either METH (1 mg/kg, i.p.) or vehicle (1 ml/kg, i.p) prime and were then placed in the chamber for 2 hours. Reinstatement conditions were identical to extinction, except for the omission of a maximum infusion criterion.

Experiment treatment conditions

Experiment 1

Prior to their reinstatement test session, rats in experiment 1 (n = 15) received local administration of one of three doses of oxytocin (0.5 pmol, 1.5 pmol or 4.5 pmol/side) or vehicle followed by a METH prime. A Latin square design was used to counter any possible ordering effects of intracranial treatment. On the last reinstatement test session, rats received a bilateral microinjection of the highest oxytocin dose (4.5 pmol) and a vehicle prime to ensure oxytocin alone did not alter lever press activity.

Experiment 2

Experiment 2 was conducted on the basis of local administration of oxytocin into the NAc core producing a significant reduction of METH-induced reinstatement. Upon identifying a significant reduction, reinstatement testing in experiment 2 involved rats (n = 15) receiving a bilateral microinjection of oxytocin (1.5 pmol dose from experiment 1 to facilitate the action of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT), a cocktail of oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (1 nmol in cocktail 1, 3 nmol in cocktail 2) or vehicle followed by a METH prime. Oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2, Thr^4]OVT were co-administered as we have previously

found that desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT successfully reversed the oxytocin effect when microinjected with oxytocin (Baracz & Cornish 2013). Again, a Latin square design was used to counter ordering effects of drug administration. On the last reinstatement test session, rats received a bilateral microinjection of the highest OTR antagonist dose (3 nmol) followed by a vehicle prime to determine that the OTR antagonist alone did not alter lever press activity.

Histology

Following the completion of testing, rats were deeply anaesthetized with sodium pentobarbitone (135 mg in 1 ml, i.p.) and underwent intracardiac perfusion with 50 ml of 0.9% saline followed by 50 ml of 10% formalin. Brains were extracted, post-fixed in a 10% formalin solution for 7 days, and sliced into 60 µm-thick coronal sections using a cryostat. Sections were mounted on gel slides. The rat brain atlas of Paxinos & Watson (1997) was used to verify cannulae placement. Only data from rats with correct bilateral cannulae placement were analysed.

Statistical analysis

Data are displayed as the mean ± SEM. Daily rates of active and inactive lever pressing during self-administration were analysed using a two-way repeated measures ANOVA. Number of infusions and active lever pressing across the 20-day period were also compared using a repeated measures ANOVA to ensure rats acquired METH self-administration. Locomotor activity throughout self-administration was analysed using a repeated measures ANOVA. To assess whether rats extinguished METH-paired responses, mean active lever pressing from the last three METH self-administration sessions was compared with active lever pressing during the extinction sessions using a repeated measures ANOVA, as was changes in locomotor activity. Analysis of reinstatement data was performed on the first hour of the 2-hour sessions. To determine that rats reinstated METH-paired lever responding, active lever pressing for the first hour of the last day of extinction prior to reinstatement was compared with active lever pressing during the first hour of reinstatement using a two-way repeated measures ANOVA. Active and inactive lever pressing on reinstatement were compared using a two-way repeated measures ANOVA to ensure that increased lever pressing activity was not due to hyperactivity. Locomotor activity was also analysed between extinction and reinstatement test days using a two-way repeated measures ANOVA, and across reinstatement sessions using a repeated measures ANOVA. Tests comparing reinstatement test sessions were considered separate a priori hypotheses, and so planned pairwise comparisons were conducted to compare treatment doses with vehicle (experiment 1) as well as to oxytocin treatment (experiment 2; Field 2009). Statistical analyses were performed using SPSS 20 Graduate Student Version for Mac (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

RESULTS

METH self-administration and extinction

Analysis of active [$F(1, 14) = 0.266$, $P = 0.614$] and inactive lever presses [$F(1, 14) = 0.041$, $P = 0.843$] as well as number of infusions [$F(1, 14) = 1.019$, $P = 0.330$] during intravenous self-administration and active lever presses during extinction [$F(1, 14) = 0.571$, $P = 0.462$] showed no significant difference across rat groups in experiments 1 and 2. Locomotor activity across self-administration [$F(1, 14) = 0.571$, $P = 0.462$] and extinction [$F(1, 14) = 0.070$, $P = 0.795$] sessions was also not significantly different across rat groups in both experiments. As such, the two experimental groups were analysed together. Rats acquired intravenous METH self-administration, as indicated by a significant increase in METH intake over the 20-day period [day 1 $M = 13$, SEM = 2; day 20 $M = 33$, SEM = 4; $F(19, 285) = 7.450$, $P < 0.001$; Fig. 1a]. Active lever pressing also increased significantly over the self-administration period [day 1 $M = 19$, SEM = 5; day 20 $M = 58$, SEM = 17; $F(19, 285) = 3.135$, $P < 0.001$]. A significant difference between active and inactive lever responses was measured, indicating rats were able to differentiate between the active lever delivering METH and the inactive lever [$F(1, 15) = 41.476$, $P < 0.001$; Fig. 1b]. Locomotor activity was not significantly different across the 20-day period [$M = 2724.934$, SEM = 326.983; $F(19, 285) = 0.874$, $P = 0.616$; Fig. 1c].

Over the course of extinction sessions, active lever pressing reduced from an average of 30 presses (SEM = 4) on day 1 to two presses (SEM = 1) by the last extinction session (Fig. 1a). Rats in cohort 1 achieved extinction criteria after 16 sessions, whereby a significant reduction in active lever presses, as well as locomotor activity was present across extinction sessions compared with the mean of the last three self-administration sessions [$F(16, 224) = 14.489$, $P < 0.001$; $F(16,112) = 3.934$, $P < 0.001$ respectively].

In cohort 2, rats achieved extinction criteria after 13 sessions where a significant reduction in active lever presses, as well as locomotor activity, occurred across extinction sessions compared with the mean of the last three self-administration sessions [$F(13, 91) = 6.001$, $P < 0.001$; $F(13, 91) = 3.146$, $P = 0.001$ respectively].

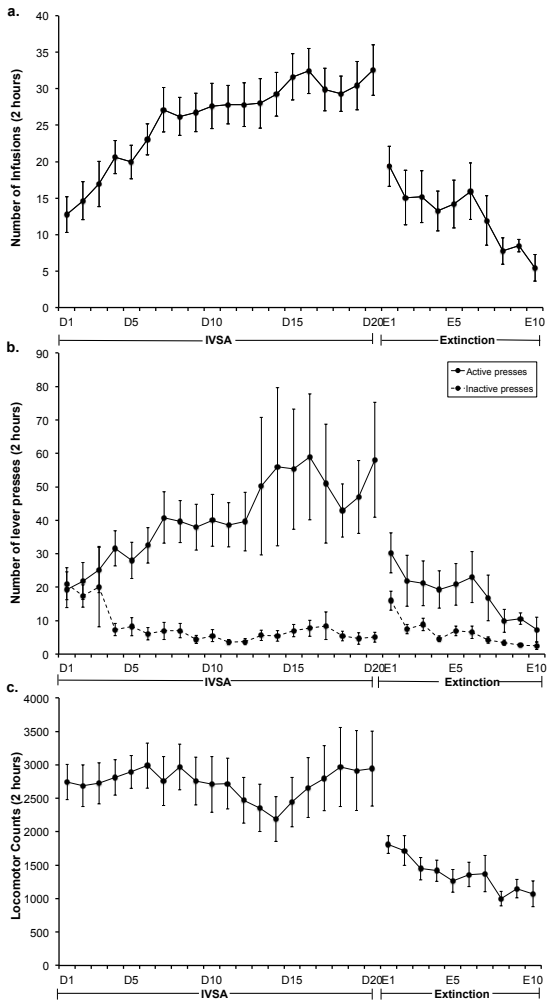

Figure 1 Mean (± SEM) number of a) infusions, b) active and inactive lever presses and c) mean (± SEM) locomotor activity across the 20 days of intravenous METH (0.1 mg/kg) self-administration and extinction. Extinction was conducted for a minimum of 10 days and until less than 25 lever presses were made per session for 2 consecutive days. Only the data from the first 10 days of extinction are displayed

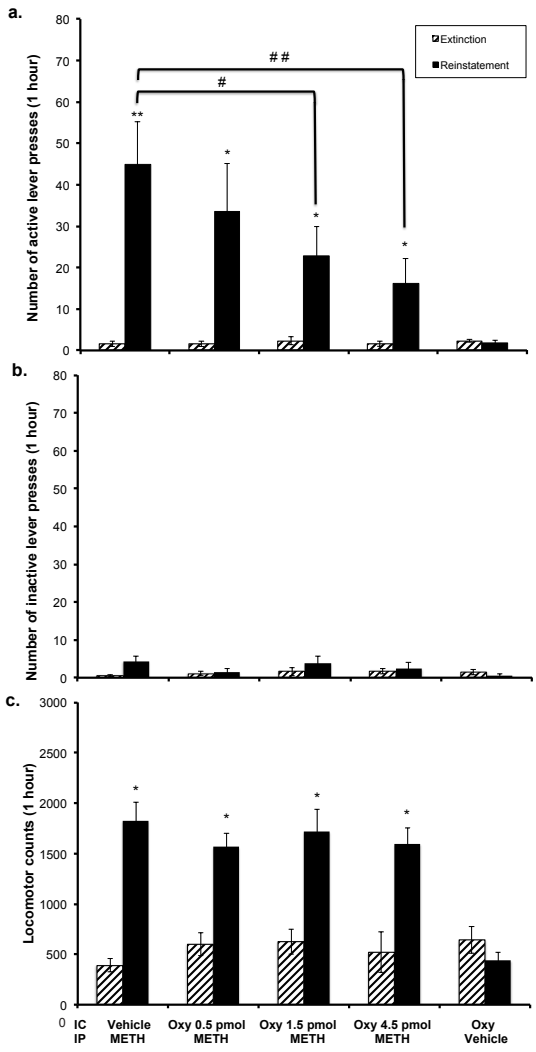

Figure 2 Effects of oxytocin or vehicle microinjection in the NAc core on a) active lever presses, b) inactive lever presses and c) locomotor activity during METH (1 mg/kg, i.p.) primed reinstatement sessions ($n = 8$). #$P < 0.05$, ##$P < 0.005$ versus saline + METH condition; *$P < 0.05$, **$P < 0.01$ versus prior extinction day. Data are presented as mean ± SEM

Experiment 1

Effect of oxytocin on METH-induced reinstatement

METH-primed reinstatement produced active lever presses at a similar level to intravenous METH self-administration, significantly greater than active lever pressing produced on the last extinction day [$F(1, 7) = 14.752$, $P = 0.006$; Fig. 2a]. When comparing each oxytocin dose to vehicle treatment in the NAc core, a significant reduction in active lever pressing was evident when rats received the 1.5 pmol [$F(1, 7) = 9.005$, $P = 0.020$] and 4.5 pmol doses into the NAc core prior to a METH prime [$F(1, 7) = 21.225$, $P = 0.002$]. The 0.5 pmol oxytocin dose did not significantly reduce reinstatement when microinjected prior to the METH prime [$F(1, 7) = 1.813$, $P = 0.220$]. Oxytocin microinjection alone (oxy 4.5 pmol + VEH treatment) into the NAc core did not significantly alter active lever pressing compared with the last extinction day [$t(7) = -1.160$, $P = 0.284$].

Inactive lever pressing was not significantly different across extinction and reinstatement test sessions [$F(1, 7) = 3.932$, $P = 0.088$] nor when oxytocin was administered alone [$t(7) = -1.825$, $P = 0.111$; Fig. 2b]. During reinstatement sessions, inactive lever pressing was significantly lower than active lever pressing following a

METH prime [$F(1, 7) = 12.463$, $P = 0.010$] and when oxytocin was administered alone [$t(7) = -2.393$, $P = 0.048$].

Locomotor activity was significantly higher throughout reinstatement sessions compared with extinction [$F(1, 7) = 106.729$, $P < 0.001$; Fig. 2c]. Locomotor activity was not significantly different across the treatment conditions on METH primed reinstatement test sessions [$F(3, 21) = 0.569$, $P = 0.642$]. Oxytocin administered alone did not significantly change locomotor activity during reinstatement in comparison with the prior extinction session [$t(7) = -1.309$, $P = 0.232$].

Experiment 2

Effect of co-administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT and oxytocin on METH-induced reinstatement

Active lever pressing activity significantly increased during reinstatement testing following a METH priming injection, when compared with the last extinction day [$F(1, 7) = 84.587$, $P < 0.05$; Fig. 3a]. When compared with vehicle treatment in the NAc core, local administration of oxytocin significantly reduced active lever pressing activity produced by a METH prime [$F(1, 7) = 7.783$, $P = 0.027$]. The co-administration of either desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT doses with oxytocin were unable to reverse the modulating effects of oxytocin on METH lever pressing activity when compared with oxytocin microinjection alone [cocktail 1: $F(1, 7) = 0.785$, $P = 0.405$; cocktail 2: $F(1, 7) = 0.188$, $P = 0.677$]. In addition, METH lever pressing activity was not significantly different following the co-administration of either desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT doses with oxytocin in comparison with the vehicle and METH condition (cocktail 1: $F(1, 7) = 0.861$, $P = 0.384$; cocktail 2: $F(1, 7) = 0.869$, $P = 0.382$). The sole administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (3 nmol desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT + VEH treatment) did not alter active lever pressing activity when compared with the extinction day prior [$t(7) = -2.201$, $P = 0.064$].

Inactive lever pressing was significantly higher during reinstatement compared with extinction [$F(1, 7) = 8.260$, $P = 0.024$]; however, active lever pressing was significantly higher during reinstatement ($M = 59$, SEM = 6) than inactive lever pressing ($M = 18$, SEM = 5), suggesting that rats differentiated between the two levers [$F(1, 7) = 96.447$, $P < 0.05$; Fig. 3b] and that a METH prime successfully reinstated previous active lever pressing activity. Inactive lever pressing following the sole administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT was not significantly different to active lever pressing [$t(7) = 0.919$, $P = 0.338$], or to inactive lever pressing on the extinction day prior [$t(7) = -2.084$, $P = 0.076$].

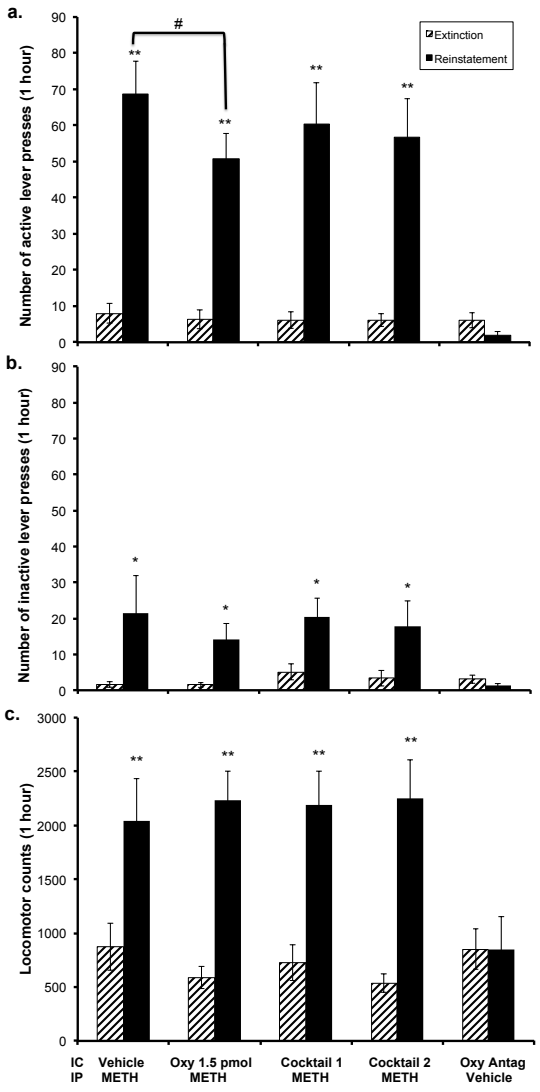

Figure 3 Effects of oxytocin, cocktail 1 (oxytocin and oxytocin antagonist 1 nmol dose) and 2 (oxytocin and oxytocin antagonist 3 nmol dose) or vehicle microinjection in the NAc core on a) active lever presses, b) inactive lever presses and c) locomotor activity during METH (1 mg/kg, i.p.) primed reinstatement sessions ($n = 8$). #$P < 0.05$; *$P < 0.05$, **$P < 0.01$ versus prior extinction day. Data are presented as mean ± SEM

Locomotor activity was significantly higher throughout reinstatement sessions compared with extinction [$F(1, 7) = 36.823$, $P = 0.001$; Fig. 3c]. Across reinstatement test sessions prior to which a drug prime was administered, locomotor activity was not significantly different [$F(3, 21) = 0.212$, $P = 0.887$]. DesGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT administered alone was also unable to significantly increase locomotor activity during reinstatement in comparison with the prior extinction session [$t(7) = -0.012$, $P = 0.991$].

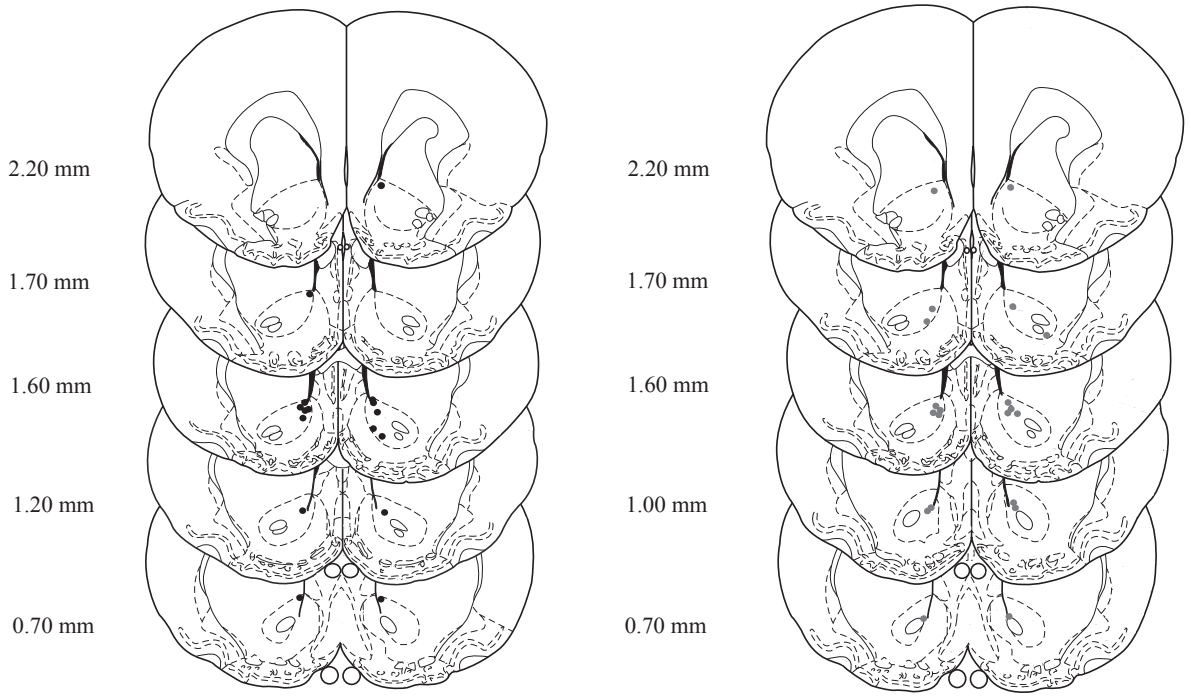

Figure 4 Anatomical coronal diagrams depicting the microinjection sites in the NAc core. The coronal diagram on the left shows the injection sites for experiment 1, and the diagram on the right shows the injection sites for experiment 2. The numbers to the left of the image depict the distance in mm from bregma

Histological analysis

Histological examination of cannulae placement mandated the removal of five rats from the sample because of one or both misplaced guide cannulae (three from experiment 1 and two from experiment 2). Figure 4 shows the correctly located cannulae for experiments 1 and 2.

DISCUSSION

The main finding of this study is that oxytocin microinjected at doses of 1.5 pmol and 4.5 pmol/side into the NAc core reduces reinstatement to METH-seeking behaviour caused by a METH prime. Somewhat surprisingly, the co-administration of an OTR antagonist failed to substantially prevent this action of oxytocin. That is, the effect of oxytocin plus OTR antagonist combination (cocktail) did not significantly differ from oxytocin treatment alone or, from vehicle treatment on METH-induced reinstatement. This suggests a tentative conclusion that the effect of oxytocin to reduce METH priming on reinstatement may be independent of the OTR.

The effectiveness of oxytocin administration in modulating METH-related behaviours is increasingly well documented (Qi *et al*. 2008, 2009; Carson *et al*. 2010a; Baracz *et al*. 2012). The NAc core has been identified as an important brain region involved in acute METH and oxytocin interactions (Carson *et al*. 2010b; Baracz *et al*. 2012). However, the involvement of the NAc core in this interaction following chronic METH administration had not been previously documented. As such, the current study directly examined the involvement of the NAc core in mediating the effects of oxytocin on METH-induced reinstatement, following a period of intravenous METH self-administration (20 days). We show here that oxytocin microinjected into this region reduced METH-primed reinstatement of drug-seeking behaviour in a dose-dependent manner.

To gain a greater understanding of the selectivity of the oxytocin effect on modulating METH-seeking behaviour in the NAc core, we co-administered the selective OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin prior to the METH prime. This OTR antagonist is 95 times more selective for the OTR than for the vasopressin V1a receptor (Manning *et al*. 2008), and so provides insight into the receptor specificity of oxytocin's effects in reducing METH-primed reinstatement. The same antagonist has been used to determine OTR involvement in pain modulation (Yang *et al*. 2011b) and anxiety (Figueira, Peabody & Lonstein 2008), and was previously used by our group to examine OTR involvement in acute METH reward (Baracz & Cornish 2013).

In the present study, co-administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin in the NAc core did not specifically prevent the inhibitory effect of oxytocin on METH priming. Importantly, the OTR antagonist reinstatement test did not alter lever press activity under extinction conditions when administered

into the NAc core alone, suggesting that endogenous oxytocin in this region does not play a role in maintaining extinction. The ineffectiveness of the OTR antagonist is intriguing given that the same compound administered alone (Yang *et al*. 2011a) or in combination with oxytocin (Baracz & Cornish 2013) can be effective. However, conflicting findings on the existence of the OTR in the NAc core have been reported with some studies reporting low densities (Freund-Mercier *et al*. 1987), transient presence through adolescence (Shapiro & Insel 1989) or none at all in this region (Tribollet *et al*. 1988; Adan *et al*. 1995). Studies differentiating between the NAc shell and core have only identified OTRs in the shell region (Veinante & Freund-Mercier 1997). It might be concluded then that OTRs are present, at best, in this region at very low levels, with the caveat that receptor autoradiography is perhaps a problematic technique for identifying OTRs, particularly at presynaptic locations (Dolen *et al*. 2013). In any case, it might be proposed that receptors other than the OTR may be involved in oxytocin effects in the NAc core.

The existence of an additional, currently uncharacterized, OTR subtype has been proposed (Adan *et al*. 1995; Chan *et al*. 2003) and might explain the somewhat ambivalent effect of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT on oxytocin modulation of METH reinstatement. Alternatively, oxytocin is known to bind to vasopressin V1a receptors with reasonable affinity (Tribollet *et al*. 1988) and the vasopressin V1a and V1b receptor subtypes have been located within the core region of the NAc (Hernando *et al*. 2001; Stoop 2012). V1a receptors are increasingly linked to various functional effects of oxytocin (Hicks *et al*. 2014) and it would clearly be useful to explore their role in the effects of oxytocin reported here.

An additional possibility is that oxytocin is modulating METH seeking in the NAc core by activating dopamine D_1 or D_2 receptors. Dopamine terminals, as well as D_1 and D_2 receptors, are distributed greatly throughout the NAc (Boyson, McGonigle & Molinoff 1986). Moreover, oxytocin and dopamine interact to modulate various social and sexual behaviours (Baskerville *et al*. 2009; Shahrokh *et al*. 2010) in addition to drug reward. We have also previously shown that oxytocin microinjected into the subthalamic nucleus blocked the formation of a dopamine-induced place preference, an effect mediated by the OTR (Baracz & Cornish 2013). Indeed, at a cellular level, functional receptor–receptor interactions between dopamine D_2 receptors and OTR have recently been described (Romero-Fernandez *et al*. 2013). This data suggests that oxytocin and dopamine directly interact and may have implications for reciprocal interactions between these two signalling systems in the NAc and with METH-primed reinstatement.

A final possibility is that oxytocin is interacting with delta-subunit-containing gamma-aminobutyric acid-A (GABA-A) receptors in the NAc to modulate METH seeking. Our group has recently found that oxytocin can occupy an ethanol-sensitive site on these receptors to prevent various ethanol effects (unpublished data). These findings highlight a promiscuity of oxytocin at additional receptor types and invites further investigation of regional receptor-specific actions that might influence METH-related behaviours.

Interestingly, the effects of oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT to modulate METH-seeking behaviour were independent of any modulation of METH-induced hyperactivity. METH-induced hyperactivity is a durable and consistent finding (Pontieri *et al*. 1990; Qi *et al*. 2008; Carson *et al*. 2010a,b) and the present study demonstrates higher levels of locomotor activity during the acquisition and maintenance stage of METH self-administration than during extinction (when no METH is present). Unlike previous studies where systemic or icv administration of oxytocin attenuated METH-induced hyperactivity (Qi *et al*. 2008; Carson *et al*. 2010a,b), this hyperactivity was unaffected by oxytocin when directly administered in the NAc core in the current study. This agrees with previous findings, where oxytocin microinjection into the NAc core did not attenuate METH-induced hyperactivity during the conditioning session of the conditioned place preference paradigm (Baracz *et al*. 2012). This suggests that an effect of oxytocin to reduce METH-induced hyperactivity is not occurring through the core region of the NAc and highlights the likely different neural substrates that are involved in stimulant versus reward processes (Gong, Justice & Neill 1997).

The ability for oxytocin to reduce METH-related reward and relapse suggests its potential as a pharmacological intervention for METH abuse. As oxytocin also reduced the behavioural effects of other psychostimulants such as cocaine (Kovacs *et al*. 1990), reduced cannabis withdrawal symptoms (Cui *et al*. 2001), and physical tolerance and dependence on morphine in rodents (Kovacs *et al*. 1985), its applicability as a pharmacotherapy extends beyond METH to include other drugs of abuse. In addition, we (Baracz *et al*. 2012; Baracz & Cornish 2013) and others (Qi *et al*. 2009) have demonstrated that oxytocin, given centrally, does not have a rewarding effect, further emphasizing the potential of oxytocin as a pharmacotherapy. Oxytocin is already being administered intranasally to human clinical populations, including in studies examining its efficacy in reducing stress responses of drug-dependent individuals. A number of clinical trials are listed on the National Institute of Health Clinical Trials registry (USA), EU Clinical Trials registry (Europe) and the Australian New Zealand Clinical Trials registry.

In conclusion, the present study is the first to demonstrate that oxytocin directly administered into the NAc core reduces METH-seeking behaviour and that this may not specifically involve the OTR. This effect of oxytocin is selective for METH-induced priming effects and does not influence the locomotor hyperactivity caused by METH administration. These results highlight a potentially important direction for future pharmacotherapies aimed at assisting in the recovery of METH-dependent individuals.

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Authors Contribution

SJB and JLC were responsible for study concept and design. SJB and NAE collected animal behavioural data. SJB drafted the manuscript. JLC and ISM provided critical revision of the manuscript. All authors critically reviewed content and approved the final version of the manuscript for publication.

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Chapter 4: Oxytocin microinjected into the subthalamic nucleus of the rat reduces reinstatement of methamphetamine-seeking behaviour

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Co-Author Contribution

Cornish, J.L.

Contributed to research design, provided technical assistance and manuscript editing	5%

Everett, N.A.

Provided assistance with running self-administration	2%

4.1. Introduction

The psychostimulant methamphetamine (METH) is a potent and addictive illicit drug that is frequently abused worldwide (United Nations Office on Drugs and Crime, 2010). Chronic METH use can result in serious and pronounced cognitive (Ornstein et al., 2000), neurological (McCann et al., 1998; Volkow et al., 2001) and psychiatric dysfunction (Dyer and Cruickshank, 2007; Harris and Batki, 2000) in addition to physical health problems (Turnipseed et al., 2003; Westover et al., 2007). The reinforcing properties of METH are associated with prolonged and enhanced functionality of the monoamine neurotransmitter dopamine within the mesocorticolimbic circuit (Koob, 1992; Meredith et al., 2005). Currently, the availability of effective pharmacotherapies for METH dependence is limited (Ciketic et al., 2012).

The neuropeptide oxytocin has been proposed as a potential pharmacotherapy for drug dependence. Oxytocin administration has been shown to reduce the rewarding effects and addictive potential of various illicit drugs, one of which being METH (Baracz and Cornish, 2013; Baracz et al., 2012; Carson et al., 2010a; Carson et al., 2010b; Cox et al., 2013; Qi et al., 2009; Qi et al., 2008). In particular, intracerebroventricular (icv) administration of oxytocin prevented the acquisition of a place preference for METH and blunted METH-induced hyperactivity (Qi et al., 2009). In addition, Carson et al. (2010a) showed that intraperitoneal (i.p.) injections of oxytocin reduced the self-administration of METH, reinstatement to METH-seeking behaviour and METH-induced hyperactivity.

Recently, it was discovered that the subthalamic nucleus (STh) is involved in oxytocin modulation of the cellular and behavioural effects produced by acute METH exposure. Specifically, peripherally administered oxytocin reduced METH-induced cellular activation as measured by Fos expression in the STh (Carson et al., 2010b), and a microinjection of oxytocin into this region attenuated the formation of a conditioned place preference for METH (Baracz et al., 2012).

The STh has only recently been associated with drug and natural reward (Baunez et al., 2005; Carson et al., 2010b; Lardeux et al., 2009; Rouand et al., 2010). In particular, lesions to the STh decrease motivation for cocaine and alcohol whilst increasing motivation for sucrose rewards (Baunez et al., 2005; Lardeux and Baunez, 2008). Furthermore, different neuronal populations in this brain region have been shown to selectively respond to cocaine or sucrose reward (Lardeux et al., 2013), as well as code for reward-related predictions and reward magnitude (Darbaky et al., 2005; Lardeux et al., 2009). Considering the published literature, the STh has received little attention for its involvement in reward. In accordance, minimal research has been published examining oxytocin modulation of acute METH reward in the STh (Baracz et al., 2012; Carson et al., 2010b). Furthermore, no published studies have investigated whether oxytocin is also modulating the effects of chronic exposure to METH within this brain region.

The purpose of the present study was to investigate the ability of oxytocin to modulate reinstatement to METH-seeking behaviour in the STh using the reinstatement model of intravenous METH self-administration. Firstly, we examined whether oxytocin microinjected into the STh would reduce responding on the METH-paired lever when exposed to a METH priming injection after a period of extinction of lever press activity (no METH access). Secondly, we examined if oxytocin modulation of METH lever pressing activity was occurring through the activation of the oxytocin receptor (OTR) by the concomitant antagonism of the OTR in the STh.

4.2. Materials and methods

4.2.1. Animals

32 male Sprague Dawley rats (weighing 200-250 g) were obtained from the Animal Research Centre (Perth, WA, Australia). Rats were housed in pairs (cage size: 40 x 27 x 16

cm until week 6 when they were relocated to larger cages: 64 x 20 x 40 cm) with the exception of a two-day postoperative period of individual housing. Food and water were available *ad libitum* in the home cages and not during experimental procedures. Lighting was kept on a 12-hour light/dark cycle (lights on 06:00), with all experiments conducted during the light cycle. Housing room temperature was maintained at 21°C (±1°C). Prior to the start of experimentation, rats were acclimatised to the facility for seven days and were handled daily for a further seven days. All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7^{th} edition, 2004) and were approved by the Macquarie University Animal Ethics Committee.

4.2.2. Drugs

Methamphetamine hydrochloride (METH) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). Oxytocin was synthesised by AusPep Ltd (Parkville, VIC, Australia). The selective OTR 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). All drugs were dissolved in saline (0.9%) for injection purposes with the oxytocin and OTR antagonist cocktail solutions freshly prepared for each reinstatement session. Vehicle administration was a 0.9% saline solution.

4.2.3. Apparatus

Testing was conducted in 16 standard operant response chambers (32 x 25 x 34 cm; Med Associates, St Albans, VT, USA), which were housed in sound-attenuating boxes (41 x 56 x 56 cm) equipped with a fan for masking noise and to provide ventilation. Each chamber

was equipped with two retractable levers (1 active, 1 inactive) and a house light. The chambers also contained a metal arm with an adjustable weight and a spring connector, which were attached to a swivel. Polyethylene tubing threaded through the spring connector was connected to a 10 ml syringe attached to an infusion pump (Med Associates) located outside of the sound-attenuating chamber. The tubing exiting from the base of the spring connector was connected to the back mount of the intravenous catheter.

Four infrared photobeam detectors were also positioned on the sidewall of each operant chamber to measure locomotor activity. Active and inactive lever presses, number of infusions and locomotor activity was collected and recorded using MED-PC software.

4.2.4. Surgery

Rats were firstly implanted with a chronic indwelling catheter in the right jugular vein, followed by insertion of bilateral intracranial cannulae to 1 mm above the STh. To achieve this, rats were anaesthetised with isoflurane gas (3% in oxygen 2 l/min) and aseptic surgical techniques were used. Catheter implantation, as well as catheter construction is as previously described (Motbey et al., 2013). Subsequently, rats were placed in a stereotaxic apparatus for bilateral implantation of guide cannulae (26 gauge; 14 mm) to 1 mm above the STh (with nosebar = - 3.3 mm, measured from bregma: anterior/posterior, - 3.8 mm; lateral, + 2.5 mm; dorsal/ventral, - 7.0 mm), similar to our previous study (Baracz et al., 2012). Co-ordinates were adapted from the rat brain atlas of Paxinos and Watson (1997). Rats were treated with 0.2 ml of the antibiotic cephazolin sodium (100 mg/ml) intravenously and the analgesic carpofen (5 mg/kg) subcutaneously at the time of surgery and daily for the following two days. Following this, catheter patency was maintained by a daily intravenous flush of 0.2 ml of cephazolin sodium in heparinised saline (300 IU/ml). Rats were allowed 5-7 days to recover from surgery before experimentation began.

4.2.5. Acquisition and maintenance of METH self-administration

Rats were allowed to acquire self-administration of METH during 2-hour fixed ratio 1 scheduled sessions conducted 5 days a week. At the beginning of each session, catheters were flushed with 0.1 ml heparinised saline (10 IU/ml) and were connected to the infusion line. Lever extension and house light illumination indicated the initiation of the session. Levers were allocated as active or inactive, where the location of the active lever was counterbalanced across chambers. Depression of the active lever delivered a 3 s infusion of METH (50 µl, 0.1 mg/kg/infusion), immediately followed by the house light extinguishing and a 20 s time out period, during which depression of the active lever was recorded, yet had no consequences. Depression of the inactive lever had no programmed consequences at any time. To avoid overdose, each rat was limited to a maximum of 60 infusions per session. The session ended when either 2 hours had elapsed or the rat had received 60 infusions of METH, and was indicated by lever retraction and the house light turning off. At the end of each session, the infusion line was disconnected and catheters were flushed with 0.2 ml of cephazolin sodium in heparinised saline solution. Self-administration sessions were undertaken for 20 days.

4.2.6. Extinction

Following the last day of METH self-administration, rats were exposed to daily 2-hour extinction sessions. Depression of the active lever resulted in a saline infusion. Otherwise, the sessions were identical to self-administration sessions. Rats continued under extinction conditions for a minimum of ten days and until < 25 lever presses were made per session for two consecutive days. During the second week of extinction, rats were given one sham saline microinjection into the STh and one i.p. (1 ml/kg saline) injection before the 2-hour session.

4.2.7. Reinstatement and microinjection procedure

Once extinction criteria were met, rats underwent reinstatement testing. Each reinstatement test session was separated by three extinction days. Prior to reinstatement, rats received a bilateral infusion of treatment or vehicle into the STh (200 nl/side delivered over 1 minute; as previously described in (Baracz et al., 2012). Both microinjectors (33 gauge; 16 mm) were attached by polyethylene tubing to a 1 µl Hamilton syringe with infusions being driven by a microinjection pump (Harvard Apparatus, USA). The microinjectors remained in position for 30 s after the completion of the microinjection to ensure the entire dose had infused into the brain region. Five minutes following, rats received either a METH (1 mg/kg, i.p.) or vehicle (0.9% saline, i.p.) priming injection and were then placed in the chamber for 2 hours to measure lever pressing activity. Reinstatement conditions were identical to extinction, except for the omission of a max out criteria.

4.2.8. Experiment treatment conditions

4.2.8.1. Experiment 1

During reinstatement, rats (n = 16) received local administration of one of three doses of oxytocin (0.2 pmol, 0.6 pmol, and 1.8 pmol/side) or vehicle followed by METH (1 mg/kg, i.p.). A Latin square design was used to counter the ordering effects of treatment. On the last reinstatement test session, rats received a bilateral microinjection of the highest oxytocin dose (1.8 pmol/side) and a vehicle i.p. injection to test if oxytocin alone was unable to reinstate lever press activity.

4.2.8.2. Experiment 2

Experiment 2 was conducted on the basis of local administration of oxytocin into the STh producing a significant reduction in METH-induced reinstatement. Upon identifying a significant reduction, reinstatement testing in experiment 2 involved rats (n = 16) receiving a bilateral microinjection of oxytocin (most effective dose from experiment one), a cocktail of oxytocin and the OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (3 nmol/side) or vehicle followed by a METH i.p. injection. The dose of OTR antagonist used was based on an effective dose to reduce METH reward in the STh from our previous study (Baracz and Cornish, 2013). On the last reinstatement test session, rats received a bilateral microinjection of the OTR antagonist dose (3 nmol/side) followed by a vehicle i.p. injection to determine that the OTR antagonist alone did not alter reinstatement of lever press activity.

4.2.9. Histology

Following the completion of testing, rats were deeply anaesthetised with sodium pentobarbitone (135 mg in 1 ml, i.p.) and underwent intracardiac perfusion with 50 ml of 0.9% saline followed by 50 ml of 10% formalin. Brains were extracted, post-fixed in a 10% formalin solution for seven days, and sliced into 60 µm thick coronal sections using a cryostat. Sections were mounted on gel slides. The rat brain atlas of Paxinos and Watson (1997) was used to verify cannulae placement. Only data from rats with correct bilateral cannulae placement were analysed.

4.2.10. Statistical analysis

Data is displayed as the mean ± SEM. Daily rates of active and inactive lever pressing during self-administration was analysed using a two-way repeated measures analysis of variance (ANOVA). Number of infusions and active lever pressing across the 20-day period were also compared using a repeated-measures ANOVA to ensure rats acquired METH self-administration. Locomotor activity throughout self-administration was analysed using a repeated measures ANOVA. To assess whether rats extinguished METH-paired responses, mean active lever pressing from the last three METH self-administration sessions was compared to active lever pressing during the extinction sessions using a repeated measures ANOVA, as was changes in locomotor activity. Analysis of reinstatement data incorporated the first hour of the two-hour sessions. To determine that rats reinstated METH-paired lever responding, active lever pressing for the first hour of the last day of extinction prior to reinstatement was compared to active lever pressing during the first hour of reinstatement using a two-way repeated measures ANOVA. Active and inactive lever pressing on reinstatement were compared using a two-way repeated measures ANOVA to ensure that the increased lever pressing activity was not due to hyperactivity. Locomotor activity was also analysed across extinction and reinstatement using a two-way repeated measures ANOVA, and across reinstatement sessions using a repeated measures ANOVA. Tests comparing reinstatement test sessions were considered separate a priori hypotheses, and so planned pairwise comparisons were conducted to compare treatment doses to vehicle (experiment 1) as well as to oxytocin treatment (experiment 2; (Baracz et al., in press; Field, 2009). Statistical analyses were performed using SPSS 20 Graduate Student Version for Mac. Statistical significance was set at $P < 0.05$.

4.3. Results

4.3.1. METH self-administration and extinction

Analysis of active ($F(1,18) = 1.526$, $p = 0.233$) and inactive lever presses ($F(1,18) = 0.442$, $p = 0.515$) as well as number of infusions ($F(1,18) = 1.656$, $p = 0.214$) during intravenous METH self-administration and active lever presses during extinction ($F(1,18) = 0.975$, $p = 0.336$) showed no significant difference across rat groups in experiment 1 and 2. Additionally, locomotor activity during intravenous METH self-administration ($F(1,18) = 1.486$, $p = 0.239$) and extinction ($F(1,18) = 0.269$, $p = 0.610$) was not significantly different across rat groups in experiment 1 and 2. Considering this, the two experimental groups were analysed together.

Rats acquired intravenous METH self-administration, as indicated by a significant increase in METH infusions across the 20 day period (day 1 $M = 15$, $SEM = 3$, day 20 $M = 39$, $SEM = 3$; $F(19,361) = 16.582$, $p < 0.005$; see figure 1a). A significant increase in active lever pressing was also evident (day 1 $M=24$, $SEM = 5$; day 20 $M = 55$, $SEM = 7$; $F(19,361) = 8.842$, $p < 0.005$). Active and inactive lever pressing was significantly different, indicating rats were able to differentiate between the two levers ($F(1,19) = 151.937$, $p < 0.005$; see figure 1b). Locomotor activity across the self-administration period was not significantly different across the 20-days ($F(3.539, 67.245) = 0.728$, $p = 0.560$; see figure 1c).

Figures 1a and 1b show the mean number of active and inactive lever presses and locomotor activity over the extinction period for rats in both experiment 1 and 2. Throughout the duration of the extinction period, active lever pressing reduced from an average of 43 ($SEM = 5$) on day 1 to 10 ($SEM = 2$) on the last extinction session. Rats in experiment 1 achieved extinction criteria after 19 sessions, where a significant reduction in active lever pressing, as well as locomotor activity was evident across extinction sessions compared to the average of the last three self-administration sessions ($F(13, 130) = 17.049$, $p < 0.005$; $F(13, 130) = 5.988$, $p < 0.005$ respectively). In experiment 2, rats achieved extinction criteria

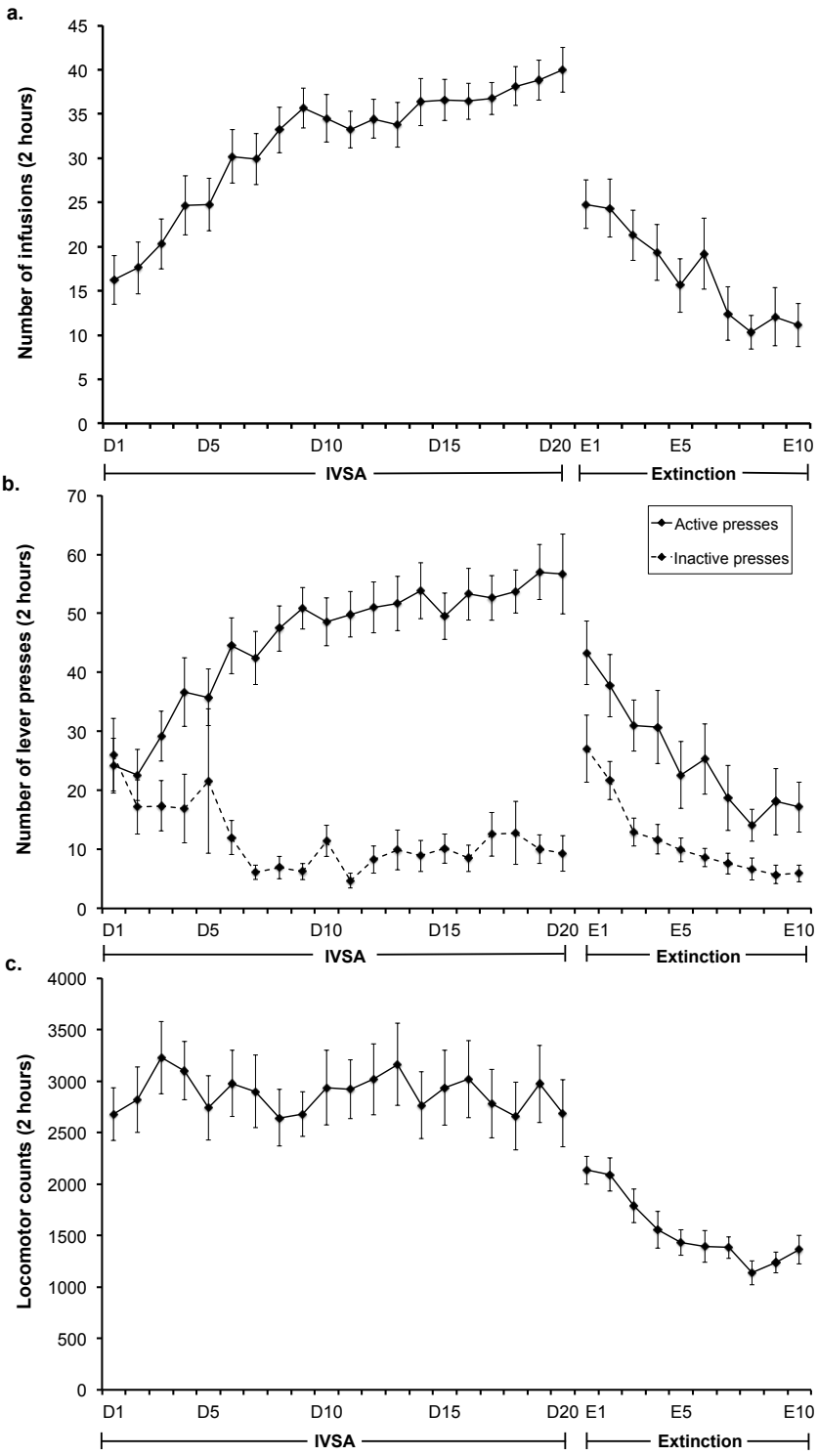

Fig 1. Mean (± SEM) number of a. infusions, b. active and inactive lever presses, as well as c. mean (± SEM) locomotor activity across the 20 days of intravenous METH (0.1mg/kg) self-administration and extinction. Extinction was conducted for a minimum of 10 days and until less than 25 lever presses were made per session for two consecutive days. Only the data from the first 10 days of extinction is displayed in comparison to the mean of the last three days of self-administration.

after 13 sessions. A significant reduction in active lever presses, as well as locomotor activity, was present across extinction sessions compared to the mean of the last three self-administration sessions ($F(16, 368) = 22.021$, $p < 0.005$; $F(16, 128) = 5.823$, $p = < 0.005$ respectively).

4.3.2. Experiment 1

4.3.2.1. Effect of oxytocin on METH-induced reinstatement

Rats reinstated to their previous active (METH-paired) lever pressing activity following a METH priming injection when compared to active lever pressing on the last extinction session ($F(1,10) = 29.289$, $p < 0.005$; figure 2a). When considering the microinjection treatments administered prior to the METH priming injection, active lever pressing following the 0.2 pmol, 0.6 pmol and 1.8 pmol/side oxytocin doses were not significantly different to vehicle ($F(1,10) = 0.522$, $p = 0.486$; $F(1,10) = 2.197$, $p = 0.169$; $F(1,10) = 1.233$, $p = 0.293$ respectively). Oxytocin microinjected alone (oxytocin 1.8 pmol/side + vehicle i.p. injection) did not significantly alter active lever pressing compared to the last extinction day ($t(10) = 0.186$, $p = 0.856$).

Inactive lever pressing was significantly higher during reinstatement compared to extinction ($F(1, 10) = 16.076$, $p = 0.002$; figure 2b), although active lever pressing was significantly higher during reinstatement ($M = 59.227$, $SEM = 7.242$) than inactive lever pressing ($M = 14.205$, $SEM = 3.211$), suggesting rats continued to differentiate between the two levers ($F(1,10) = 29.289$, $p < 0.005$). Inactive lever pressing during reinstatement testing was not significantly different to active lever pressing after oxytocin was solely administered ($t(10) = 0.887$, $p = 0.396$), or to inactive lever pressing on the extinction day prior ($t(10) = 0.647$, $p = 0.532$).

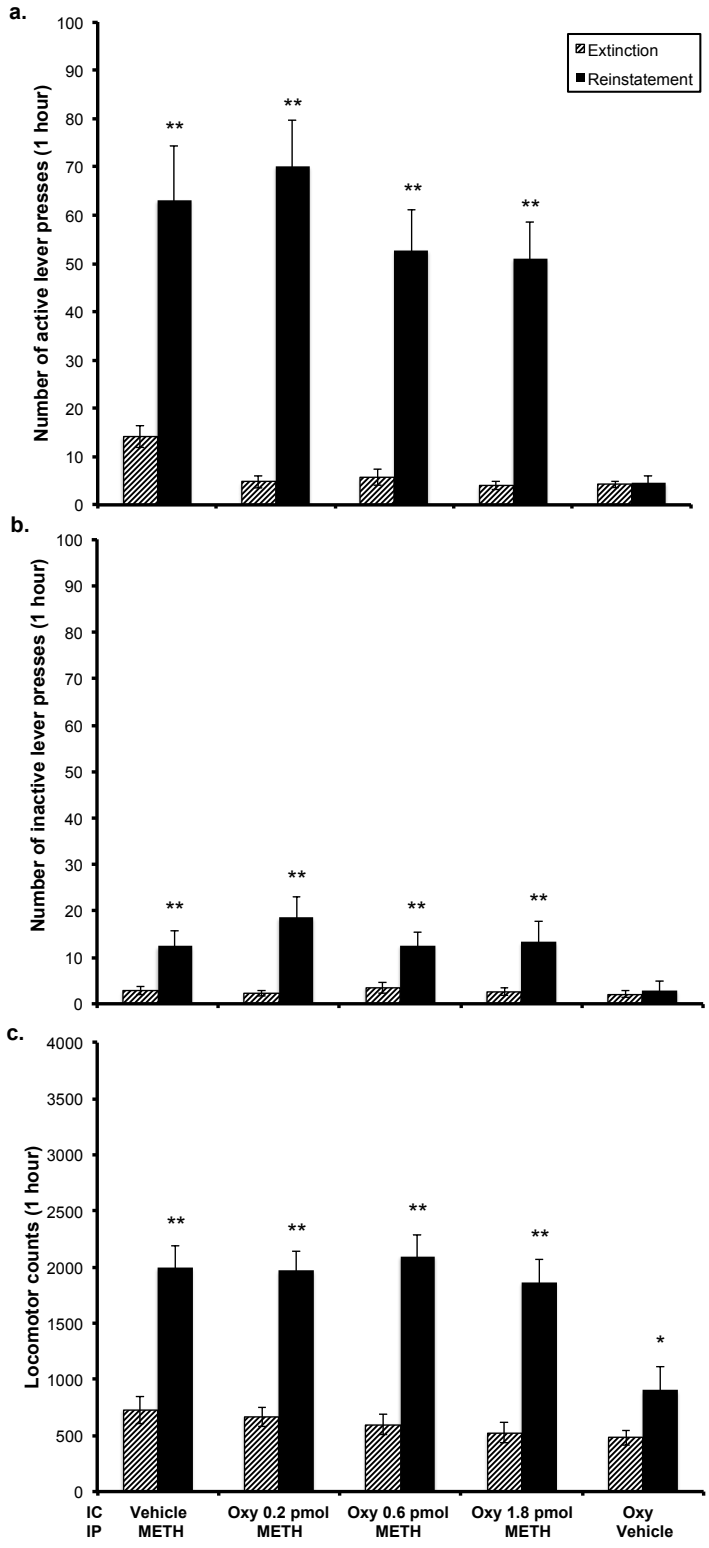

Fig 2. Effects of oxytocin or vehicle microinjection in the STh on a. active lever presses, b. inactive lever presses, and c. locomotor activity during METH (1mg/kg, i.p.) primed reinstatement sessions (n=8). * $p < 0.05$, ** $p < 0.01$ vs prior extinction day. Data are presented as mean ± SEM.

Locomotor activity was significantly higher during reinstatement sessions in comparison to extinction sessions ($F(1, 10) = 81.755$, $p < 0.005$; figure 2c). Across reinstatement test sessions prior to which a METH prime was administered, locomotor activity was not significantly different ($F(3, 30) = 1.763$, $p = 0.175$). Oxytocin administered alone significantly increased locomotor activity during reinstatement in comparison to the prior extinction session ($t(10) = 2.577$, $p = 0.028$).

4.3.3. Experiment 2

On the basis of a slight trend in oxytocin reducing METH-induced reinstatement being identified in experiment 1, we tested a higher oxytocin dose of 3.6 pmol/side for the antagonist study. If successful, further reinstatement testing incorporating desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT would be conducted. As such, the first two reinstatement sessions involved counterbalancing of the vehicle and oxytocin microinjections, and on the third session, all rats would receive a cocktail of oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (3 nmol/side).

4.3.3.1. Effect of higher oxytocin dose and co-administered desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin on METH-induced reinstatement

Rats lever pressing activity significantly increased during reinstatement sessions following a METH prime compared to the extinction day prior to test ($F(1, 8) = 83.773$, $p < 0.005$; figure 3a). In comparison to vehicle, a microinjection of the 3.6 pmol/side dose of oxytocin into the STh significantly reduced active lever pressing when administered prior to a METH i.p. injection ($F(1,8) = 6.118$, $p = 0.039$). The co-administration of this oxytocin dose with desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT into the STh, however, did not significantly alter

METH lever pressing activity when compared to the vehicle microinjection treatment (*F(1,*8) = 1.306, $p = 0.286$). Additionally, the co-administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin was unable to reverse the modulating effect of oxytocin on METH lever pressing activity when compared to the oxytocin microinjection treatment (*F(1,*8) = 1.533, $p = 0.251$). The sole administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT did not significantly alter active lever pressing activity when compared to the extinction day prior ($t(8) = 0.693$, $p = 0.508$).

Inactive lever pressing activity was significantly higher during reinstatement compared to extinction ($F(1,8) = 16.764$, $p = 0.003$; figure 3b), although active lever pressing was significantly higher ($M = 75.704$, $SEM = 7.958$) than inactive lever pressing ($M = 34.222$, $SEM = 8.093$) on reinstatement suggesting rats continued to differentiate between the two levers ($F(1,8) = 26.002$, $p = 0.001$). Inactive lever pressing was significantly higher when desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT was administered alone on reinstatement in comparison to extinction ($t(8) = 3.507$, $p = 0.008$), although was not significantly different to active lever pressing on reinstatement ($t(8) = 2.111$, $p = 0.068$).

Locomotor activity was significantly higher during reinstatement compared to extinction ($F(1,8) = 184.158$, $p < 0.005$; figure 3c). Motor activity induced by METH administration was not significantly different across the reinstatement sessions ($F(1.038,16) = 3.867$, $p = 0.083$). DesGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT administered alone did not significantly alter locomotor activity throughout reinstatement compared to the prior extinction session ($t(8) = 0.550$, $p = 0.598$).

4.3.4. Histological analysis

Histological examination of cannulae placement mandated the removal of five rats from the sample due to one or both misplaced guide cannulae (three from experiment 1 and

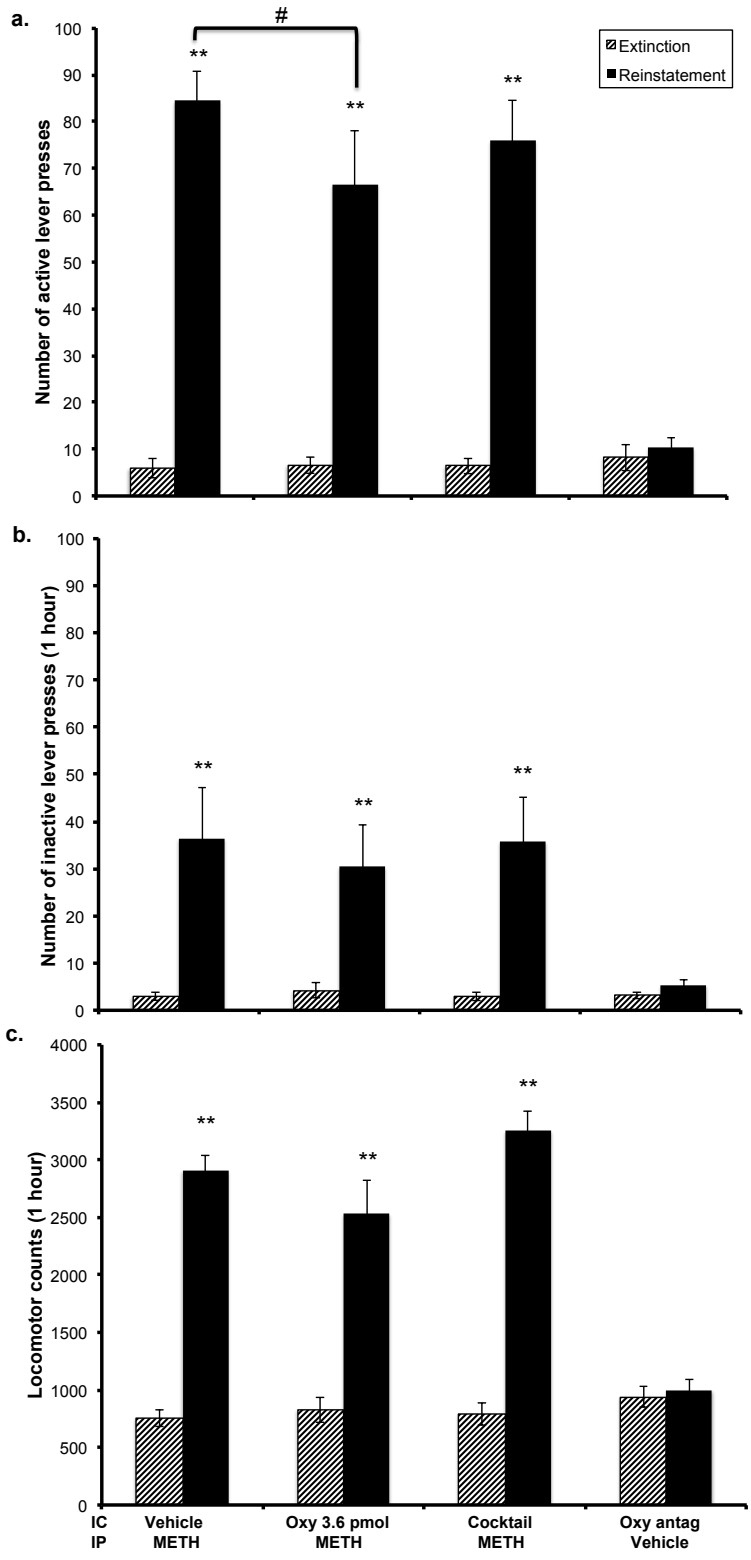

Fig 3. Effects of oxytocin, cocktail, or vehicle microinjection in the STh on a. active lever presses, b. inactive lever presses, and c. locomotor activity during METH (1mg/kg, i.p.) primed reinstatement sessions (n=8). # $p < 0.05$ vs. saline + METH condition; ** $p < 0.01$ vs prior extinction day. Data are presented as mean ± SEM.

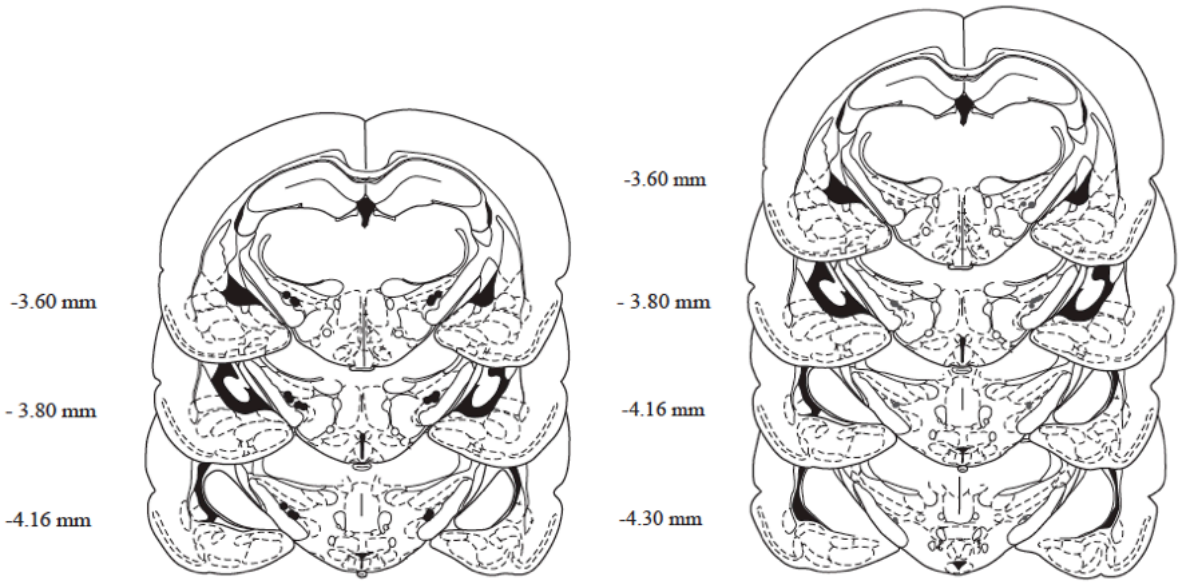

Fig 4. Anatomical coronal diagrams depicting the microinjection sites in the STh. The coronal diagram on the left shows the injection sites for experiment one, and the diagram on the right shows the injection sites for experiment two. The numbers to the left of the image depict the distance in mm from bregma.

two from experiment 2). Figure 4 shows the correctly located cannulae for experiment 1 and 2.

4.4. Discussion

In the present study, oxytocin administered at the highest dose of 3.6 pmol/side into the STh significantly decreased reinstatement to METH-induced lever pressing activity. The co-administration of the selective OTR antagonist with oxytocin, however, did not significantly change METH lever pressing activity when compared to both vehicle and oxytocin treatment. This suggests that oxytocin modulation of relapse to METH-seeking behaviour may be independent of activity at the OTR.

The ability of oxytocin administration to attenuate METH-related behaviours after acute and chronic drug exposure has previously been documented (Baracz et al., 2012; Carson et al., 2010a; Qi et al., 2009; Qi et al., 2008). The STh has also been identified as a key region involved in acute METH and oxytocin interactions (Baracz et al., 2012; Carson et al., 2010b), however the importance of this neuropeptide in regulating the STh in METH-seeking behaviour has not yet been reported. The current study is the first to directly examine the involvement of the STh in mediating the effects of oxytocin on METH-induced reinstatement. At the doses initially examined (0.2 pmol, 0.6 pmol, and 1.8 pmol/side), a significant reduction in lever pressing activity was not identified when oxytocin was microinjected into the STh prior to a METH prime. This result was surprising considering we have previously found the 0.6 pmol/side dose microinjected into the STh to be effective in attenuating the formation of a conditioned place preference to a single pairing with METH (Baracz et al., 2012). On testing of a higher oxytocin dose (3.6 pmol/side), we found oxytocin to be effective in significantly reducing METH-primed reinstatement.

It has previously been shown that the oxytocin system is dysregulated following chronic illicit drug exposure. You and colleagues (2000) identified that rodents chronically exposed to morphine experienced a decrease in oxytocin synthesis in the hypothalamus. Additionally, a decrease of oxytocin content in blood plasma, the hippocampus, and the hypothalamus was evident in rats following a 4-day treatment schedule of twice daily cocaine injections (Sarnyai et al., 1992b). In the current study we have shown that a dose of oxytocin (0.6 pmol/side) that is effective in preventing acute METH reward (Baracz et al., 2012) did not reduce METH-seeking behaviour in rats that have been exposed to chronic intravenous METH self-administration for 20 days. This suggests that the chronic exposure to METH has likely altered the oxytocin system to necessitate higher doses of oxytocin administration to reduce METH-related behaviours. We are currently investigating the effect of chronic METH self-administration on the oxytocin system and OTR distribution to determine the mechanisms behind this functional change.

To further our understanding of the selectivity of oxytocin activity in the STh on attenuating METH-seeking behaviour, we co-administered the high affinity OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin (Manning et al., 2008). This potent OTR antagonist has been used to determine the involvement of the oxytocin receptor in various behaviours, namely pain modulation (Yang et al., 2011) and anxiety (Figueira et al., 2008), and has previously been used by our group to determine oxytocin mechanisms in acute METH reward (Baracz and Cornish, 2013) and chronic METH exposure (Baracz et al., in press).

The co-administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin into the STh produced an interesting outcome. DesGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT did not specifically reverse the oxytocin effect on METH-induced lever pressing activity. This was quite surprising considering we have previously examined the effectiveness of this dose in the STh and found it was sufficient to block the modulating effect of oxytocin on the formation of a conditioned place preference for dopamine (Baracz and Cornish, 2013). However, we have

recently reported that the effect of oxytocin administration into the nucleus accumbens to reduce METH-primed reinstatement was also not specifically affected by OTR antagonism (Baracz et al., in press). As such, it is likely that OTR functioning has changed with chronic METH exposure. Indeed, Zanos and colleagues (2014) showed that after 10 days of METH i.p. injections in rodents, the OTR was upregulated in the amygdala and hypothalamus. Changes to the functionality or expression of the OTR in the STh offers a potential theory for the limited effect desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT administration had on reversing the oxytocin effect on METH lever pressing activity following chronic METH administration.

The effect of oxytocin to reduce METH primed reinstatement was not specifically affected by antagonism of the OTR to suggest that oxytocin may be activating alternate receptors beyond the currently identified OTR to modulate METH-induced reinstatement. An additional, currently uncharacterised OTR subtype has been proposed (Adan et al., 1995; Chan et al., 2003) and could help explain the inhibitory effect of oxytocin on relapse to METH-seeking behaviour. Alternatively, oxytocin may have acted through vasopressin receptors. Oxytocin is known to bind to vasopressin receptors with reasonable affinity (Tribollet et al., 1988) and the vasopressin V1a receptor has been associated with a number of functional effects of oxytocin (Caldwell et al., 2008; Hicks et al., 2014; Ramos et al., 2013; Song et al., 2014). However, neither the vasopressin V1a or V1b receptors, or the OTR have been localised in the STh using traditional methods of receptor autoradiography, although this is a problematic technique with limited sensitivity (Freund-Mercier et al., 1988). While we have shown a functional effect of oxytocin, through the OTR, to reduce METH reward (Baracz and Cornish, 2013) it would clearly be of benefit to examine whether oxytocin is also acting through a functioning V1a receptor in the STh to modulate METH primed reinstatement.

The effects of oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT administration to the STh on METH-seeking behaviour were in the absence of any changes to METH-induced hyperactivity. Methamphetamine administration has consistently been shown to increase

locomotor activity in rodents (Carson et al., 2010a; Carson et al., 2010b; Pontieri et al., 1990; Qi et al., 2008). In line with this, we found that rats exhibited greater locomotor activity during intravenous METH self-administration than in the absence of METH during extinction. Systemic or icv administration of oxytocin has previously been shown to reduce METH-induced hyperactivity (Carson et al., 2010a; Carson et al., 2010b; Qi et al., 2008). Our current findings demonstrate that METH hyperactivity was not affected by a microinjection of oxytocin or the co-administration of oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT into the STh. This is in agreement with our previous studies where we examined the involvement of specific brain regions in oxytocin modulation of METH reward and abuse. Namely, we found that oxytocin microinjected into the STh was unable to attenuate METH-induced hyperactivity during the conditioning stage of the conditioned place preference paradigm (Baracz et al., 2012) and that co-administration of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin into the nucleus accumbens core did not reduce METH-induced hyperactivity on reinstatement to METH-seeking behaviour (Baracz et al., in press).

Systemically administered oxytocin in the absence of drug administration typically causes sedation or no changes to rodent locomotor activity (Qi et al., 2008; Shirley et al., 2011; Uvnas-Moberg et al., 1994). Our findings, however showed locomotor activity increased in the oxytocin and vehicle condition when compared to the prior extinction session. This increase in locomotor activity was in the absence of an increase in active or inactive lever pressing. Interestingly, we have previously found that oxytocin solely microinjected into the STh increased locomotor activity in the absence of a conditioned place preference forming (Baracz et al., 2012). Our current findings, in combination with our previous results highlights the likely different neural substrates that are involved in stimulant and reward processes (Gong et al., 1997).

The reduction in METH-related reward and relapse to METH-seeking behaviour following oxytocin administration emphasizes the potential effectiveness of oxytocin as a pharmacotherapeutic treatment for METH dependence. The applicability of oxytocin as a

pharmacotherapy also extends to other drugs of abuse as oxytocin administration reduces the abuse and addictive properties of cocaine, opiates, cannabis, and alcohol in rodents (Cui et al., 2001; Kovacs et al., 1985; Kovacs et al., 1990; McGregor and Bowen, 2012). Further, oxytocin itself does not elicit rewarding effects following administration (Baracz and Cornish, 2013; Baracz et al., 2012; Qi et al., 2009), providing additional support for its use as a pharmacotherapy. It is also possible that repeated intranasal oxytocin administration may help restore the dysregulated oxytocin system following drug abuse (Baskerville and Douglas, 2010). Clinical trials examining the effectiveness of intranasal oxytocin administration in human populations abusing various illicit drugs such as cannabis, opiods, and alcohol are currently listed on the National Institute of Health Clinical Trials registry (USA), EU Clinical Trials registry (Europe), and the Australian New Zealand Clinical Trials registry. However, clinical trials examining oxytocin intranasal administration on reducing METH dependence are yet to be listed.

In conclusion, the present study is the first to show that oxytocin microinjected into the STh reduces METH-seeking and that oxytocin modulation of this behaviour does not appear to specifically involve the OTR. The oxytocin effect is specific for METH reward and not METH-induced hyperactivity. These findings highlight an important direction for research into pharmacotherapeutic treatment for METH abuse and addiction.

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Chapter 5: Changes to oxytocin receptor expression in the nucleus accumbens core and subthalamic nucleus following chronic intravenous methamphetamine self-administration

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5.1. Introduction

Methamphetamine (METH) is a highly addictive, illicit psychostimulant that is commonly abused on a global scale (United Nations Office on Drugs and Crime, 2010). Chronic METH abuse is associated with a range of pronounced cognitive (Ornstein et al., 2000) and neurological deficits (Volkow et al., 2001), psychiatric symptoms (Dyer & Cruickshank, 2007), and physical health problems (Turnipseed, Richards, Kirk, Diercks, & Amsterdam, 2003; Westover, McBride, & Haley, 2007). The reinforcing properties of METH are related to the prolonged and enhanced functionality of the monoamine neurotransmitter dopamine within the mescorticolimbic circuit following drug administration (Koob, 1992; Rose & Grant, 2008). At present, current pharmacological treatments for METH dependence have limited efficacy (Ciketic, Hayatbakhsh, Doran, Najman, & McKetin, 2012).

The neuropeptide oxytocin has been proposed as a potential pharmacotherapy for drug dependence as it has been shown to modulate the rewarding effects and abuse potential of various illicit and licit drugs including alcohol (McGregor & Bowen, 2012; Szabo, Kovacs, & Telegdy, 1989), heroin (Kovacs, Borthaiser, & Telegdy, 1985a), morphine (Kovacs, Horvath, Sarnyai, Faludi, & Telegdy, 1985b; Kovacs, Sarnyai, Szabo, & Telegdy, 1986), cocaine (Kovacs, Sarnyai, Babarczi, Szabo, & Telegdy, 1990; Morales-Rivera et al., 2014; Sarnyai et al., 1991; Sarnyai et al., 1992a; Sarnyai & Kovacs, 1994) and more recently METH. When centrally administered in rodents, oxytocin reduced METH-induced hyperactivity (Qi et al., 2008), the acquisition of a conditioned place preference to METH and stress-induced relapse to METH-seeking behavior (Qi et al., 2009; although see Subiah, Mabandla, Phulukdaree, Chuturgoon, & Daniels, 2012). Systemic injections of oxytocin have also been shown to attenuate the intravenous self-administration of METH and METH primed reinstatement (Carson, Cornish, Guastella, Hunt, & McGregor, 2010a). The ability of oxytocin to modulate METH-related behaviours extends to female rodents, whereby regardless of the stage of the estrous cycle, oxytocin reduced the self-administration of METH, as well as cue-, drug-, and

stress-induced reinstatement (Cox, Young, See, & Reichel, 2013) and when pretreated during adolescence, reduced the motivation to lever press for METH and attenuated METH primed reinstatement in adulthood (Hicks, Cornish, Baracz, Suraev, & McGregor, in press).

The subthalamic nucleus (STh) and nucleus accumbens (NAc) core have recently been identified as brain regions involved in oxytocin modulation of acute METH reward and relapse to METH use. A peripheral injection of oxytocin reduced acute METH-induced c-Fos expression in the NAc core and STh (Carson et al., 2010b) and direct administration of oxytocin into either region blocked the formation of a conditioned place preference to METH following a single conditioning session (Baracz et al., 2012). A recent examination of oxytocin modulation of chronic METH abuse also showed that oxytocin microinjected into the accumbens core (Baracz, Everett, McGregor, & Cornish, in press) or STh (Baracz et al., under review) reduced drug-primed reinstatement to METH-seeking behaviour.

Investigation into oxytocin modulation of METH-related reward has shown that oxytocin attenuates the increased dopamine neurotransmission that is evident following METH administration. Specifically, intracerebroventricular administration of oxytocin reduced the increased utilisation of dopamine in the dorsal and ventral striatum following METH administration (Qi et al., 2009). Further, it has been shown that oxytocin directly administered into the STh blocked the formation of a conditioned place preference to dopamine following a single conditioning session and that was reversed by co-administration of des-Gly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT, a selective oxytocin receptor (OTR) antagonist (Baracz & Cornish, 2013). This suggests that oxytocin modulates dopamine reward within the STh through activation of the OTR.

The involvement of the OTR in oxytocin modulation of METH-seeking behaviour following chronic METH self-administration however, was surprisingly modest. The local administration of des-Gly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT combined with oxytocin in the NAc core (Baracz et al., in press) or the STh (Baracz et al., under review) had a non-specific effect on blocking the inhibitory action of oxytocin on METH primed relapse to drug-seeking

behaviour. This suggests that oxytocin may act to reduce METH-seeking behaviour via additional receptors to the OTR. Previous studies that have examined oxytocin attenuation of cue or stress-induced reinstatement of cocaine or METH use have either failed to examine the involvement of the OTR (Morales-Rivera et al., 2014) or have used the non-selective OTR antagonist atosiban (Han et al., 2013; Qi et al., 2012; Qi et al., 2008), which has limited discriminability between the OTR and the V1a receptor for the structurally similar neuropeptide vasopressin (Manning, Stoev, Cheng, Wo, & Chan, 2001). Altogether, this highlights a limited understanding of the involvement of oxytocin at the OTR in modulating psychostimulant-induced behaviours.

Dysregulation of the oxytocin system has been documented following repeat drug administration in brain regions other than the NAc core and STh. Chronic exposure to morphine has been associated with a reduction in oxytocin synthesis within the rodent hypothalamus (You, Li, Song, Wang, & Lu, 2000) and rats that were treated with twice daily cocaine injections for four days experienced a decline in oxytocin content in the hippocampus and hypothalamus, and in blood plasma (Sarnyai et al., 1992a; Sarnyai et al., 1992b). Recently the NAc was examined in rats following exposure to two moderate-to-high doses of METH spaced one month apart, whereby an increase in the expression of oxytocin mRNA was identified (Cadet et al., 2014). Altered functioning of the OTR has also been reported following 10 days of systemic METH administration in rodents, whereby levels of the OTR were upregulated in the amygdala and hypothalamus (Zanos et al., 2014). In contrast, female dams that had been administered cocaine twice daily for 20 days had lower OTR binding density in the ventromedial hypothalamus and bed nucleus of the stria terminalis (Jarrett, McMurray, Walker, & Johns, 2006). Chronic exposure to the related psychostimulant 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") or the depressant ϒ-hydroxybutyrate (GHB) also alters oxytocin function as 10 days exposure to the peripheral administration of MDMA was associated with increased oxytocin mRNA expression and equal exposure to GHB was associated with an increase in OTR mRNA expression (van Nieuwenhuijzen, Long,

Hunt, Arnold, & McGregor, 2010). Considering that chronic METH administration appears to change the level of oxytocin or OTR, yet a role for the OTR in mediating the effect of oxytocin to reduce relapse is not clear (Baracz et al., in press; Baracz et al., under review), the current study will examine changes to OTR density in the NAc core and STh following chronic intravenous METH self-administration.

In addition to alterations in the oxytocin system following chronic drug exposure, disturbances to stress-regulatory mechanisms are also evident and are associated with increased susceptibility to abusing illicit drugs (Bisagno & Cadet, 2014; Sarnyai, Shaham, & Heinrichs, 2001). Following psychostimulant administration, the neuropeptide corticotropin-releasing factor (CRF) and corticosterone (CORT) are activated to initiate a stress response. Indeed, increases in plasma CORT levels have been reported immediately following 20 days of intraperitoneal (i.p.) METH injections during adolescence (Zuloaga, Siegel, Acevedo, Agam, & Raber, 2013). Following exposure to restraint stress, plasma CORT levels as well as CRF mRNA in the paraventricular nucleus of the hypothalamus have been shown to increase during acute withdrawal from chronic cocaine i.p. administration (Mantsch et al., 2007). CRF also acts in extrahypothalamic brain regions to influence adaptive and homeostatic processes in addition to moderating behavioural and subjective stress responses (Bisagno & Cadet, 2014).In the Nac, augmented mRNA expression of CRF and its receptors CRF1 and CRF2 were evident following two METH i.p. injections spaced one month apart (Cadet et al., 2014). Moreover, increased CRF concentrations in the amygdala have been identified in rats experiencing withdrawal from METH administration (Nawata, Kitaichi, & Yamamoto, 2012). Oxytocin has been shown to reduce plasma CORT levels when systemically administered acutely or repeatedly for 5 days (Petersson, Hulting, & Uvnas-Moberg, 1999) and to inhibit CRF expression in the hypothalamus (Bülbül et al., 2011; Windle, 2004; Zheng et al., 2010). Typically, studies examining oxytocin modulation of CRF have been restricted to the hypothalamus. CRF receptors are widely located within the brain; including within the NAc and STh (Wynn, 1984). Taken together, changes to the oxytocin system during METH self-

administration and following cessation of chronic METH administration may impair the anti-stress effects of oxytocin to reduce plasma CORT levels and may also interact to reduce CRF levels in cells of the NAc core and STh.

The primary purpose of the present study was to investigate changes to the endogenous oxytocin system centrally within the NAc core and STh, as well as peripherally in blood plasma following chronic self-administration of METH and after a period of extinction from drug taking. As oxytocin interacts with CRF and CORT, an additional aim was to examine whether changes to central CRF levels and CORT in plasma were also apparent. We utilised the extinction model of intravenous METH self-administration to examine the impact of actively administering METH over a 20-day period as well as following behavioural extinction on OTR and CRF fibre density in the NAc core and STh and on oxytocin and CORT blood plasma levels.

5.2. Materials and methods

5.2.1. Animals

32 male Sprague Dawley rats (weighing 200-250 g) were obtained from the Animal Research Centre (Perth, WA, Australia). Rats were housed in pairs (cage size: 40 x 27 x 16 cm) with the exception of a two-day postoperative period of individual housing. Food and water were available *ad libitum* in the home cages and not during experimental sessions. Lighting was kept on a 12-hour light/dark cycle (lights on 06:00), with all experiments conducted during the light cycle. Housing and laboratory room temperature was maintained at 21°C (±1°C). Prior to the start of experimentation, rats were acclimatised to the facility for seven days and were handled daily for a further seven days. All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th edition, 2013) and were approved by the Macquarie University Animal Ethics Committee.

5.2.2. Drugs

Methamphetamine hydrochloride (METH) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia) and was dissolved in saline (0.9%) for treatment administration. Vehicle administration was a 0.9% saline solution.

5.2.3. Apparatus

Testing was conducted in 16 standard operant response chambers (32 x 25 x 34 cm; Med Associates, St Albans, VT, USA) as described previously (Baracz et al., in press). Briefly, the operant response chambers were housed in sound-attenuating boxes (41 x 56 x 56 cm) equipped with a fan for masking noise and to provide ventilation. Each chamber was equipped with two retractable levers (1 active, 1 inactive) and a house light. The chambers also contained a metal arm with an adjustable weight and a spring connector, which were attached to a swivel. Polyethylene tubing threaded through the spring connector was attached to a 10 ml syringe driven by an infusion pump (Med Associates) located outside of the sound-attenuating chamber. The tubing exiting from the base of the spring connector was connected to the back mount of the intravenous catheter. Four infrared photobeam detectors were also positioned on the sidewall of each operant chamber to measure locomotor activity. Active and inactive lever presses, number of infusions and locomotor activity was recorded using MED-PC software (Med Associates).

5.2.4. Surgery

Rats were implanted with a chronic indwelling catheter in the right jugular vein. To achieve this, rats were anaesthetised with isoflurane gas (3% in oxygen 2 l/min) and aseptic

surgical techniques were used. Catheter implantation, as well as catheter construction is as previously described (Motbey et al., 2013). Rats were treated with 0.2 ml of the antibiotic cephazolin sodium (100 mg/ml) intravenously and the analgesic carpofen (5 mg/kg) subcutaneously at the time of surgery and daily for the following two days. Following this, catheter patency was maintained by a daily intravenous flush of 0.2 ml of cephazolin sodium in heparinised saline (300 IU/ml). Rats were allowed 5-7 days to recover before experimentation began.

5.2.5. Treatment conditions

The 32 rats underwent experimentation as a single cohort yet were allocated to either the intravenous self-administration (IVSA) group (n = 16; group 1) or the IVSA + extinction group (n = 16; group 2). Within each group, 10 rats were assigned to lever press for METH, whilst the remaining 6 were yoked saline controls to 6 of the 10 IVSA METH rats. The rats in the IVSA group were perfused 24 hours following the 20th day of METH self-administration, and the rats in the IVSA + extinction group were perfused 24 hours following the 15th day of extinction.

5.2.6. Acquisition and maintenance of METH self-administration

Prior to the initiation of the self-administration procedure, 12 rats from the cohort were paired with 1 rat each, which served as yoked controls. The yoked rat was run simultaneously with the IVSA METH rat and passively received an infusion of saline that was contingent on the active lever pressing of the IVSA METH rat. The remaining 8 rats in the cohort self-administered METH and were not paired with a yoked control.

IVSA METH rats were allowed to acquire self-administration of METH during 2-hour fixed ratio 1 schedule sessions conducted 5 days a week. At the beginning of each session,

catheters were flushed with 0.1 ml heparinised saline (10 IU/ml) and were connected to the infusion line. Lever extension and house light illumination indicated the initiation of the session. Levers were allocated as active or inactive, where the location of the active lever was counterbalanced across chambers. Depression of the active lever delivered a 3 s infusion of METH (0.1 mg/kg/infusion, 50 μL) to the IVSA METH rat, and an infusion of saline (0.9%) to the yoked rat. Following each infusion, the house light extinguished and a 20 s time out period occurred, during which depression of the active lever was recorded, yet had no consequences. Depression of the inactive lever had no programmed consequences at any time. To avoid overdose, each rat was limited to a maximum of 60 infusions per session. The session ended when either 2 hours had elapsed or the IVSA METH rat had received 60 infusions of METH, and was indicated by lever retraction and the house light turning off. At the end of each session, the infusion line was disconnected and catheters were flushed with 0.2 ml of cephazolin sodium in heparinised saline solution. Self-administration sessions were undertaken for 20 days.

5.2.7. Extinction

Following the last day of METH self-administration, rats in the IVSA+ extinction cohort were exposed to daily 2-hour extinction sessions. Depression of the active lever resulted in a saline infusion. Otherwise, the sessions were identical to self-administration sessions. Rats continued under extinction conditions for a minimum of ten days and until < 25 active lever presses were made per session for two consecutive days (Baracz et al., in press).

5.2.8. Blood collection and assay

Prior to intracardiac perfusion, approximately 5 ml of cardiac blood was collected in pre-chilled heparinised tubes and plasma was separated by centrifugation at 1600 g for 15

minutes at 4°C. Plasma was aliquoted into eppendorfs and stored at -80°C until the samples were assayed. Plasma oxytocin and CORT were assayed using commercially available oxytocin and CORT ELISA kits (ENZO Life Sciences, Ann Arbor, MI). Oxytocin samples were extracted using solid-phase extraction as previously described (ENZO Life Sciences, manual). One ml of plasma was mixed with an equal volume of 0.1% trifluoracetic acid (TFA) in water. The solution was then centrifuged and the supernatant added to a 200 mg C18 Sep-Pak column which had been pre-conditioned with acetonitrile (ACN; 1 ml) and 0.1% TFA in water. The samples were slowly eluted through the application of a 3 ml solution of 95% ACN and 5% of 0.1% TFA in water. The eluate was collected and evaporated to dryness using a centrifugal concentrator under vacuum (Genevac, EZ-2 Series). For the immunoassay, the samples were reconstituted with 250 µl of assay buffer, which was included in the ELISA kit. The oxytocin ELISA kit has a sensitivity of 15.0 pg/ml, intra-assay coefficient of variation below 13.3% and inter-assay coefficient of variation below 20.9%.

In terms of the CORT assay, plasma samples were diluted in a 1:100 solution with steroid displacement reagent (SDR) in deionised water. The samples were then diluted 1:50 times in an assay buffer included in the ELISA kit. The CORT ELISA kit has a sensitivity of 26.99 pg/ml, intra-assay coefficient of variation below 8.4%, and inter-assay coefficient of variation below 13.1%.

5.2.9. Tissue Collection and Immunofluorescence

Twenty-four hours following the last session, rats were deeply anaesthetised with sodium pentobarbitone (135 mg in 1 ml, i.p.; Clifford Hallam Healthcare, Roselands, NSW, Australia), cardiac blood was collected, and intracardiac perfusion was conducted with 300 ml of ice cold Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, Castle Hill, NSW, Australia) followed by 300 ml of ice cold 4% PFA. Brains were extracted and fixed overnight in 4% PFA at 4 °C prior to sectioning. 50 µm thick coronal sections were sliced using a

vibrating microtome (VT1200 S, Leica Microsystems, North Ryde, NSW, Australia) in a 1:3 sequential series and added to 25 ml pots containing phosphate buffered saline (PBS) with 0.1% Tween 20 (Sigma-Aldrich). Following this, free-floating sections were transferred to cryoprotectant solution and stored at -20°C until immunofluorescence was conducted.

The immunofluorescence procedure was used to measure OTR and CRF expression in 5 brains from each of the four treatment conditions. Firstly, tissue sections were washed three times for 30 min in Tris phosphate buffered saline (TPBS; Tris-HCL 10mM + sodium phosphate buffer 0.1 M + 0.9% NaCl), followed by a 30 min wash in Tris (10mM, pH 10) + Tween 20 (0.01%) at 80°C to enhance antigen retrieval. The tissue was then left to cool for at least 1 hour before three 5 min washes in TPBS. The tissue sections were then pre-incubated with TPBSm (TPBS with 0.05% merthiolate) and 10% normal horse serum for 1.5 hours, after which tissue sections were incubated with primary antibodies for 6 - 8 hours at room temperature and 40 - 42 hours at 4°C. The primary antibody used for detecting the OTR was goat anti-oxytocin receptor (1:500 dilution, Santa Cruz Biotechnology sc-8102), which is an affinity purified goat polyclonal antibody raised against a peptide which maps at the c-terminus of the OTR of human origin. In addition, the polyclonal guinea pig anti-CRF antibody was used (1:4000 dilution, T-5007, Peninsula Laboratories). Following three 30 min post-primary antibody washes, sections were again pre-incubated in TPBSm and 10% normal horse serum for 1.5 hours before incubation with secondary antibodies (all dilutions 1:500, CY3-conjugated Donkey anti goat, Jackson Immunoresearch; CY5-conjugated Donkey anti guinea pig, Jackson Immunoresearch) overnight at 4°C. After washing off the secondary antibodies, sections were mounted on glass slides and coverslipped with mounting medium (Vectashield Hardset, Vector Labs, CA, USA), which were then viewed on a fluorescence microscope (Axioimager Z2, Carl Zeiss, Germany). A control experiment was performed by omitting the primary or secondary antibodies and resulted in an absence of immunostaining, indicating that the primary and secondary antibodies had high binding specificity.

5.2.10. Image acquisition and density analysis

Coronal sections were imaged and adjusted for brightness and contrast in an identical manner using Zen Software (Carl Zeiss, Germany). The density from five equidistant sections from the NAc core (2.5 mm and 0.5mm to bregma) and three equidistant sections from the STh (between -3.12 and -3.72 from bregma) were analysed bilaterally. Anatomical identification of the NAc and STh was based on the rat brain atlas of Paxinos and Watson (2005). Each image was processed in an identical manner using Photoshop CS5 (Adobe Systems Software, Ireland). Optical density measurements of OTR and CRF staining in the NAc core and STh were calculated using mean grey values. The mean grey values of the anterior commissure was also collected as a measure of non-specific background staining in sections containing the NAc core and the mean grey value of the olivary pretectal nucleus was collected in sections containing the STh. Mean optical density was then calculated by subtracting background staining so that the final value represented the specific signal of OTR- or CRF-immunoreactive (ir) fibres (Austin, Beyer, Bembrick, & Keay, 2010; Pardey et al., 2012).

5.2.11. Statistical analysis

Data is displayed as the mean ± SEM. Daily rates of active and inactive lever pressing during self-administration were analysed using a two-way repeated measures analysis of variance (ANOVA). Number of infusions and active lever pressing across the 20-day period were also compared using a repeated-measures ANOVA to ensure IVSA METH rats acquired METH self-administration and that non-consequential lever pressing activity of yoked saline rats did not change over the 20-day period. Locomotor activity throughout self-administration

was analysed using a repeated measures ANOVA. To assess whether rats extinguished METH-paired responses, mean active lever pressing from the last three METH self-administration sessions was compared to active lever pressing during the extinction sessions using a repeated measures ANOVA, as was changes in locomotor activity during this period. Additionally, locomotor activity of IVSA METH rats was compared to the yoked saline rats across the 20-day self-administration period as well as across extinction sessions using a two-way repeated measures ANOVA.

Oxytocin and CORT plasma samples were analysed using a one-way ANOVA, followed by post-hoc pairwise comparisons with Fisher's least significant difference test. Samples that were above or below 2 standard deviations from the mean were excluded from plasma analyses.

Mean optical density for OTR- and CRF- ir fibres were analysed using a mixed methods ANOVA. Separate analyses were undertaken for the STh and NAc core, with a between subjects factor of treatment and a within subjects factor of coronal section relative to bregma. Posthoc pairwise comparisons were conducted using Fisher's least significant difference test. Statistical analyses were performed using SPSS 20 Graduate Student Version for Macintosh (SPSS Inc., Chicago, IL. USA). Statistical significance was set at $P < 0.05$ for all analyses.

5.3. Results

Of the original 32 rats that underwent jugular vein catheter implantation, 4 were removed from the study due to: non-acquisition of METH IVSA (2), lack of lever press extinction (1) and loss of catheter patency (1).

5.3.1. METH self-administration

Analysis of active ($F(1,14) = 0.188$, $p = 0.671$) and inactive lever presses ($F(1,14) = 0.878$, $p = 0.365$), number of infusions ($F(1,14) = 0.790$, $p = 0.389$), and locomotor activity ($F(1,14) = 0.062$, $p = 0.808$) during intravenous self-administration showed no significant difference across IVSA METH rats in group 1 and 2. Yoked saline rats across the two groups did not show a significant difference in active ($F(1,10) = 0.274$, $p = 0.612$) and inactive lever presses ($F(1,10) = 0.065$, $p = 0.804$), number of infusions received ($F(1,10) = 0.790$, $p = 0.389$) or locomotor activity ($F(1,10) = 0.027$, $p = 0.872$) during the 20-day period. As such, the IVSA METH rats from the IVSA group and the IVSA + extinction group were analysed together as were the yoked saline rats from the IVSA and IVSA + extinction groups.

IVSA METH rats acquired intravenous METH self-administration, as indicated by a significant increase in METH intake over the 20-day period ($F(19,285) = 17.853$, $p < 0.05$; Figure 1a). A significant increase in active lever pressing was also evident from day one to day 20 ($F(19, 285) = 19.271$, $p < 0.05$). A significant difference between active and inactive lever responses was measured across the 20 day period, indicating IVSA METH rats differentiated between the METH-paired lever and the inactive lever ($F(1,15) = 60.940$, $p < 0.05$; Figure 1b). Yoked saline rats did not show an increase in active lever pressing over the 20 day period ($F(19, 209) = 1.031$, $p = 0.427$), nor was a significant difference present between active and inactive lever pressing ($F(1,11) = 3.56$, $p = 0.086$). In terms of locomotor activity, the IVSA METH rats had significantly higher locomotor activity than the yoked saline rats ($F(1, 26) = 63.326$, $p < 0.05$; Figure 1c) demonstrating the stimulatory effects of METH administration.

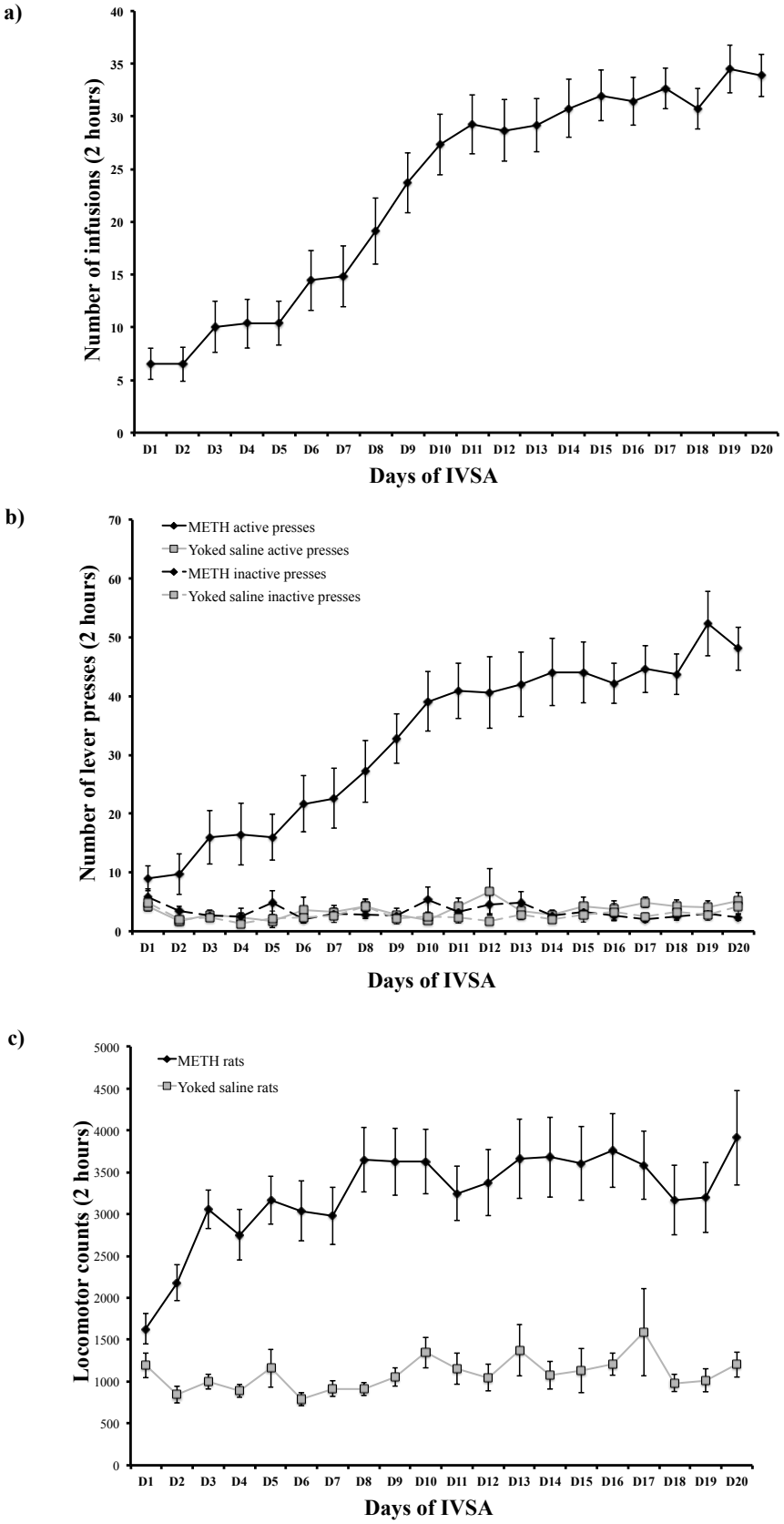

Figure 1. Combined data of groups 1 and 2 to show the mean (± SEM) number of a) infusions, b) active and inactive lever presses and c) locomotor activity across the 20 days of intravenous METH (0.1 mg/kg) self-administration for IVSA METH and yoked saline rats.

5.3.2. Behavioural extinction

Over the course of extinction sessions the METH IVSA rats in the IVSA + extinction group showed a decrease in active lever pressing from the first to the last extinction session (see figure 2a). Active lever presses for the yoked saline rats was consistently low across extinction session. Extinction criteria for the METH experienced rats was met after 15 sessions, whereby a significant reduction in active lever presses was present across extinction sessions compared to the mean of the last three self-administration sessions ($F(15,105) = 6.60$, $p < 0.05$). Locomotor activity also significantly reduced across extinction sessions compared to the mean of the last three self-administration sessions for the IVSA + extinction METH rats ($F(15,105) = 10.721$, $p < 0.05$). Locomotor activity for the yoked saline rats was not significantly different across extinction sessions compared to the mean of the last three self-administration sessions ($F(15,75) = 1.206$, $p = 0.287$), nor was their activity different to the METH experienced rats ($F(1,12) = 0.076$, $p = 0.788$; Figure 2b).

5.3.3. Plasma oxytocin and CORT concentration

The levels of extracted plasma oxytocin and CORT following METH self-administration and extinction are shown in figure 3. A significant main effect of treatment on extracted plasma oxytocin was evident ($F(3, 25) = 4.979$, $p = 0.009$). Post-hoc testing showed that plasma oxytocin concentration (pg/ml) was significantly higher in the IVSA METH treatment group than the IVSA yoked saline group ($p = 0.007$) and the IVSA + extinction yoked saline group ($p = 0.003$). In addition, plasma oxytocin levels were higher in the IVSA + extinction METH group than in the IVSA + extinction yoked saline group ($p = 0.041$). Plasma oxytocin concentration was not significantly different between IVSA METH and IVSA + extinction METH groups ($p = 0.191$) and IVSA yoked saline and IVSA + extinction yoked

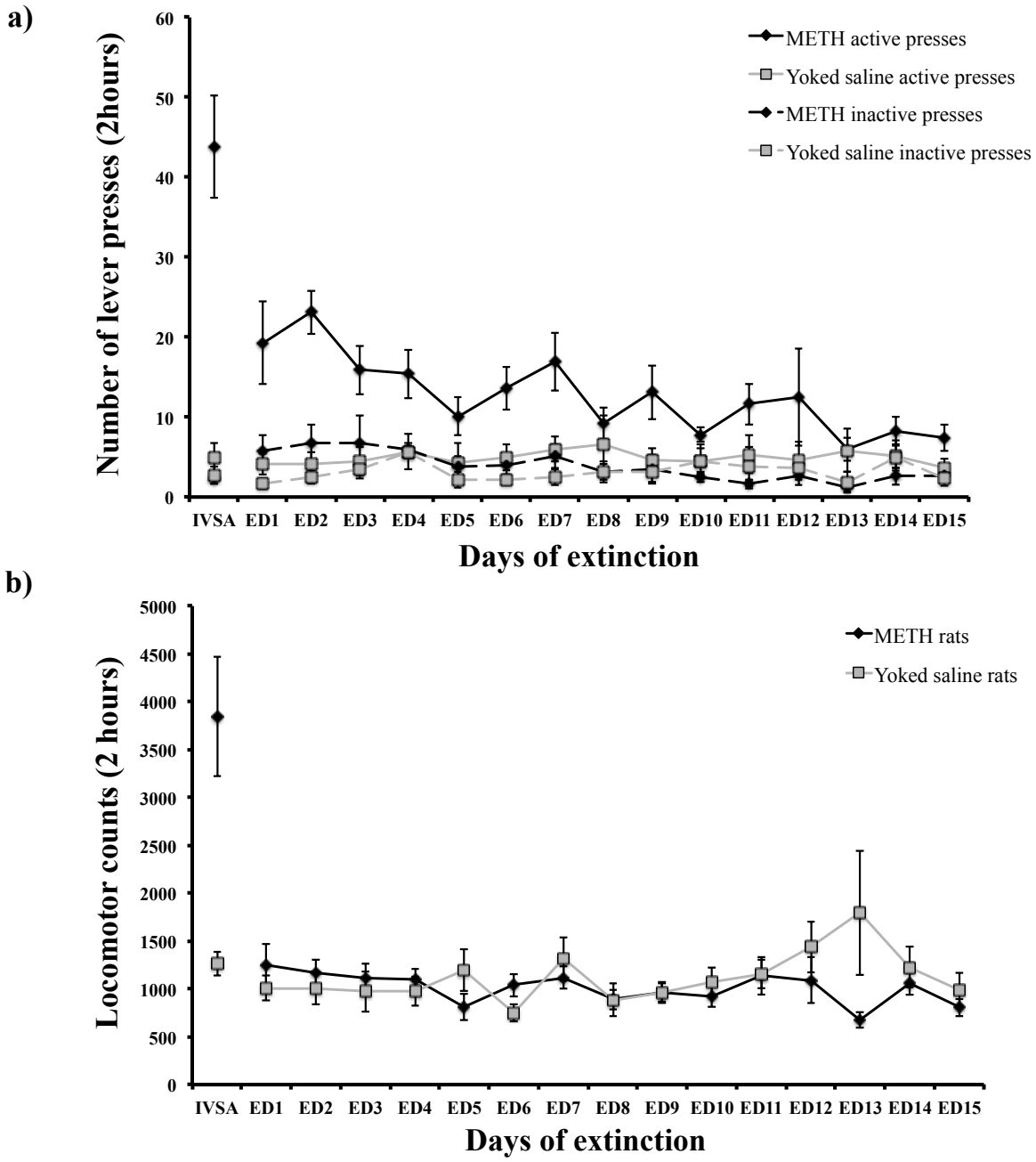

Figure 2. Mean (± SEM) number of a) active and inactive lever presses and b) locomotor activity across extinction for IVSA + extinction METH and IVSA + extinction yoked saline rats. Rats in the IVSA + extinction METH group met criteria after 15 days of extinction.

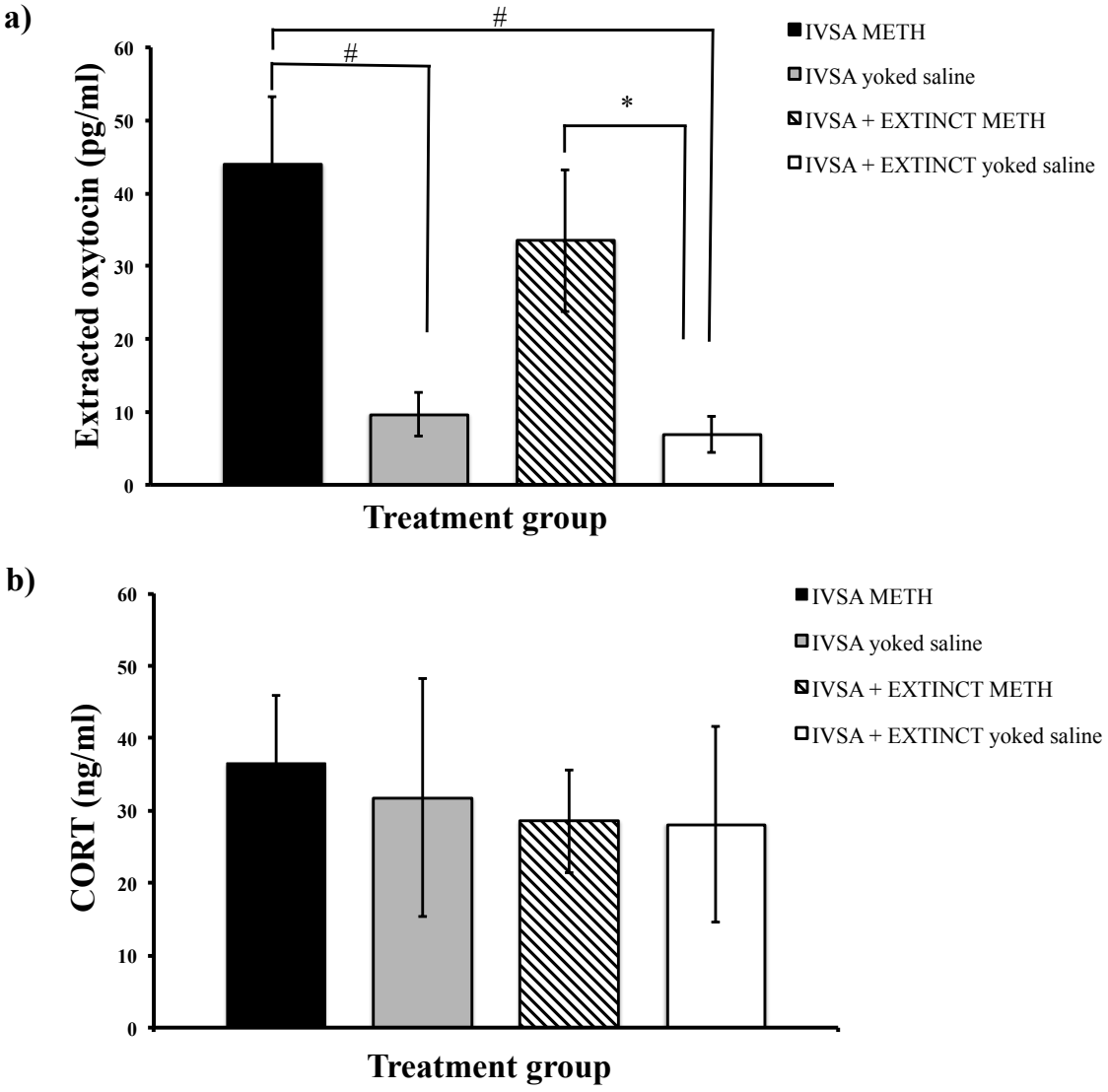

Figure 3. Mean (± SEM) basal plasma levels of a) extracted oxytocin (pg/ml) and b) CORT (ng/ml) in METH IVSA and yoked saline rats from groups 1 and 2.

# $p < 0.05$ compared to IVSA METH, * $p < 0.05$ compared to IVSA + EXTINCT METH

saline groups ($p = 0.823$). No significant main effect of treatment on plasma CORT concentration (ng/ml) was identified ($F(3,23) = 2.090$, $p = 0.134$). Post hoc tests did not reveal any significant differences in plasma CORT concentration between groups ($p > 0.05$).

5.3.4. OTR optical density analysis

The images in figures 4a and 5a show the staining for OTR-ir fibres in the NAc core and STh across the four treatment groups. The analysis of mean optical density (figure 4b) showed a significant difference in OTR-ir fibres across the NAc core coronal sections, with a higher density of ir fibres identified at 2.52 mm from bregma ($F(4,64) = 22.092$, $p < 0.05$). Comparisons of the treatment groups revealed significantly more OTR-ir staining in the IVSA yoked saline group than in the IVSA METH group ($p = 0.016$), which was particularly prevalent at 1.5 mm from bregma ($p = 0.001$). There was no significant difference in the IVSA + extinction METH and IVSA + extinction yoked saline groups ($p = 0.424$), yet there was a trend towards a significant increase in OTR-ir staining in the IVSA + extinction METH group in comparison to the IVSA METH group ($p = 0.080$). The optical density of OTR-ir fibres was not significantly different across yoked saline groups ($p = 0.589$).

An examination of OTR-ir fibre staining in the STh across treatment groups revealed that across the coronal sections, there was no significant difference in staining for OTR-ir fibres ($F(2, 32) = 2.200$, $p = 0.127$; Figure 5b). Comparisons of OTR-ir fibre staining across treatment groups did not show any significant differences ($p > 0.05$). After a close examination of the data, a comparison of treatment groups only at the coronal section -3.12 mm from bregma was conducted. OTR-ir fibre staining did not significantly differ between the IVSA METH and IVSA yoked saline groups ($p = 0.294$), and was significantly higher in the IVSA + extinction METH group compared to the IVSA + extinction yoked saline group ($p = 0.015$) and the IVSA METH group ($p = 0.008$). No significant difference in OTR-ir fibre density was apparent across the yoked saline groups ($p = 0.446$).

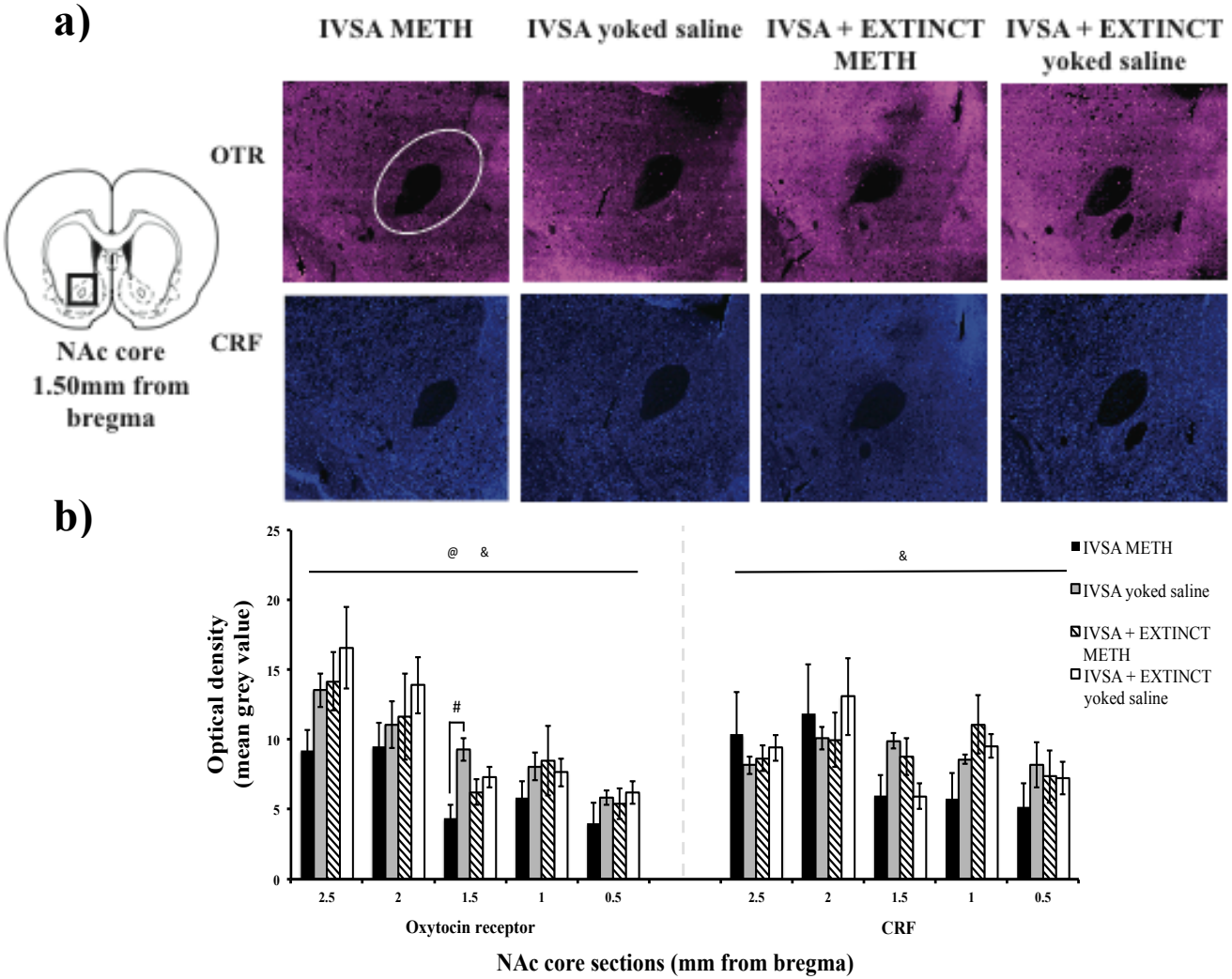

Figure 4. OTR-ir and CRF-ir fibres in the NAc core of METH IVSA rats and yoked saline rats in groups 1 and 2 (a). Purple staining represents OTR-ir fibres and blue staining represents CRF ir-fibres. Images have been adjusted for presentation. The white square represents the approximate region of interest. (b) Mean (± SEM) OTR and CRF immunoreactivity in the NAc core across the four treatment groups. The optical density of OTR-ir fibres was higher in the IVSA yoked saline group compared to the IVSA METH group controlling for coronal sections. A significant difference in OTR optical density across coronal sections was evident controlling for treatment. In terms of CRF-ir fibres, a significant difference in optical density across coronal sections was evident, although no significant differences across treatment groups were apparent. @: Mixed methods ANOVA main effect of group $p < 0.05$, &: Mixed methods ANOVA main effect of coronal sections $p < 0.05$, # $p < 0.05$ compared to the IVSA METH group.

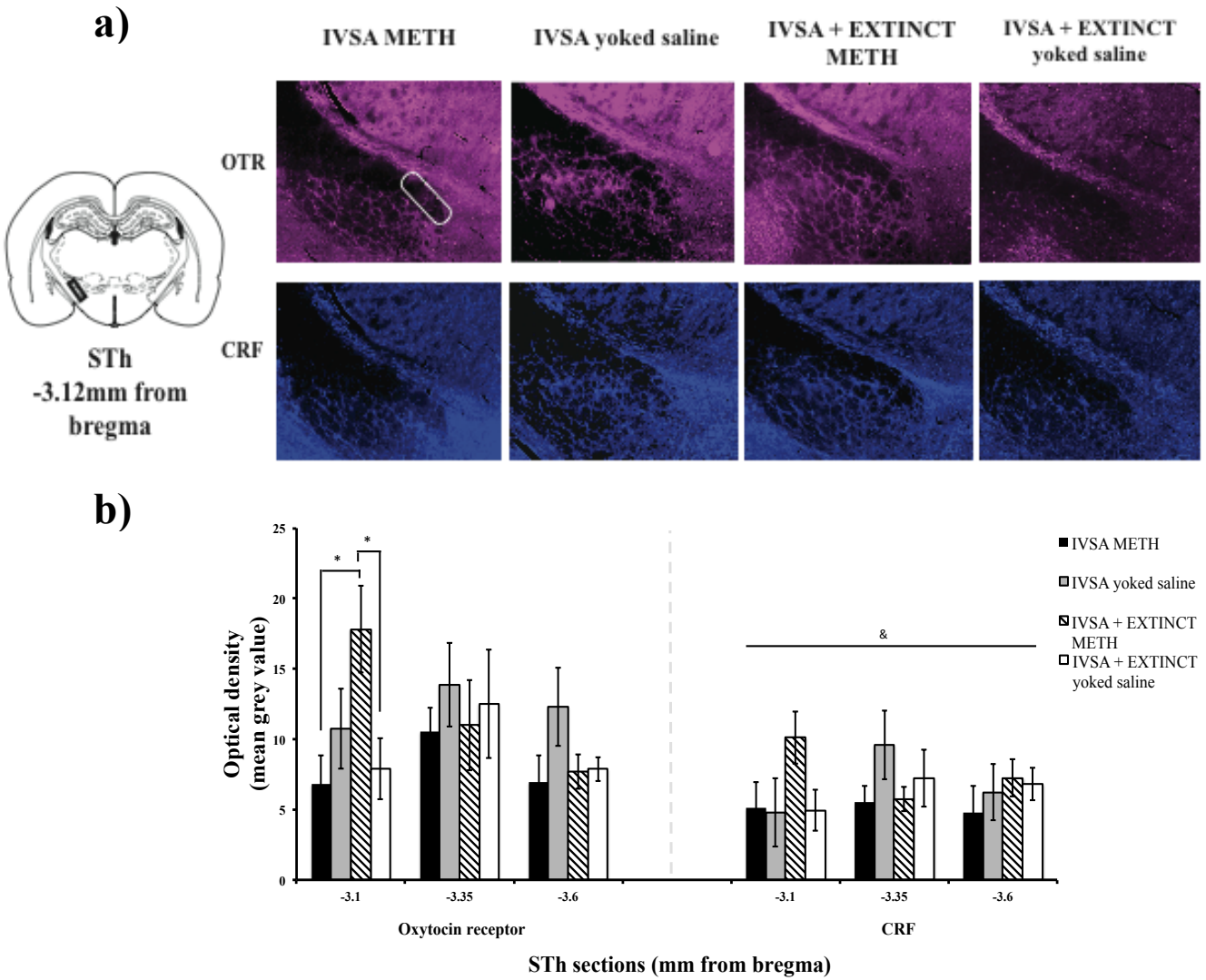

Figure 5. OTR-ir and CRF-ir fibres in the STh of METH IVSA rats and yoked saline rats in groups 1 and 2 (a). Purple staining represents OTR ir-fibres and blue staining represents CRF-ir fibres. Images have been adjusted for presentation. The white square represents the approximate region of interest. (b) Mean (± SEM) OTR and CRF immunoreactivity in the STh across the four treatment groups. In the anterior STh, OTR-ir fibre density was higher in the IVSA + extinction METH group compared to the IVSA METH group and the IVSA + extinction yoked saline group. In terms of CRF-ir fibres, a significant difference in optical density across coronal sections was evident, although no significant differences across treatment groups were apparent. &: Mixed methods ANOVA main effect of coronal sections, $p < 0.05$, * $p < 0.05$ compared to the IVSA + extinction METH group.

5.3.5. CRF optical density analysis

The staining for CRF-ir fibres are shown across treatment groups in the NAc core and STh in figures 4a and 5a. Optical density analysis showed a significant difference in CRF-ir fibre staining across coronal sections of the NAc core ($F(4,64) = 4.488$, $p = 0.003$; figure 4b). However, no significant difference was apparent in CRF-ir fibre staining across treatment groups ($p > 0$ .05). In the STh, there was also a significant difference in optical density across coronal sections ($F(2, 32) = 5.967 = p = 0.006$; figure 5b), although no significant difference in staining was evident when comparing treatment groups ($p > 0$ .05).

5.4. Discussion

The aim of this study was to primarily examine changes to the endogenous oxytocin system centrally and peripherally following chronic METH self-administration and after a period of extinction. Additionally, due to the known regulation of CRF by oxytocin, changes to central CRF levels and plasma CORT levels were also investigated. Rats that self-administered METH chronically over a 20-day period (IVSA METH group) had increased plasma oxytocin levels and reduced OTR-ir fibre staining in the NAc core in comparison to rats that were yoked to receive saline infusions (IVSA yoked saline group). Following 15 days of extinction from METH IVSA, oxytocin plasma levels remained elevated, OTR expression in the anterior STh was augmented, and a trend towards normalization of OTR-ir fibre density in the NAc core was evident in the rats whom had previously self-administered METH (IVSA + extinction METH group) compared to the IVSA METH group. No changes in CRF-ir fibre staining or plasma CORT concentration were identified when comparing the rats that self-administered METH and the yoked saline rats.

Plasma oxytocin levels have previously been examined following acute MDMA or chronic MDMA, METH, and cocaine exposure (Hicks et al., in press; Sarnyai et al., 1992a;

Sarnyai et al., 1992b; Thompson, Callaghan, Hunt, Cornish, & McGregor, 2007; Williams et al., 2012). Our study demonstrated that, compared to controls, METH IVSA significantly increased plasma oxytocin levels, which remained elevated following 15 days of extinction. This is suggestive of a chronic increase in oxytocin plasma levels with, and following, chronic METH exposure. Our baseline levels of oxytocin are similar to those measured previously (Suraev et al., 2014), with chronic METH IVSA sustaining a four-fold increase in this level. The effect of acute METH administration on plasma oxytocin levels is yet to be reported, however our data and others suggest that acute (MDMA, Thompson et al., 2007) and chronic (cocaine, Williams et al., 2012) psychostimulant administration can increase plasma oxytocin levels. In contrast to these findings, Sarnyai et al. (1992a) found that four days of twice daily cocaine injections decreased plasma oxytocin levels. The discrepancy in findings may reflect differences in the length of time during which the rodents were exposed to cocaine (4 days compared to 20 days as in Williams et al., 2012).

Prior studies that have investigated plasma oxytocin levels in association with METH exposure have incorporated pretreatments or concurrent treatment of oxytocin with METH IVSA. Recently, Hicks et al. (in press) measured extracted oxytocin plasma levels in female rats that had been pretreated with oxytocin during adolescence and exposed to METH through the self-administration paradigm in adulthood. Oxytocin plasma levels were higher in rats that had been pretreated with oxytocin than vehicle in METH experienced rats. In addition, male rats that received seven injections of oxytocin over the course of METH IVSA also had higher oxytocin plasma levels than rats that were administered vehicle when measured after 14 days of extinction (McGregor & Bowen, 2012). No difference in oxytocin plasma levels, however, was identified between the vehicle treated rats that self-administered METH and the control rats. These discrepant findings may be due to differences in rat gender (Hicks et al., in press), the omission of a control baseline oxytocin group (Hicks et al., in press) as well as the different approach in measuring plasma oxytocin levels, as oxytocin was not extracted from the plasma prior to undertaking the enzyme immunoassay (McGregor & Bowen, 2012). It has

recently been argued that without firstly extracting oxytocin, the assay is tagging additional molecules beyond oxytocin and so is not providing an accurate measure (McCullough, Churchland, & Mendez, 2013). Even so, tentative inferences that can be drawn suggest that oxytocin plasma levels are chronically increased after extinction from chronic METH exposure, and that oxytocin plasma levels may be increased to a greater extent when oxytocin is administered either intermittently during, or before METH exposure. If this is the case, it appears that the resulting increase in endogenous plasma oxytocin in conjunction with additional oxytocin administration may reduce the impact of METH administration and METH-related behaviours. Indeed, numerous studies have shown attenuation of a range of METH-related behaviours after systemic oxytocin administration in rodents that have received either acute or chronic METH injections (Baracz et al., 2012; Carson et al., 2010a; Carson et al., 2010b; Cox et al., 2013; Hicks et al., in press). Altogether, this data is suggestive of a protective mechanism of oxytocin against the adverse effects of METH administration on behaviour.

Both the NAc core and STh have been identified as important brain regions involved in oxytocin modulation of METH reward and abuse (Baracz et al., in press; Baracz et al., 2012; Carson et al., 2010b). Oxytocin appears to modulate acute dopamine-mediated reward through activating the OTR within the STh (Baracz & Cornish, 2013), although the OTR does not appear to be solely driving oxytocin attenuation of reinstatement to METH-seeking behaviour within either the NAc core or STh (Baracz et al., in press, Baracz et al., under review). However, the current study demonstrates changes to OTR density in the NAc core and STh across IVSA and extinction to METH.

In the NAc core, the optical density of OTR-ir fibres was reduced in rats that self-administered METH (IVSA METH group) compared to yoked saline rats (IVSA yoked saline and IVSA + extinction yoked saline groups). Further, a trend towards normalization of OTR-ir fibre density to baseline-yoked levels was evident in the IVSA + extinction METH group compared to the IVSA METH group. Taken together, this is suggestive of a decline in OTR-ir

fibres in the NAc core during chronic METH exposure and a partial recovery in OTR-ir fibre density following 15 days extinction from METH IVSA. Differences in OTR-ir fibre staining were also measured in the STh (restricted to the measured anterior sections). Significantly more OTR-ir fibres were stained in the STh of IVSA + extinction METH rats than in the IVSA METH rats and the IVSA + extinction yoked saline rats. In addition, no difference in OTR-ir fibre density was evident between the IVSA METH and IVSA yoked saline rats. Altogether, these findings suggest that OTR fibre density in the STh is typically low, yet increase following extinction from METH IVSA.

The changes to OTR density in the NAc core and STh with METH self-administration and extinction may suggest a positive role for oxytocin administration in the reduction of relapse to METH-seeking behaviour. Local microinjection of oxytocin into these brain regions has been shown to reduce relapse to METH-induced reinstatement (Baracz et al., in press; Baracz et al., under review). However, these previous studies failed to find a significant effect of the co-administration of a selective OTR antagonist to block the effect of oxytocin on METH-seeking behaviour, which may be due to the concurrent administration of METH to prime reinstatement behaviour. The re-administration of METH could have produced an elevated level of circulating oxytocin to compete with the dose of the antagonist used. An additional consideration is that the examination of OTR-ir fibre density is not a direct reflection of the number of OTRs, or indeed, the change in number of available OTRs. It is also possible that the decline in OTR-ir fibre density is due to a reduction in protein expression rather than a decrease in fibres. Moreover, it may be that additional receptors, particularly dopamine, GABA-A and V1a receptors are involved in regulating the effect of oxytocin on relapse to METH-seeking behaviour (Baracz et al., in press) with further research required to provide insight into the mechanistic action of oxytocin on METH-related behaviours.

The level of OTR expression in the STh over the duration of METH IVSA and following extinction followed a different pattern to that of the NAc core, whereby OTR

expression decreased throughout METH IVSA, whilst in the STh, OTR expression increased throughout extinction from METH IVSA. The NAc core and the STh are connected by an indirect, multisynaptic circuit, which originates in the medial prefrontal cortex and extends to the output regions of the basal ganglia (Maurice, Deniau, Glowinski, & Thierry, 1998). The interconnected nature of this circuit allows for the possibility that the increase in OTR-ir fibres in the STh during extinction is a downstream consequence of changes occurring in the NAc core with chronic METH exposure and the transition from drug-taking to the extinction of drug-seeking behaviour. Further investigation into changes to the oxytocin system within the indirect cortico-striato-pallidal-subthalamic circuit across METH self-administration, extinction and relapse will usefully determine the mechanisms by which oxytocin can modulate METH abuse and dependence.

The visualisation of the OTR using traditional techniques of receptor autoradiography has previously provided inconsistent findings in the NAc core and a lack of imaging in the STh. In terms of the NAc core, studies reported low densities (Freund-Mercier et al., 1987), transient presence throughout the adolescent period (Shapiro & Insel, 1989) or no measure of OTRs at all in this region (Adan et al., 1995; Tribollet, Barberis, Jard, Dubois-Bauphin, & Dreifuss, 1988). The present findings provide further support for the localisation of the OTR in the rat NAc core through the use of immunofluorescence. In relation to the STh, the only prior identification of OTR localisation in this region was through measures of OTR mRNA (Vaccari, Lolait, & Ostrowski, 1998). It is possible that the limited sensitivity of receptor autoradiography for this receptor (Freund-Mercier, Stoeckel, Dietl, Palacios, & Richard, 1988) has produced negative findings for OTR levels in the STh. Indeed, a functional effect of oxytocin, through the OTR in the STh, to reduce acute METH reward has been demonstrated (Baracz & Cornish, 2013). The findings of the present study provide support at a cellular level for the localization of the OTR in the STh.

A particular limitation of the current study is the technical issue surrounding the use of commercially available OTR antiserums. Inconsistent staining using OTR antiserum from

different lot numbers produced from the same company as well as OTR antiserums from different companies has been reported when the same tissue preparation methods have been used (Boccia, Petrusz, Suzuki, Marson, & Pedersen, 2013; Yoshida et al., 2009). Considering this, our choice of OTR antiserum was carefully evaluated using pilot brain sections to ensure consistent and specific OTR staining in positive control regions as well as a lack of visualisation in negative control regions. Failure to visualise the OTR in the STh in previous studies using receptor autoradiography may be due to the OTR being of low binding affinity in the STh. Even though we were able to visualise the OTR using immunohistochemistry, it is unclear if the OTR antiserum that was utilised in this study is sensitive for low-affinity OTRs and so may have impacted on the identification of OTR-ir fibres in the STh.

It has long been argued that peripheral and central oxytocin systems are largely independent of each other. However there is some evidence, from both animal and human clinical studies that changes to peripheral plasma oxytocin levels are synchronous with changes to central oxytocin levels (Carson et al., 2014; Wang et al., 2013; Wotjak et al., 1998 although see Amico, Challinor, & Cameron, 1990; Kagerbauer, Schuster, Blobner, Kochs, & Landgraf, 2013). If such a relationship is present, it is likely, based on the findings of this study, that there was also an increase in oxytocin levels within the central nervous system. In line with this, an increase in endogenous oxytocin levels within the brain, particularly in the NAc could correspond with a decrease in OTR-ir fibres in the NAc core, reflecting a compensatory homeostatic mechanism. However, this relationship is tenuous as the sustained increase in plasma oxytocin levels during extinction is not reflected in sustained reductions in receptor level in the NAc. Moreover, *increases* in OTR in the STh are measured following extinction from METH IVSA. In order to understand the relationship between peripheral and central oxytocin levels, and indeed any interaction between oxytocin regulation of the two brain regions, future studies should measure real time oxytocin levels and the associated regional receptor changes that occur during METH IVSA and drug extinction.

The dysregulation of the endogenous oxytocin system has been documented following repeated injections of cocaine (Sarnyai et al., 1992a; Sarnyai et al., 1992b), morphine (You et al., 2000), MDMA, GHB (van Nieuwenhuijzen et al., 2010), and recently, METH (Cadet et al., 2014; Zanos et al., 2014). The examination of changes to the OTR following chronic psychostimulant exposure has largely resulted in discrepant findings, although different brain regions have typically been investigated. We are the first to show reduced OTR-ir fibre density in the NAc core following chronic METH administration. This is in agreement with Cadet and colleagues (2014) investigation into the NAc following repeat METH injections, as an increase in oxytocin mRNA was evident, consistent with a compensatory decrease in the OTR. In contrast, Zanos et al. (2014) showed that immediately following 10 days of METH administration, an upregulation of the OTR was evident in the amygdala and hypothalamus with no change to the NAc. The discrepancy may be related to the differing methodology (receptor autoradiography), the shortened period of METH exposure, or the means of administration (passive i.p. injection). Our findings also revealed an increase in OTR-ir fibres in the STh as well as a trend towards a significant increase in OTR-ir fibres in the NAc core in the IVSA + extinction METH group compared to the IVSA METH group. This suggests a partial recovery of the oxytocin system in the NAc core and a modulatory response in the STh to the absence of METH administration and provides important insights into the neuroplasticity of the OTR. Continued investigation into alterations to the oxytocin system is clearly needed to fully understand the impact of chronic METH exposure on oxytocin.

Interestingly, METH IVSA and yoked saline rats did not significantly differ in plasma CORT levels nor in optical density of CRF-ir fibres. Previous studies have shown increased plasma CORT levels (Tomita et al., 2013; Zuloaga et al., 2013), higher CRF levels in plasma and the amygdala (Nawata et al., 2012), and increased CRF mRNA expression in the NAc (Cadet et al., 2014) following repeat exposure to psychostimulant drugs. In contrast, and in agreement with our findings, a lack of an effect on CORT plasma levels after repeat exposure to METH has also been reported (Hicks et al., in press; Shirayama et al., 1999). The similar

CORT plasma levels in METH IVSA and yoked saline rats may be related to the time that CORT was measured. In the present study, we collected blood plasma 24 hours following the last METH IVSA or extinction session, Hicks et al. (in press) collected blood plasma at approximately the same time point following drug-primed reinstatement, and Shirayama and colleagues (1999) collected blood plasma three days following METH administration. In studies that found increased CORT levels, CORT was measured between 1 and 2 hours after METH injection (Tomita et al., 2013; Zuloaga et al., 2013). Indeed, Tomita and colleagues (2013) reported that CORT plasma levels peaked at 60 minutes following chronic METH administration, after which, CORT levels gradually returned to basal levels. A similar effect was also reported when measuring CRF in the brain, whereby changes to CRF were evident when samples were extracted within 2 hours following the last METH injection (Cadet et al., 2014; Nawata et al., 2012; Tomita et al., 2013). This data suggests that METH administration results in a short-lived increase in CORT plasma levels and CRF-ir fibres that is not exaggerated or extended with chronic METH exposure.

Overall, the present study is the first to show a decrease in OTR-ir fibres in the NAc core coupled with an increase in plasma oxytocin levels following chronic METH exposure. An additional novel finding of the present study indicates that following extinction from METH IVSA, oxytocin plasma levels remained elevated, OTR-ir fibres in the STh increased, and OTR-ir fibre density partially recovered to baseline levels in the NAc core. CRF-ir fibre density in the NAc core and STh, as well as CORT plasma levels, did not change across treatment groups. These results further our understanding of a modulatory role for the oxytocin system in METH abuse, which provides insight into the applicability of oxytocin or related compounds as future pharmacotherapies for METH dependence.

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5.5. References

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Chapter 6: General Discussion

6.1. Major findings

Oxytocin has previously been shown to reduce the rewarding effects and abuse potential of the psychostimulant methamphetamine (METH), although an understanding of the mechanisms of oxytocin action within the brain were lacking. This thesis examined: i) the mechanisms by which oxytocin interacted with dopamine (DA) to reduce METH-related reward, ii) the involvement of the subthalamic nucleus (STh) and nucleus accumbens (NAc) core in oxytocin attenuation of chronic METH use and iii) the neural changes to the oxytocin system at a cellular level in the NAc core and STh following chronic METH use. Overall, this thesis provides several novel findings pertaining to oxytocin modulation of METH-related behaviours within both brain regions and furthers our understanding of oxytocin action as a possible pharmacotherapy for drug dependence.

An initial investigation determined that oxytocin locally administered into the STh and NAc core attenuated the formation of a conditioned place preference (CPP) for METH (Baracz et al., 2012). This provoked a more detailed investigation into the mechanisms by which oxytocin modulates acute METH reward with a specific focus on the less-examined STh. The interaction between oxytocin and DA was examined, as it was suggested that oxytocin modulates the increase in DA neurotransmission that occurs following METH administration although the exact mechanisms were unclear (McGregor & Bowen, 2012; Qi et al., 2009; Qi et al., 2008). The CPP paradigm was used to examine acute reward processes through the application of a single conditioning session. As outlined in Chapter 2, this study showed that DA microinjected into the STh resulted in the formation of a CPP for dopamine, which was attenuated by the co-administration of oxytocin. Moreover, when a cocktail of DA, oxytocin and desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT, a highly selective oxytocin receptor (OTR) antagonist was directly administered into this brain region, desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT blocked the inhibiting effect of oxytocin on place preference formation. Altogether, this study was the first to show that local administration of DA within the STh

produced reward-related behaviour, which was modulated by oxytocin at the level of the OTR.

To further identify the involvement of the NAc core and STh in oxytocin modulation of METH reward and addiction, Chapters 3 and 4 examined the direct application of oxytocin solely and in combination with desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT in the NAc core or STh on METH-primed reinstatement in rats that had previously been experienced at intravenous METH self-administration. The intravenous self-administration/reinstatement paradigm was investigated to determine whether the oxytocin system also modulates behaviours associated with chronic METH use in these two key brain regions.

Chapter 3 reported on findings pertaining to the NAc core, showing that direct oxytocin administration into the core subregion (0.5 pmol, 1.5 pmol, 4.5 pmol/side) dose dependently reduced reinstatement to METH-seeking behaviour. Further, this study showed that the co-administration of the selective OTR antagonist did not specifically inhibit the effect of oxytocin to reduce METH-induced lever pressing activity. This suggests that in the NAc core, oxytocin attenuated METH-related behaviours through additional receptors beyond the OTR.

The effect of oxytocin on METH-primed reinstatement in the STh yielded similar findings (Chapter 4). Only the highest oxytocin dose (3.6 pmol/side) microinjected into the STh significantly reduced reinstatement to METH-seeking behaviour. As one of the lower oxytocin doses (0.6 pmol/side) tested had previously been shown to reduce acute METH reward when microinjected into the STh (Baracz et al., 2012), this suggests that the endogenous oxytocin system may be dysregulated following chronic exposure to METH. Again, the co-administered OTR antagonist (desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT) had a non-specific effect on relapse to METH use. This too, suggests dysregulation of the OTR in the STh, as it appears that in terms of chronic METH exposure, oxytocin modulated METH-related behaviours through additional receptors beyond the OTR.

Previous studies have suggested that oxytocin and its receptor are dysregulated following chronic psychostimulant exposure (Cadet et al., 2014; Sarnyai et al., 1992a; Sarnyai et al., 1992b; Zanos et al., 2014), yet the involvement of the OTR in oxytocin modulation of relapse to METH-seeking behaviour, as outlined in Chapters 3 and 4, is unclear. Following on from these studies, Chapter 5 investigated changes in OTR fibre density in both key brain regions following chronic intravenous self-administration (IVSA) of METH and after extinction from METH IVSA. In addition, oxytocin plasma levels were also examined to gain a general understanding of changes to the endogenous oxytocin system following chronic METH use. Rats that chronically self-administered METH had increased oxytocin plasma levels coupled with decreased OTR-immunoreactive (ir) fibre staining in the NAc core compared to rats that were yoked to receive saline infusions. Following extinction from METH IVSA, rats that had lever pressed for METH had persistently elevated oxytocin plasma levels, increased OTR-ir fibre density in the STh and a trend towards normalisation of OTR-ir fibre staining in the NAc core compared to rats that had self-administered METH chronically and were not exposed to extinction conditions. This suggests a partial recovery of the OTR in the NAc core and a modulatory response in the STh to the absence of METH IVSA. Altogether, the alterations to the oxytocin system, both centrally within the NAc core and STh, and peripherally in blood plasma levels following chronic exposure to METH as well as after extinction from METH IVSA highlights the neural changes to the oxytocin system and how it becomes altered by repeated exposure to METH.

As METH is the second most commonly abused illicit drug worldwide (United Nations Office on Drugs and Crime, 2013), is highly addictive, and is associated with numerous long-term adverse effects when repeatedly administered (Dyer & Cruickshank, 2007; Ornstein et al., 2000; Turnipseed, Richards, Kirk, Diercks, & Amsterdam, 2003; N. D. Volkow et al., 2001), the development of effective pharmacotherapeutic treatments is crucial. Overall, this thesis has furthered understanding of the mechanisms of oxytocin action within two brain regions that have been identified as important regulators of METH reward and

abuse. This furthering of knowledge of oxytocin mechanisms of action ultimately assists in providing additional insight into the applicability of oxytocin as a pharmacotherapy for METH dependence.

6.2. Implications

6.2.1. Oxytocin and dopamine interactions

Through the application of pharmacological techniques, oxytocin has been shown to modulate DA neurotransmission following illicit drug administration. Oxytocin, when administered either centrally or systemically, reduced the increased utilisation of DA specifically within the NAc after an acute cocaine injection (Kovacs, Sarnyai, Babarczi, Szabo, & Telegdy, 1990; Sarnyai, Szabo, Kovacs, & Telegdy, 1990) as well as in the dorsal striatum and NAc after an acute METH injection (Qi et al., 2008). Further, local administration of oxytocin in the prelimbic cortex attenuated amphetamine-induced increases in DA levels in the NAc (Young, Liu, Gobrogge, Wang, & Wang, 2014). However, the mechanisms by which oxytocin reduced chronic drug abuse were uncertain.

The study reported in Chapter 2 of this thesis explored the relationship between oxytocin and DA within the STh; a brain region that is emerging as an important modulator of drug abuse. Through the use of the most selective OTR antagonist currently available (Manning et al., 2012), it was shown that oxytocin attenuated DA-mediated reward in the STh through the OTR. This study was the first to implicate the OTR in oxytocin modulation of DA activity in reward processes. As DA is a primary neurotransmitter involved in reward processes (Hyman, Malenka, & Nestler, 2006; Koob, 2009; Schultz, 2000) and is largely implicated in METH-related reward (Schultz, 2000), the findings of this study postulate that within the STh, oxytocin attenuates METH reward by reducing DA activity through the activation of the OTR. Considering that the studies reported in Chapters 3 and 4 found that oxytocin modulation of relapse to METH-seeking behaviour is not specifically driven by the

OTR, the logical progression in subsequent studies would have been to examine whether oxytocin was instead acting through DA receptors to reduce METH-primed reinstatement. However, the co-administration of a mixed DA D_1/D_2 receptor antagonist with oxytocin would have directly blocked METH action and subsequently, lever pressing activity, ultimately confounding the interpretation of the study findings.

Dopamine, however, is not the only neurotransmitter involved in psychostimulant addiction, nor is it the only neurotransmitter that interacts with oxytocin to modulate behaviour. The amino acids glutamate and gamma-aminobutyric acid (GABA) are also involved in acute psychostimulant effects as well as drug dependence and craving through the dysregulation of their respective systems. Further, oxytocin has been shown to interact with both glutamate and GABA to modulate a range of behaviours. When exposed to forced swim conditions, oxytocin release in the central amygdala of the rat inhibits glutamate release to modulate stress responses (Bosch, Sartori, Singewald, & Neumann, 2007). Intrathecal oxytocin administration in the dorsal horn of the spinal cord has also been shown to block glutamate-mediated sensory transmission in cold swim and restraint-stress induced antinociception (Robinson et al., 2002). In relation to GABA, oxytocin neurons in the supraoptic nucleus are innervated by GABAergic projections (Theodosis, Paut, & Tappaz, 1986) which inhibit oxytocin neuronal electrical and secretory activity when under social defeat stress (Engelmann et al., 2004). Recently, an interaction between oxytocin and GABA was shown to be involved in modulating alcohol-related behaviours, whereby oxytocin reduced motor impairment and GABAergic activity induced by ethanol consumption at δ subunit-containing GABA-A receptors (Bowen et al., 2015).

An interaction of oxytocin with glutamate and GABA in METH-related behaviours has also been examined specifically in the medial prefrontal cortex (PFC) and dorsal hippocampus. Qi and colleagues (2012) showed that intracerebroventricular (icv) administration of oxytocin prior to an acute METH injection inhibited the METH-induced reduction in extracellular GABA levels in the dorsal hippocampus, and stimulated an increase

in extracellular GABA levels, as well as inhibited an increase in glutamate levels, in the medial PFC. The same research group also demonstrated, using the reinstatement model of CPP, that central oxytocin administration inhibits restraint stress-induced reinstatement to METH-seeking behaviour partially by inhibiting stress-induced increases in extracellular glutamate levels in the medial PFC (Qi et al., 2009).

It is plausible that oxytocin also interacts with the amino acids in the NAc or the STh to modulate METH related-behaviours. The NAc receives glutamatergic input from numerous brain regions (Ikeda, Kamei, Koshikawa, & Cools, 2012), is largely composed of GABAergic medium spiny neurons (Liang et al., 2014), and consists of metabotropic (mGluR5) and ionotropic (AMPA and NMDA) glutamate receptors, and GABA-A and -B receptors (Ikeda et al., 2012; Liang et al., 2014; Roohi, Sarihi, Shahidi, Zarei, & Haghparast, 2014). Similarly, glutamatergic and GABAergic fibres project to the STh (Wilson & Bevan, 2011), and both GABA-A and -B receptors and metabotropic (mGluR1 and mGluR5) glutamate receptors have been located in the STh (Boyes & Bolam, 2007; Marino, Awad-Granko, Ciombor, & Conn, 2002). As DA typically modulates GABA and glutamate neurotransmission (Beaulieu & Gainetdinov, 2011), it is possible that a complex process is involved in oxytocin modulation of METH-related reward and particularly relapse to METH-seeking behaviour that incorporates an interaction of DA with GABA and glutamate regulation of the NAc core and STh. Finally, considering the recent discovery of oxytocin acting through δ subunit-containing GABA-A receptors to modulate ethanol-related effects, specifically investigating whether oxytocin is modulating relapse to METH-seeking behaviour through this mechanism in both brain regions would be a logical consideration.

6.2.2. Integrating oxytocin activity at the nucleus accumbens core and subthalamic nucleus in drug addiction circuitry

Carson and colleagues (2010b) demonstrated in their pivotal study that oxytocin attenuated acute METH-induced cellular activation *only* within the NAc core and STh. This suggests that oxytocin plays an important role within these two brain regions in acute METH reward and that the NAc core and STh may be closely linked. Indeed, one of the many multi-synaptic circuits that connect the cerebral cortex with the output nuclei of the basal ganglia has projections from the NAc core through to the STh (see Figure 1; Maurice, Deniau, Glowinski, & Thierry, 1998). This indirect cortico-striato-pallido-subthalamic circuit incorporates projections from the medial PFC to the NAc core, which then project to the ventral pallidum and finally to the medial STh. The STh influences the activity of the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr); the output nuclei of the basal ganglia (Maurice et al., 1998). Activation of the indirect circuit results in late or slower disinhibition of the STh (Kolomiets et al., 2001). Specifically, stimulation of the medial PFC activates glutamatergic projections to the NAc core, whose GABAergic projections have an inhibitory effect on the ventral pallidum, which leads to the inhibition of the GABAergic projections to the medial STh, resulting in the disinhibition of the STh (Maurice et al., 1998). An additional hyperdirect pathway incorporating glutamatergic input from the medial PFC provides early or immediate excitation of the STh. As STh neurons are glutamatergic, excitation of these neurons through either the hyperdirect or indirect pathway has an excitatory effect on the GPi and SNr. Neuronal fibres of the output nuclei are GABAergic, and so their excitation has an inhibitory effect on thalamo-cortical circuits to initiate behaviour. Ultimately, the STh suppresses any undesired or inappropriate behaviours

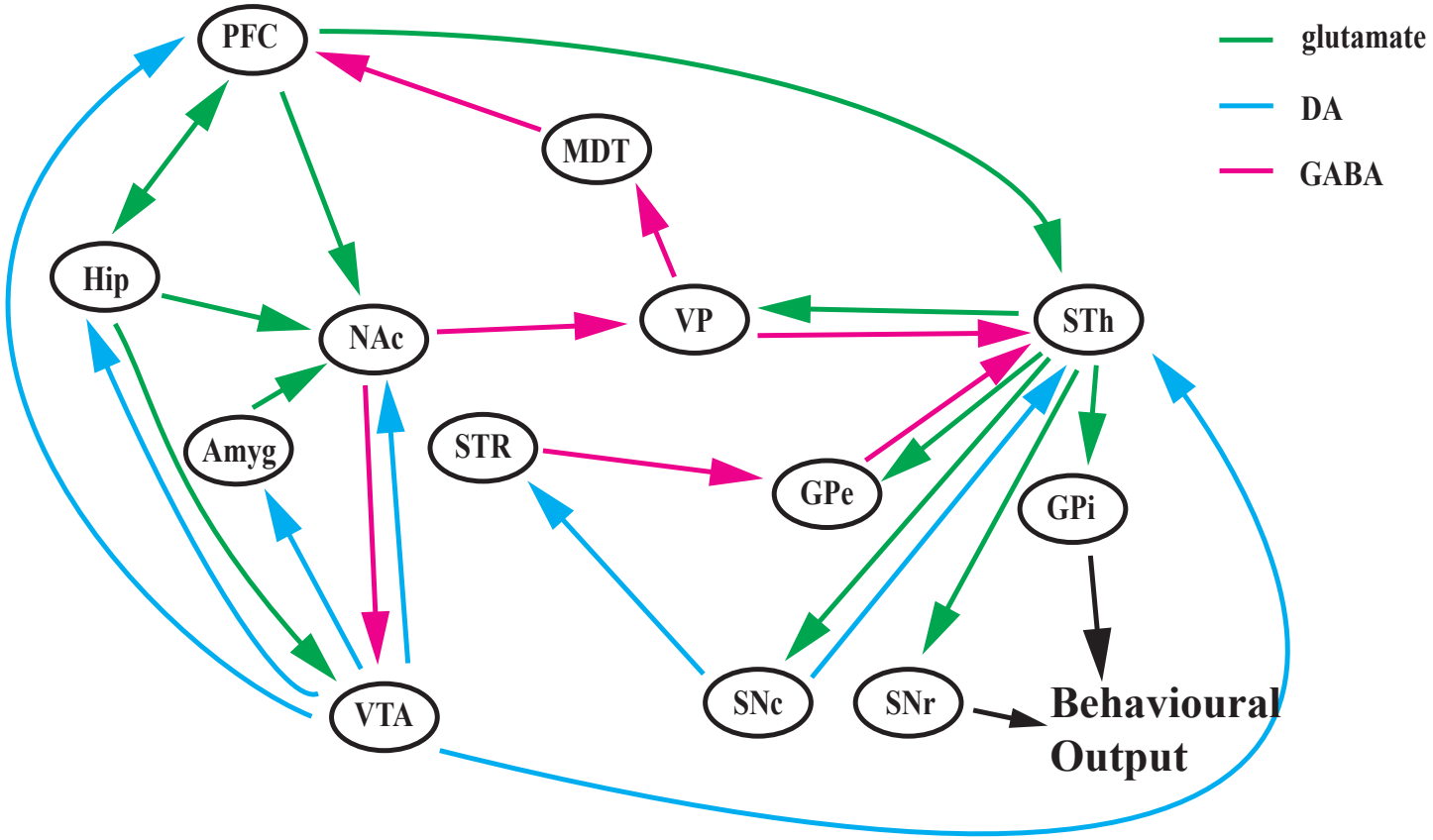

Figure 1. Circuitry involved in METH reward and abuse. The mesocorticolimbic, cortico-striato-pallido-subthalamic and nigrostriatal pathways are depicted. Amgy, amygdala; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; hip, hippocampus; MDT, medial dorsal thalamus; NAc, nucleus accumbens; PFC, prefrontal cortex; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STh, subthalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

through activating the inhibitory fibres of the output nuclei of the basal ganglia (Hamani, Saint-Cyr, Fraser, Kaplitt, & Lozano, 2003; Shen, Zhu, Munhall, & Johnson, 2003; Smith, Bevan, Shink, & Bolam, 1998).

In addition to glutamatergic and GABAergic input to the cortico-striato-pallido-subthalamic circuit, DA is also involved in modulating the activity of this pathway. Dopaminergic fibres innervate the NAc, ventral pallidum and STh, and DA receptors, either D_1-like, D_2-like, or a combination have been located in all of the regions comprising this circuit (Magill, Bolam, & Bevan, 2001; Smith & Kieval, 2000). In relation to the NAc, pre-synaptic D_1-like receptors attenuate afferent glutamatergic fibres from the PFC (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004), and D_1-like and D_2-like receptors on GABAergic efferents to the ventral pallidum modulate inhibitory control (Smith & Kieval, 2000). Further, the dopaminergic tone of the STh is critically involved in regulating activity of the nucleus and its influence on output regions of the basal ganglia, whereby D_2 receptorsmodulate the local release of both glutamate and GABA (Shen et al., 2003). Overall, the regulation of the cortico-striato-pallido-subthalamic circuit is quite complex and involves an intricate interplay between numerous neurotransmitters.

Importantly, the mesocorticolimbic reward pathway connects with the cortico-striato-pallido-subthalamic circuit at the NAc core and STh. The mesocorticolimbic reward pathway extends from the ventral tegmental area (VTA) to the NAc, ventral pallidum, amygdala, hippocampus and PFC (Koob, 1992). The NAc is a key region of the reward circuit, integral for experiencing reward-related effects after exposure to drugs of abuse (Volkow et al., 2010). In addition to a connection to the reward circuit through the NAc core, the STh also receives direct excitatory projections from the medial PFC, that have proven to be functionally relevant (Chudasama, Baunez, & Robbins, 2003; Maurice et al., 1998). The mesocorticolimbic pathway is the main site of action following acute administration of psychostimulants (Luscher & Malenka, 2011). With chronic or repeated use of psychostimulants, the dorsal striatum and wider nigrostriatal pathway are recruited, which are

integral for habit formation of compulsive drug taking (Mameli & Luscher, 2011). This pathway communicates with the NAc through the dorsal striatum and the STh through the substantia nigra pars compacta and the external segment of the globus pallidus (Phillips, 1984). In addition to recruiting the nigrostriatal pathway, the glutamatergic neurons of the PFC are also implicated in chronic psychostimulant use, as impairments in glutamate regulation are associated with increased motivation to seek and administer psychostimulants, and reduced inhibitory control (Kalivas & Volkow, 2005; Volkow, Wang, Fowler, Tomasi, & Telang, 2011). As the STh receives input from the PFC as well as the mesocortiocolimbic and nigrostriatal circuits, it appears that the STh is in a central position to integrate information from regions implicated in chronic drug use to influence cognitive and behavioural outcomes.

The findings of Chapters 3 and 4 demonstrating that exogenous oxytocin attenuated relapse to METH-seeking behaviour in the NAc core and STh, together with the known connections between neural circuits involved in drug addiction, suggests that local administration of oxytocin in these brain regions rescued the functioning of these circuits. Considering the complex activity of each circuit, it is difficult to discern the particulars surrounding their interaction and specifically how oxytocin restores functioning. Exposure to METH following a period of withdrawal is known to activate the VTA and subsequently DA release in the NAc (Robinson & Berridge, 1993). This appears to activate the projections to the PFC, contributing to glutamate release in this region. This would increase the excitation of the glutamatergic projections to the NAc and STh. The activation of the NAc also seems to inhibit the cortico-striato-pallido-subthalamic circuit through activating D2 receptors, inhibiting the STh. Finally, the STh would receive inhibitory input from the nigrostriatal pathway. Considering that the STh would also be receiving increased dopaminergic input from the VTA and SNc (Shen & Johnson, 2000) and more GABAergic input than glutamatergic input, the tonic dopaminergic level would likely be altered, potentially reducing activity in the STh. As the STh would be receiving more inhibitory than excitatory input, the

STh would have less control over inhibiting the motivation to, and engagement in, seeking and administering METH.

The local administration of oxytocin into the NAc core may also restore the normal functioning of the cortico-striato-pallido-subthalamic circuit, whereby the STh is disinhibited and able to suppress engagement in compulsive behaviours associated with METH-seeking. Additionally, as suggested above, oxytocin microinjected into the STh may help recover the normal activity of the region, so that the STh stimulates the output regions of the basal ganglia to inhibit engagement in compulsive drug-seeking behaviours (see Figure 2). The results of this thesis suggest an important role for the NAc core and STh in the regulation of METH behaviour by oxytocin, however the basis of their investigation was identified through the study by Carson et al (2010b) using an acute METH administration procedure. It is possible that other regions are affected by systemic oxytocin administration in chronic METH models and further measures of the effect of oxytocin on cellular activation following repeated METH administration would aim to elucidate additional areas of interest.

It has been suggested that the integrity of the endogenous oxytocin system is important for resilience against addiction and possibly maintaining the regular functioning of the mesocorticolimbic, cortico-striato-pallido-subthalamic and nigrostrial circuits. In Chapter 5, it was reported that chronic METH IVSA increased oxytocin plasma levels. The increase of oxytocin in plasma at 24 hours after the final IVSA session and the likely synchronous increase in central extracellular oxytocin levels (Carson et al., 2014; Wang et al., 2013; Wotjak et al., 1998) may be a means of trying to restore the depleted oxytocin system following chronic drug exposure. Polymorphisms to the OTR (Tost et al., 2010) and environmental factors, including exposure to trauma or stressors (Opacka-Juffry & Mohiyeddini, 2012; Unternaehrer et al., 2012), dysfunctional parental attachment (Wismer Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005) and limited or poor social interactions (Branchi et al., 2013) alters the functioning of the oxytocin system and has been associated

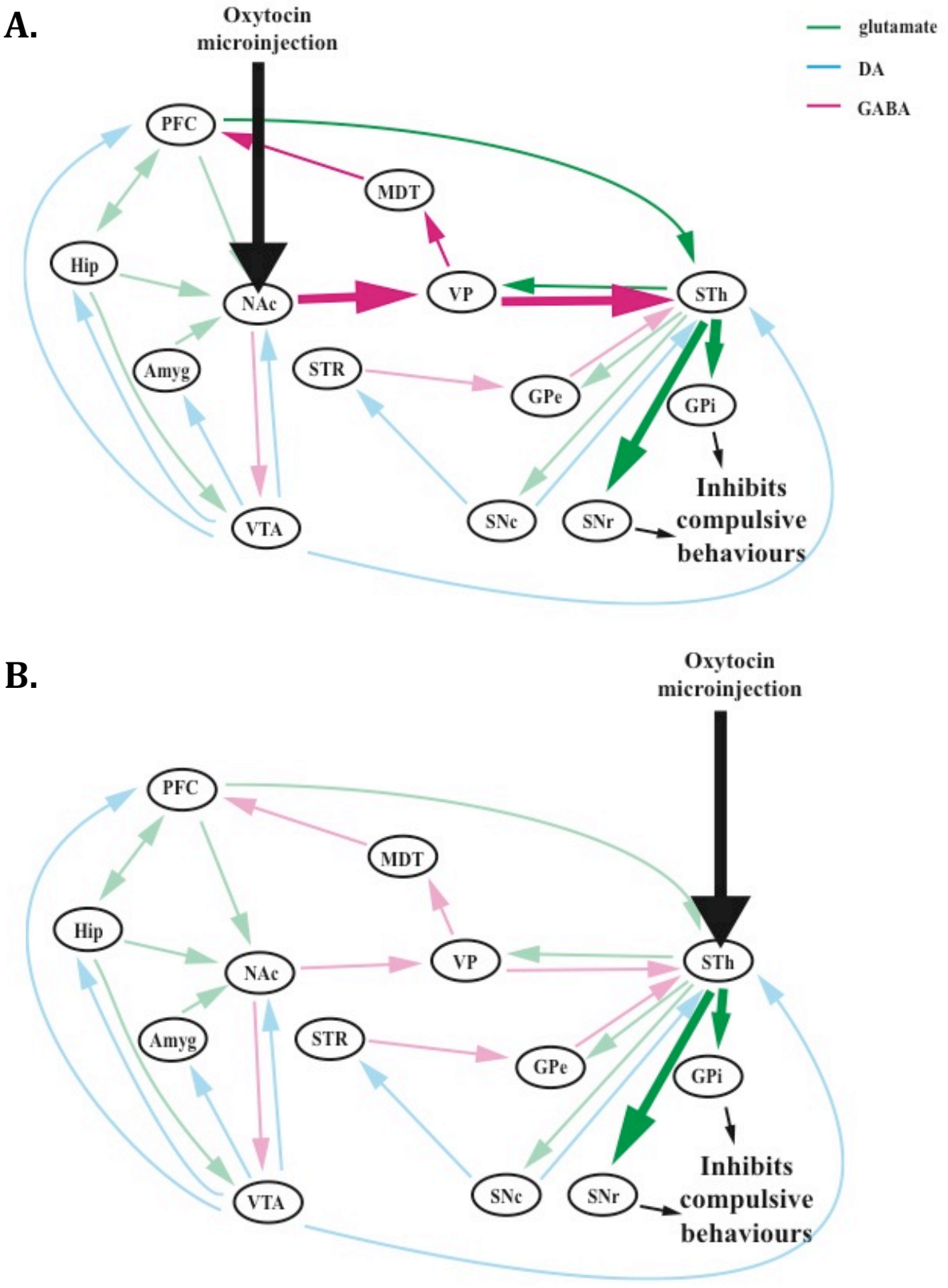

Figure 2. The effect of microinjecting oxytocin into the A) NAc core and B) STh prior to a METH-priming injection on behavioural output. Amgy, amygdala; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; hip, hippocampus; MDT, medial dorsal thalamus; NAc, nucleus accumbens; PFC, prefrontal cortex; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STh, subthalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

with increased susceptibility for becoming addicted to drugs of abuse (for a review see Buisman-Pijlman et al., 2014). Considering this, it is possible that oxytocin intranasal administration could replenish diminished oxytocin levels in drug-addicted individuals. Indeed, oxytocin pretreatment in adolescent rodents appeared to be protective against the development of addictive behaviours in adulthood (Bowen, Carson, Spiro, Arnold, & McGregor, 2011; Hicks, Cornish, Baracz, Suraev, & McGregor, in press) and concurrent oxytocin treatment during METH IVSA in adult rats reduced motivation to self-administer METH (Carson, Cornish, Guastella, Hunt, & McGregor, 2010a). Laboratory rodents live in environments that do not typically replicate the range of social experiences normally engaged in, or completely model their natural environment. Coupled with repeat activation of the hypothalamic-pituitary-adrenal axis with exposure to drugs of abuse, this could potentially be modelling changes to the oxytocin system of humans at risk of repeated drug use. Even though no changes to CORT plasma levels were reported 24 hours following the final METH IVSA session in Chapter 5, it is likely that if blood plasma was collected immediately following the METH IVSA session, increased CORT plasma levels would have been identified, consistent with studies that found increased CORT levels immediately following a toxic METH dose (Tomita et al., 2013) and immediately after 10 days of chronic METH administration (Zuloaga, Siegel, Acevedo, Agam, & Raber, 2013). Taken together, it appears that CORT and the endogenous oxytocin system are activated after METH administration to affect addiction circuitry, and while an interaction is likely, a temporal assessment of this interaction is yet to be described.

6.3. Limitations and future directions

6.3.1. Oxytocin and arginine vasopressin

Oxytocin and arginine vasopressin (AVP) are structurally similar neuropeptides. In terms of their chemical structure, they only differ by two amino acids at positions 3 and 8, and the genes for the neuropeptides, which are highly homogenous, share the same chromosomal locus, although are transcribed in opposite directions (see Figure 3; Gimpl & Fahrenholz, 2001; Lee, Macbeth, Pagani, & Young, 2009). In accordance, the OTR and AVP receptors show high sequence homology, whereby oxytocin can bind to the AVP receptors at a slightly lower affinity than the OTR (Chini & Manning, 2007; Tribollet, Barberis, Jard, Dubois-Bauphin, & Dreifuss, 1988). Considering this, and the results of Chapters 3 and 4, it is possible that oxytocin is acting through AVP receptors to modulate relapse to METH-seeking behaviour.

Like the OTR, AVP receptors are G-protein coupled (Chini & Manning, 2007). Currently, three AVP receptors have been identified; the V1a, V1b, and V2 receptors. The V2 receptor has been located almost solely within the kidneys, whilst V1 receptors have been identified both peripherally as well as centrally within the brain, with the V1a receptor being the most widely expressed (Hernando, Schoots, Lolait, & Burbach, 2001; Stoop, 2012). In the rat brain, the V1a receptor is located within the substantia nigra, NAc, central amygdala, lateral and ventromedial hypothalamus, lateral septum, ventral tegmental area, olfactory system, dentate gyrus, superior colliculus, dorsal raphe, nucleus of the solitary tract and inferior olive whilst the V1b receptor is located within the caudate putamen, hippocampus, NAc, preoptic area of the hypothalamus, anterior hypothalamus, suprachiasmatic nucleus, olfactory bulb, cerebellum, mammillary bodies and red nucleus (Caldwell, Lee, Macbeth, & Young, 2008; Hernando et al., 2001; Stoop, 2012). The distribution pattern of AVP V1

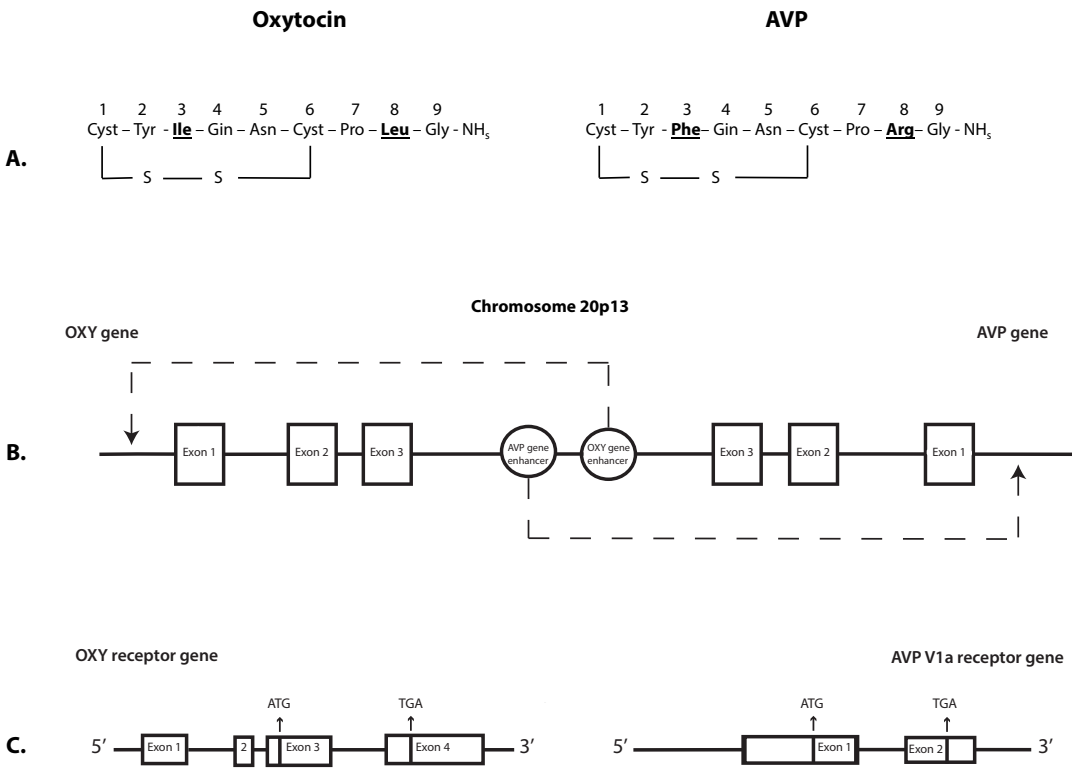

Figure 3. The structural similarities between the oxytocin and AVP systems. A) The amino acid structure of oxytocin and vasopressin, which only differ by 2 amino acids at positions 3 and 8. B) The genetic structure of the oxytocin and vasopressin gene. Both genes are located on the human chromosome 20p13 and are transcribed in opposite directions. C) The genetic structure of the oxytocin receptor and AVP V1a receptor, which share high sequence homology. Diagram adapted from Gimpl and Fahrenholz (2013) and Kumsta, Hummel, Chen, and Heinrichs (2013).

receptors largely differs to the OTR and where both neuropeptide receptors are located in the same brain region, they are typically expressed in different subsections (Gimpl & Fahrenholz, 2001; Tribollet, Dubois-Bauphin, Dreifuss, Barberis, & Jard, 1992). Altogether, it is not surprising then that oxytocin and AVP are both involved in numerous social behaviours and modulating anxiety, although typically having opposing effects. Oxytocin generally has prosocial effects, facilitating bonding, and is anxiolytic (Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000; Young, Lim, Gingrich, & Insel, 2001). In contrast, AVP is associated with intermale aggression, courtship, and is anxiogenic (Litvin, Murakami, & Pfaff, 2011; Thompson, George, Walton, Orr, & Benson, 2006).

Recently, a number of prosocial and autonomic effects that were traditionally thought to be solely driven by the oxytocin system were found to also involve the AVP system. Adjacent lying, which is increased following an acute systemic injection of oxytocin, was reduced when rats were pretreated with SR49059, a V1a receptor antagonist (Ramos et al., 2013). Oxytocin-induced defensive aggregation in groups of rats was also reduced by administration of SR49059, indicating that oxytocin activated the V1a receptor to modulate social response to threatening stimuli (Bowen & McGregor, 2014). In syrian hamsters, flank marking, a form of social communication, was increased by icv administration of oxytocin, and attenuated by a V1a receptor antagonist (Song et al., 2014). Further, oxytocin injections in an animal model of autism rescued social deficits as well as cognitive flexibility in oxytocin receptor knock-out mice, where these effects were reversed by SR49059 (Sala et al., 2011). In terms of autonomic effects, Hicks et al. (2014) showed that systemic oxytocin injections reduced body temperature and heart rate, with this effect also being blocked by SR49059. Altogether, this suggests that in addition to the OTR, oxytocin acts through the V1a receptor to modulate numerous social and autonomic actions.

Considering the strong involvement of the V1a receptor in numerous oxytocin-mediated effects, future studies should elucidate the role that AVP plays in psychostimulant abuse and addiction. Understanding this will help inform appropriate

treatment development in terms of targeting either the oxytocin system exclusively or in combination with the AVP system. This is particularly relevant considering the findings of Chapters 3 and 4, where co-administration of the selective OTR antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT with oxytocin into either the NAc core or STh was not able to significantly reverse the modulating effect of oxytocin on METH-seeking behaviour. It would be of interest to investigate the involvement of the V1a receptor in METH IVSA through the co-administration of oxytocin and SR49059. This will help elucidate the targets of the oxytocin system in reducing METH-induced relapse to drug-seeking behaviour.

6.3.2. Localisation of the oxytocin receptor

The visualisation and subsequent localisation of the OTR within the brain has been hampered by a number of technical difficulties. Traditionally, exploratory studies investigating the location of the OTR throughout the rat brain used *in vitro* receptor autoradiography. This technique involves the use of radioactive ligands (typically [^{125}I]-OTA in the case of the OTR) to label a particular receptor type or radioactively label amino acids or neurotransmitters to trace neuronal fibres. In general, this technique has several shortcomings including long exposure time, requiring careful manipulation of radioactive substances, and relatively poor resolution (Braissant & Wahli, 1998; Takakura, Hattori, Takeuchi, & Ozawa, 2012). Further, it has been particularly difficult to locate OTRs at a cellular level using this technique. This is thought to be due to the low binding potential of the OTR coupled with the limited sensitivity of the radioactive ligand (Freund-Mercier, Stoeckel, Dietl, Palacios, & Richard, 1988; Yoshida et al., 2009).

An alternative approach for visualising the OTR is through the use of immunohistochemistry. This technique allows for cellular localisation of target antigens through the application of specific antibodies that have been tagged with a specific fluorophore. However, some difficulties with the commercially available OTR targeted

antibodies have been identified. It appears that inconsistent staining using identical tissue preparation methods in different lot numbers of an antiserum from the same company as well as antiserums from different companies has been noted (Boccia, Petrusz, Suzuki, Marson, & Pedersen, 2013; Yoshida et al., 2009). Due to this, in Chapter 5, the application of the OTR antiserum was initially carefully evaluated in pilot brain sections to determine that the antibody was consistently staining the OTR in positive control regions, with no visualisation of staining in negative control regions. However, it is not known if the currently available OTR antiserums have sensitivity for low affinity OTRs, limiting the interpretation of the type of OTR measured in the current study (Moreno-Lopez, Martinez-Lorenzana, Condes-Lara, & Rojas-Piloni, 2013).

An additional difficulty with investigating the involvement of the oxytocin system in drug-related behaviours is the likely existence of an additional, currently uncharacterised OTR. Considering the vast range of endocrine, behavioural and physiological actions of oxytocin, it seems unlikely that they are all mediated by a single OTR subtype and at times, the V1a receptor subtype. Indeed, support for an additional OTR subtype has been voiced within the literature (Adan et al., 1995; Chan, Wo, Stoev, Cheng, & Manning, 2003; Verbalis, 1999). As the OTR subtypes could be derived from independent genes located on different chromosomes (Verbalis, 1999) this could be hampering the discovery of additional OTR subtypes. Future investigation into similar genetically structured receptors to the currently identified OTR in addition to pharmacological analysis may be of benefit.

To improve the sensitivity for locating the currently characterised OTR, recent advances in viral vectors and transgenic animals could be used. Considering that functional OTRs were located in the STh, based on selective OTR antagonist effects reported in Chapter 2, yet have not been visualised using traditional techniques of receptor autoradiography, viral vectors could be used in OTR-Venus reporter mice to map out OTR expressing cells in the STh and determine whether they are pre- or post-synaptically located. Moreover, transgenic rats which express oxytocin fluorescent proteins (cyan fluorescent protein and monomeric red

fluorescent protein) have been developed and used to visualise oxytocin neurons within the brain (Hashimoto, Matsuura, & Ueta, 2014). The generation of transgenic rats with an OTR fluorescent protein fusion gene could greatly advance knowledge on OTR localisation in the rat brain and help characterise changes to the receptor following chronic METH exposure.

6.3.3. Oxytocin, the blood brain barrier, and implications for use as a pharmacotherapy

A major difficulty with the administration of oxytocin is that it does not readily cross the blood brain barrier. Oxytocin is a relatively large molecule, which is hydrophilic; two factors that limit its penetration (Churchland & Winkielman, 2012). In addition, if orally ingested, first pass metabolism greatly reduces the bioavailability of the peptide, further limiting the amount of active drug that reaches the blood brain barrier (Chapman et al., 2013). Administration of oxytocin directly to the brain through icv infusion, intracerebral injection, or temporary disruption of the blood brain barrier are highly invasive techniques and are not yet viable options (Lalatsa, Schatzlein, & Uchegbu, 2014). Intranasal drug delivery is a non-invasive method of administration with improved bioavailability as it avoids the blood brain barrier and first pass metabolism by the liver (Lalatsa et al., 2014). This administration approach also allows for rapid absorption of oxytocin and in turn, a quick increase of oxytocin levels in the central nervous system (Chapman et al., 2013; Parvathi, 2012). Moreover, oxytocin intranasal administration in both normal and clinical human populations has no reported adverse side effects (Anagnostou et al., 2012; Guastella et al., 2010; Guastella, Mitchell, & Dadd, 2007; Hollander, Novotny, & Hanratty, 2003; Tachibana et al., 2013). Altogether, intranasal administration is a non-invasive approach allowing rapid drug delivery to the brain with no reported adverse effects.

Evidence for central uptake of oxytocin following nasal application has been demonstrated in a rodent study conducted by Neumann's laboratory (2013). They showed that

following nasal administration, oxytocin levels in extracellular fluid extracted from the amygdala and dorsal hippocampus increased and peaked between 30 to 60 minutes after administration. Moreover, plasma oxytocin concentrations also increased 70 minutes following administration, and remained high for a further 60 minutes. This suggests that following central uptake, oxytocin crosses the blood brain barrier and also leads to enhanced peripheral effects. Even though these findings have important implications, it is difficult to extrapolate findings from rodents to humans considering structural differences of the nose (Guastella et al., 2013). In the rat, the nasal cavity is largely comprised of the olfactory epithelium (50%), whilst in humans, it is a very small portion of the nasal cavity (3%). To date, two studies have found a positive correlation between plasma and cerebrospinal (CSF) oxytocin levels, one with adults suffering from headaches (Wang et al., 2013) and the other using a convenience sample of children with serious medical conditions (Carson et al., 2014). However, all other studies that have investigated plasma and CSF oxytocin levels with a sample of pregnant women, suicide attempters or patients undergoing surgery have not found a positive relationship (Altemus et al., 2004; Jokinen et al., 2012; Kagerbauer et al., 2013). Additional investigation is clearly required to examine this relationship further in both healthy and clinical populations to determine if intranasal administration provides effective oxytocin levels in the brain.

Intranasal administration is a promising method of administration, however there are a number of factors that need to be further evaluated. To date, it is currently unclear which pathways are used to deliver oxytocin to the brain, although it is thought that the olfactory bulb and trigeminal nerve pathways are the most likely to be activated (MacDonald & Feifel, 2013). A greater understanding of the activation of pathways that result in oxytocin reaching the brain in physiologically significant levels following intranasal administration in a human population is required, as well as the brain regions that are activated. This will assist in developing more appropriate methods of administration including spray bottles that can more effectively project the oxytocin spray to target the nasal pathways responsible for central

delivery, as well as the most appropriate dose to be administered, and the most efficient number of sprays per nostril for each administration. Currently, the effect that repeated oxytocin administration has on the brain at a molecular level is unclear, as well as on physiology and behaviour. Further research into chronic treatment with intranasally administered oxytocin is needed to determine what effect oxytocin has in the brain and whether oxytocin delivered in this manner can have clinically meaningful behavioural effects. Intranasal oxytocin is currently under examination in clinical populations, including patients with autism spectrum disorder (Anagnostou et al., 2012; Guastella et al., 2010; Hollander et al., 2007; Hollander et al., 2003) schizophrenia (Bujanow, 1972, 1974; Davis et al., 2014; Gibson et al., 2014; Woolley et al., 2014), social and general anxiety (Guastella, Howard, Dadds, Mitchell, & Carson, 2009; Labuschagne et al., 2010), and alcohol and marijuana dependence (McRae-Clark, Baker, Maria, & Brady, 2013; Pedersen et al., 2012).

In addition to enhancing understanding of the long-term effects of administering oxytocin in clinical populations, the impact of context and personality factors on the outcome of oxytocin administration should be examined further. The effect of oxytocin administration appears to be context-dependent, where intranasal oxytocin administration has been shown to increase envy and gloating when engaging in a game of chance incorporating monetary gain conditions (Shamay-Tsoory et al., 2009) and increases in-group favouritism and at times out-group derogation in computer-based tasks (De Dreu, Greer, Van Kleef, Shalvi, & Handgraaf, 2011). Recent studies have also identified that intranasal oxytocin administration can enhance or amplify psychological states; such that oxytocin administration can further decrease trust in individuals with borderline personality disorder who are prone to mistrust (Bartz et al., 2011), and can increase intimate partner violence in individuals who are high in trait aggression (DeWall et al., 2014). This challenges the view of oxytocin as a purely prosocial peptide and promotes greater consideration of state and trait personality factors and the context within which oxytocin would be administered. And finally, as aforementioned, greater knowledge on mechanisms of oxytocin action for symptom improvement and attenuating drug reward could

have an impact on compound selection for intranasal application. Considering that oxytocin interacts with the AVP V1a receptor, as well as with dopamine, glutamate, and GABA, greater understanding of oxytocin action could have an impact on whether a compound that selectively targets the oxytocin system is more appropriate, or alternatively, one that acts on a combination of receptors that oxytocin targets.

6.3.4. Application of animal models of drug abuse

6.3.4.1. Conditioned place preference paradigm

Even though the CPP paradigm is considered a valid model for examining reward, there are some limitations that need to be addressed. It has been argued that providing drugs during conditioning results in state-dependent learning or alters memory. Pairing with drugs may impair memory and so block familiarisation with being exposed to that particular compartment so that on test day, animals explore the "novel" compartment more. However, when CPP has been conducted with a saline-paired, drug-paired, and novel compartment, rats spent more time in the drug-paired compartment than the novel compartment on test day, refuting this notion (Bardo & Bevins, 2000). It has also been proposed that familiarity is increased for the drug-paired compartment during conditioning and so animals spend more time in this chamber on test day. This seems unlikely, as conditioning with different agonists and antagonists produce different outcomes, which is reflective of whether the treatment is rewarding, blocks the rewarding effects of another drug when co-administered, or is aversive (Mucha, Van Der Kooy, O'Shaughnessy, & Bucenieks, 1982). A practical difficulty with CPP is the difficulty with examining a dose response curve with increasing doses of a drug, as it would be very difficult to detect the subtle effects of differing doses on the formation, or lack of formation, of a place preference. However, even considering the above-mentioned limitations, the CPP paradigm currently provides an efficient and sound approach for examining reward processes within rodents.

6.3.4.2. Intravenous drug self-administration paradigm

Experiments conducted using the intravenous drug self-administration procedure provides valuable information (Spealman & Goldberg, 1978), however the paradigm is limited in certain respects. In terms of how well this paradigm replicates human drug abuse and relapse, there are some noticeable differences. In instances where the animals undergo extinction conditions, they continue to engage in the behaviour without receiving reinforcement. Humans undergoing withdrawal are usually doing so due to a lack of drug availability, to avoid punishments (e.g. imprisonment), or because of the negative consequences of the drug (Shaham, Shalev, Lu, De Wit, & Stewart, 2003). Although in both cases, the association between the behavioural response and/or cues associated with administration of the drug diminishes with time. Differences in measuring relapse are also apparent, where in instances of measuring cue- or stress-induced reinstatement, the animals behavioural responses are not reinforced, whilst for humans who are seeking out a drug, the end result would be drug administration (Shaham et al., 2003). Considering practical limitations, the patency of intravenous catheters can limit the length of the experiment, as catheters only remain patent for a couple of months. Despite the limitations, the self-administration paradigm is a well-established and effective means for investigating numerous drug-related behaviours and brain-related changes. This procedure can help further our understanding of the myriad of behavioural, molecular and physiological changes involved in drug abuse and addiction.

6.4. Conclusions

Major findings from this thesis demonstrate that exogenous and endogenous oxytocin systems are involved in METH-related behaviours; in particular oxytocin acting within the

NAc core and STh to reduce relapse to METH-seeking behaviour. In terms of acute reward processes, oxytocin attenuated DA-mediated reward in the STh through activation of the OTR, showing that the oxytocin system was driving this effect. Direct administration of oxytocin into either the NAc core or STh reduced reinstatement to METH-seeking behaviour. However, the ability of oxytocin to attenuate METH-primed reinstatement does not appear to be driven by the oxytocin system, and likely includes additional receptors beyond the OTR. Even though the involvement of the OTR in oxytocin attenuation of relapse to METH use was weak, OTR fibre density in the NAc core and STh were differentially affected, and oxytocin blood plasma levels were chronically elevated following METH IVSA and after behavioural extinction. This shows that neural changes to the endogenous oxytocin system, centrally within the NAc core and STh as well as within the periphery, are associated with METH abuse. Altogether, these findings further highlight the importance of oxytocin in mediating METH-related reward and relapse and enhance our understanding of the brain regions and mechanisms involved. This data also provides additional support and insight into the applicability of oxytocin as a pharmacotherapeutic treatment for METH abuse and dependence.

6.5. References

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ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/014-3 **Date of Expiry:** 04 May 2013

Full Approval Duration: 05 May 2011 to 04 May 2013 (24 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and is contingent upon receipt of a Final Report at the end of this period (see Approval email for submission details).**

Principal Investigator:
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Associate Investigators:
Sarah Baracz 0410 324 069

Other people participating:
Nicholas Everett 0447 285 037

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

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

Title of the project: The rewarding effects of monoamines in the subthalamic nucleus and interactions with oxytocin administration.

Type of animal research and aims of project: 4 - Research (human or animal biology)
This project aims to further explore neurotransmitter regulation of the subthalamic nucleus (STh) in reward processes and the interaction that oxytocin has with the monoamine messengers.

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	Sex	Weight	Age	Total	Supplier/Source
Rat	SD	male	250g	3 months	144	ARC Perth
Rat	SD	male	250g	3 months	64	ARC Perth
Rat	SD	male	N/A	N/A	16	ARC Perth
				Total	**224**	

Location of research:

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

Amendments approved by the AEC since initial approval:
1. *Approved 16 June 2011: Addition of Niree Kraushaar as Associate Investigator.*
2. *Approved 20 October 2011: Increase the number of animals to 64 & inclusion of additional substance (oxytocin antagonist) to design.*
3. *Approved 02 October 2012: Re-opening protocol, addition of 16 rats & 12 months extension (to be ratified at AEC 18 October 2012)*

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.

Prof Michael Gillings (Chair, Animal Ethics Committee)

Approval Date: ***04 October 2012***

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/050-7 **Date of Expiry:** 13 November 2013
Full Approval Duration: 14 November 2011 to 14 November 2014 (36 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 / is contingent upon receipt of a Final Report at the end of this period (see Approval email for submission details).**

Principal Investigator:
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Matthew Castino	0423 181 691
Callum Hicks	0402 912 926

Other people participating:

Julia Plumb	0431 204 122
Nicholas Everett	0447 285 037

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

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:
Title of the project: The effect of oxytocin and an antagonist in the nucleus accumbens core or subthalmic nucleus on relapse to methamphetamine-seeking behaviour
Purpose: 4 - Research Human or Animal Biology
Aim: To investigate the effects of oxytocin and an oxytocin antagonist on relapse to meth-seeking behavior 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	Sex	Weight	Age	Total	Supplier/Source
Rat	Sprague- Dawley	Male	250 g	3 months	80	ARC Perth
				TOTAL	**80**	

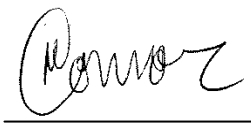
Location of research:

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

Amendments approved by the AEC since initial approval:
1. *Amendment to add additional region to be investigated using self-administration paradigm (Approved July 2012)*
2. *Amendment to add Matthew Castino as Associate Investigator with condition of adequate supervision (Approved 24 August 2012, Ratified at AEC 13 September 2012)*
3. *Amendment to add Nick Everett as Research Assistant (Approved 25 September to be ratified at AEC 18 October 2012)*
4. *Amendment to add Callum Hicks as Associate Investigator (Exec approved 16 October 2013, ratified AEC 17 October 2013)*

Conditions of Approval:
1. *The Committee agreed to approve the application subject to Saline rehydration being used.*
2. *Should any extra animals be required for testing the antagonist, an amendment would have to be submitted for this.*
3. *Mr Gates must attend the next available animal welfare and ethics course.*
4. *New rodent researchers must successfully complete the MQ rodent basic training courses*
    a. *Rodent Handling and Care*
    b. *Rodent anaesthesia with injectable and inhalant anaesthetics*
    c. *Clean and Aseptic surgical techniques*
    d. *Post-operative monitoring and pain relief*

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: 17 October 2013**

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2014/006 **Date of Expiry:** 28 February 2015

Full Approval Duration: *1 March 2014 to 1 March 2016 (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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Associate Investigators:
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Other People Participating:
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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 chronic intravenous self-administration of methamphetamine on oxytocin receptor expression in the nucleus accumbens core and subthalamic nucleus of the rat**

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

Aims: **To determine whether the oxytocin receptor is located within the nucleus accumbens core and subthalamic nucleus and to investigate whether receptor expression changes following chronic METH exposure and withdrawal from METH**

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/Sex/Weight	Total	Supplier/Source
02 – Rats	Sprague Dawley	Male / 200-250 grams on arrival	48	ARC Perth
		TOTAL	48	

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. Addition of *Ms Adriana Papallo (Approved 20 March 2014).*

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: 20 March 2014**