The effects of vasopressin and oxytocin on methamphetamine–induced place preference behaviour in rats

By

Cassandra Subiah
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Submitted in fulfillment of the requirements for the degree of Master of Medical Science (MSc) in the Department of Human Physiology, in the Faculty of Health Science at the University of KwaZulu-Natal – Westville Campus

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Co-Supervisor: Dr M. Mabandla
Date Submitted: 18 May 2012

Declaration

I, Cassandra Subiah, student number: 205503129 hereby declare that the dissertation/thesis entitled:

The effects of vasopressin and oxytocin on methamphetamine–induced place preference behaviour in rats

Is the result of my own investigation and research and that it has not been submitted in part or full for any other degree or to any other University or Tertiary Institution. Where use was made of the work of others, it is duly acknowledged. The research done in this study was carried out under the supervision of Prof W.M.U. Daniels and Dr M. Mabandla

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Cassandra Subiah Date

Acknowledgements

The following people are thanked for their guidance and support:

- Prof W.M.U. Daniels
- Dr M.V. Mabandla
- Staff at the BRU - Dr S Singh, L.Bester, Rita, Dennis, David
- Alisa Phulukdaree
- Prof Anil A. Chuturgoon
- Nerolen Soobryan
- Neuroscience Post-graduate team

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List of Abbreviations

AADC - Aromatic L-Amino Acid Decarboxylase

AMPA - 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid

BNST - bed nucleus of the stria terminalis

CA1 - Cornu Ammonis 1

CAMKII – Calcium/calmodulin-dependent protein kinases II

cAMP – cyclic adenosine monophosphate

CPP – conditioned place preference

CPu – caudate putamen

CRE - cyclic adenosine monophosphate response element

CREB – cyclic adenosine monophosphate response element binding protein

CRH - Corticotropin-releasing hormone

DARPP-32 - dopamine- and cAMP-regulated neuronal phosphoprotein

ERK - extracellular-signal-regulated kinases

HPA axis – hypothalamus - pituitary - adrenal axis

ICSS – intracranial self-stimulation

IP_3 – inositol triphosphate

LTP – long term potentiation

MAPK - Mitogen-activated protein kinases

NAc – nucleus accumbens

NMDA - N-Methyl-D-aspartate

PFC- pre-frontal cortex

PI3K - Phosphatidylinositol 3-kinases

PKA –protein kinase A

S-R – stimulus – response

TH- tyrosine hydroxylase

VMAT2 – vesicular monoamine transporter 2

VTA – ventral tegmental area

Abstract

Methamphetamine is a highly addictive stimulant drug whose illicit use and resultant addiction has become an alarming global phenomenon. The mesolimbic dopaminergic system in the brain, originating in the ventral tegmental area and terminating in the nucleus accumbens, has been shown to be central to the neurobiology of addiction and the establishment of addictive behaviour. This pathway, as part of the reward system of the brain, has also been shown to be important in classical conditioning, which is a learnt response. This common pathway has supported theories suggesting addiction as a case of maladaptive associative learning. Within the modulation of learning and memory, the neurohypophyseal hormones vasopressin and oxytocin have been seen to play a vital role. Vasopressin exerts a long- term facilitatory effect on learning and memory processes. Studies have shown that the stress responsive AVP V1b receptor systems are a critical component of the neural circuitry underlying emotional consequences of drug reward. Oxytocin, on the other hand, has an effect on learning and memory opposite to that of vasopressin. Previous studies have shown that oxytocin caused a decrease in heroin self-administration, as well as attenuated the appearance of cocaine-induced hyperactivity and stereotyped behaviour. Therefore, we adopted a reinstatement conditioned place preference model to investigate whether a V1b antagonist or oxytocin treatment would cause a decrease in methamphetamine seeking behaviour. Behavioural findings indicated that methamphetamine induced a change in the place preference in the majority of our animals. This change in preference was not seen after vasopressin administration in the extinction phase. On the other hand, the change in place preference was enhanced during the reinstatement phase in the animals treated with oxytocin. Striatal dopamine levels were determined, as methamphetamine is known to increase dopamine transmission in this area. Results showed that rats that received both methamphetamine and oxytocin had significantly higher striatal dopamine than those that received oxytocin alone. Western blot analysis for hippocampal cyclic AMP response element binding protein (CREB) was also conducted as a possible indicator of glutamatergic NMDA receptor activity, a pathway that is important for learning and memory. The Western blot analysis showed no changes in hippocampal pCREB expression. Overall our data led us to conclude that methamphetamine treatment can change place preference behaviour in rats and that this change may be partially restored by vasopressin antagonism, but exaggerated by oxytocin.

Overview

Drug addiction is a global problem causing physical, emotional and social harm, both to those addicted and those around them (Buxton and Dove, 2008). South Africa is not excluded from this statistic, with continuing escalation in the use of illicit drugs especially between the ages of 15 – 25 years being reported by the South African Community Epidemiology Network on Drug Use (SACENDU Report June 2011). In recent years, the abuse of methamphetamine in South Africa has increased, with reports from the United Nations Office on Drugs and Crime (UNODC) indicating that of the 16,300 people who sought treatment for drug abuse in South Africa in 2006, 26.9% were for methamphetamines. This makes methamphetamine one of the highest abused drugs nationally, second only to cannabis at 32.7%.

Within the paradigm of addiction many theories exist, some of which will be expanded upon in the literature review in chapter 1. However, much focus has been centred on addiction as a learnt response, with theories suggesting addiction as a case of maladaptive associative learning (Kelly and Berridge, 2002; Chao *et al*., 2004; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008). Within learning and memory, the process of long-term potentiation has been shown to play a vital role, involving the activation of cyclic adenosine monophospate response element binding protein (CREB) to initiate memory storage (Silva *et al*., 1998). This process will be discussed in more detail in chapter 1.

Regardless of the theory, one common characteristic is seen with regards to addiction, and that is that drugs of abuse all act on a specific pathway in the brain known as the mesolimbic pathway (Leff *et al*., 2000). This pathway is mediated by the neurotransmitter dopamine, which is elevated in response to all drugs of abuse, including methamphetamines (Volkow *et al.*, 2003). In the literature review of this dissertation, dopamine, methamphetamine and the relationship between the two will be discussed.

Neurohormones vasopressin and oxytocin play vital roles both peripherally as well as centrally, with its role in learning and memory being subject to much debate (Kovács *et al.,* 1979; Sahgal *et al.,* 1984; Van Ree *et al.,* 1985; Engelmann *et al.,* 1996; De Wied, 1997; Boccia *et al.,* 1998; Heinrichs *et al.,* 2004). The general consensus is that vasopressin has a stimulatory effect of learning and memory, while oxytocin has an inhibitory effect (Heinrichs *et al.,* 2004; Ring, 2005). The peripheral and central effects of both these neurohormones and their roles in learning and memory will be detailed in the literature review that follows.

With addiction being viewed as a learnt behaviour, and the suggested roles of neurohormones in learning and memory, the limited interest in the role of vasopressin and oxytocin in addiction is surprising. The few studies that have been conducted in this regard have been inconclusive and none has focused on methamphetamine addiction.

To address this shortcoming in our current knowledge we investigated the effects of vasopressin and oxytocin in an animal model of methamphetamine-induced addictive behaviour. In doing so we developed a 3 stage place preference model of addiction, comprising of acquisition, extinction and reinstatement. This model will be explained in more detail in chapter 2 of this dissertation, where an article outlining our research is included.

Given the opposite effects of vasopressin and oxytocin on learning and memory, we used a vasopressin V1b antagonist (SSR 149415) and oxytocin as treatment methods in the extinction phase. Our hypothesis was that administration of the vasopressin antagonist and oxytocin would reverse the associative learning developed in the place preference model, and result in a decrease in addictive behaviour when presented with drug cues in the reinstatement phase.

As a measure of construct validity, dopamine levels were evaluated in the striatum of methamphetamine - treated rats and controls. Similarly, CREB levels were assessed in the hippocampal tissue to determine if signalling processes within learning and memory pathways were affected in any of the rat groups. Methodology for these neurochemical analyses is outlined in chapter 2 of this dissertation.

Finally our findings are reported and discussed in the article contained in this dissertation, and are summarized in chapter 3 with recommendations for future research in this area.

The Animal Ethics Subcommittee of the University of KwaZulu-Natal approved all procedures, which were all in accordance to the guidelines of the National Institutes of Health (Ethics clearance number 044/08/Animal).

Chapter 1

Literature Review

1.1 Addiction

Drug addiction is a chronically relapsing disorder that is characterized by a compulsion to take a drug, a lack of control in limiting this intake despite knowledge of the negative consequences, and the withdrawal of the drug resulting in the emergence of a negative emotional state and motivation signs of discomfort (Roberts and Koob, 1997; Heather, 1998; Koob, 2000; Hyman and Malenka, 2001; Shippenberg and Koob, 2002; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008; Koob and Volkow, 2010).

1.1.1 Theories of addiction

Numerous theories have been proposed as to the mechanism by which addiction develops and is maintained. A few of the more prominent theories will be discussed below;

1.1.1.1 Hedonic theory:

This theory postulates that addiction develops in relation to positive and negative reinforcing properties of a drug (Chao *et al*., 2004; Feltenstein and See, 2008). The basic principle is that initial consumption of the drug results in the production of positive effects (e.g. euphoria, pleasure) or reward, which may drive subsequent drug administration in order to re-experience the reward again (positive reinforcement) (Kelly and Berridge, 2002; Chao *et al*., 2004; Feltenstein and See, 2008).

This positive reinforcement accounts for the initiation of drug taking behaviour, but the recurrent drive for reward is also mediated by the appearance of withdrawal symptoms (anhedonia and dysphoria) when drug use ceases (Kelly and Berridge, 2002; Chao *et al*., 2004; Feltenstein and See, 2008). Thus the need to alleviate these negative effects may result in continued drug use and may account for the transition from social use or experimentation to compulsive behaviour (Chao *et al*., 2004).

These opposing processes of euphoria and dysphoria are measured on a hedonic scale, and make up the components of the opponent process theory (Chao *et al*., 2004). Continued exposure to the abused drug will result in a rise in the hedonic set point which results in a

decreased hedonistic effect of the drug at a constant dose while increasing the intensity of withdrawal symptoms (Chao *et al*., 2004; Feltenstein and See, 2008). This shift from a positive to a negative hedonistic state and the dysregulation of brain reward systems leads to a loss of control over drug intake and increased vulnerability to relapse (Chao *et al*., 2004; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008).

Whilst the hedonic theory provides a mechanism for the initiation and maintenance of compulsive drug use, it fails to account for other aspects of drug abuse, such as a relapse into drug seeking and drug taking behaviour following prolonged periods of abstinence, when overt withdrawal symptoms are no longer present (Chao *et al*., 2004; Feltenstein and See, 2008). Nor does it explain the addictive nature of psychostimulant drugs, who present no physical, and variable and mild emotional withdrawal symptoms; or the fact that many non-addictive drugs may present severe withdrawal symptoms on cessation of use (Hyman and Malenka, 2001).

1.1.1.2 Incentive-sensitization:

The neural link between the ventral tegmental area (VTA) and the nucleus accumbens (NAc) plays a role in attributing salience to a stimulus, i.e. it's perceived value or attractiveness (Vanderschuren and Everitt, 2005). This theory suggests that repeated exposure to drugs of abuse leads to adaptations which results in the sensitization of this neural circuitry to the drug and drug-related stimuli (Hymen *et al*., 2001; Kelly and Berridge, 2002; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008). This sensitization causes the excessive attribution of incentive salience to the drug and associated stimuli, causing a shift from drug 'liking' to them being excessively 'wanted' and may drive compulsive drug-seeking, drug taking and relapse behaviours (Kelly and Berridge, 2002; Wolf, 2002; Chao *et al*., 2004; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008). This theory distinguishes the important difference between drug 'liking' and drug 'wanting', which separates the euphoric power of the drug from its addictive potential, i.e. drug self-administration can be maintained even in the absence of pleasure (Chao *et al*., 2004; Saah, 2005; Adinoff, 2007).

1.1.1.3 Stimulus-Response (S-R) habit learning:

Repeated exposure to drugs results in alterations in the cellular mechanism of associated S-R learning and reward predictions, with drug seeking being triggered by drug associated cues

(Kelly and Berridge, 2002; Chao *et al*., 2004; Vanderschuren and Everitt, 2005; Everitt *et al*., 2008). Accordingly, it can be said that drug taking is a learned response to conditional stimuli that results in deeply ingrained drug habits beyond the control of the addicted individual (Kelly and Berridge, 2002; Chao *et al*., 2004; Vanderschuren and Everitt, 2005; Feltenstein and See, 2008).

1.1.1.4 Frontal cortex dysfunction:

Another theory of addiction that may explain its persistent nature outlines dysfunctions within the pre-frontal cortex in response to prolonged drug exposure (Vanderschuren and Everitt, 2005; Feltenstein and See, 2008). This reduction in PFC activity reduces its inhibitory control over behaviour and decision making skills, impairing the ability of the drug user to inhibit inappropriate drug-centred behaviour, and allowing drug-associated behaviours to become dominant (Vanderschuren and Everitt, 2005; Feltenstein and See, 2008).

It is possible that a combination of all the factors suggested in these theories contribute to the neural and behavioural pathologies seen in addiction (Chao *et al.,* 2004)

1.1.2 Learning and Memory in Addiction

Drugs of abuse are both rewarding, in that they are interpreted by the brain as being positive, as well as reinforcing, meaning that behaviours that are associated with drug use tend to be repeated (Kopnisky and Hyman, 2002). This persistence of drug taking behaviour is due to the drugs ability to induce long-term neuroadaptations on a structural, cellular, molecular and genomic level (Kelly and Berridge, 2002; Kopnisky and Hyman, 2002).

These molecular and cellular adaptations seen in addiction have also been implicated in the processes of learning and memory, indicating a shared mechanism between the two (Nestler, 2001; Winger *et al*., 2005). The relationship between addiction and learning and memory is further supported by some key behavioural features of addiction that have been described as forms of memory (Nestler, 2001; Kopnisky and Hyman, 2002; Hyman, 2006). One of these core features is the conditioned aspects of addiction, which occurs due to the inappropriate recruitment of neuronal circuits and molecular mechanisms usually involved in associative learning (Nestler, 2001; Kopnisky and Hyman, 2002; Hyman, 2006). All drugs of abuse increase dopamine levels in the mesocorticolimbic system of the brain, and it is this dopamine that promotes the learning of new associations between the drug and the

environment (Kalivas and O' Brien, 2008). The role of associative learning also becomes evident during late relapse, where exposure to cues that were associated with drugs and drug use, are able to induce drug taking and relapse after a period of abstinence (Berke and Hyman, 2000; Nestler, 2001; Kopnisky and Hyman, 2002; Wolf, 2002; Saal and Malenka, 2005; Hyman, 2006). These cues may be external factors, such as the people, places or things associated with prior drug use, or internal factors such as emotions, bodily feelings or withdrawal symptoms (Berke and Hyman, 2000; Hyman, 2006). Dopamine acts within the relapse paradigm, reinforcing the prior learnt associations and cueing the drug user to carry out drug seeking behaviour (Kalivas and O' Brien, 2008).

1.1.3 Neural circuits in addiction

The neural circuitry involved in the neurobiology of addiction fall broadly into four categories, namely; reward/salience, motivation/drive, learning/conditioning and inhibitory control/emotional regulation/executive function (Volkow *et al*., 2008). Any disruption within these four circuits results in the enhanced value of a reinforcer, in this case drugs, over other reinforcers; such a natural rewards e.g. food, sex (Hyman, 2006; Volkow *et al*., 2008). This overvaluing of drug reward over natural reward contributes to compulsive drug use, as well as the marked narrowing of an addict's life goals to be focused on obtaining and using drugs (Saal and Malenka, 2005; Hyman, 2006).

The learning/conditioning circuit within the brain, when exposed repeatedly to drugs of abuse, result in the formation of new linked memories, a process which is mediated by the hippocampus and the amygdala (Volkow *et al*., 2008). Learning and memory becomes encoded via the usage of interneuronal connections, with synapses that experience frequent use being strengthened and vice versa, and the stability of these changes determining the presence of a stored memory (Wolf, 2002; Fanselow *et al*., 2005). Therefore, experience, such as repeated drug exposure, may result in a strengthened communication between neurons in the learning pathways in the brain, resulting in the long-term storage of a memory (Wolf, 2002). These linked-memories condition the drug user to anticipate pleasurable responses not only from the drug itself, but from any stimulus conditioned or associated with the drug (Volkow *et al*., 2008). This stimulus-response behaviour triggers and maintains the automatic habit responses and seeking behaviours that drive relapse in drug addicts (Everitt *et al*., 2008; Volkow *et al.*, 2008).

Synaptic plasticity is proposed to be the mechanism that underlies the formation of these associative learning behaviours, and involves (i) changes in the strength of existing neural connections, (ii) the formation, (iii) elimination and/or (iv) remodelling of dendrite-axon structures (Fanselow *et al*., 2005; Saal and Malenka, 2005; Hyman 2006). These changes occur via altered gene and protein expression within neurons that receive an enhanced or diminished signal, and produce long-term changes in neural circuits which result in long lasting alterations in behaviours (Hyman, 2006). Synaptic plasticity therefore may have an important role in mediating the behavioural consequences of drug use and the development of addiction (Thomas and Malenka, 2003).

1.1.4 The role of LTP and LTD in addiction

Long-term potentiation (LTP) and long-term depression (LTD) are mechanisms underlying synaptic plasticity that bring about neuronal changes, with LTP responding to enhanced signaling to produce changes which increase synaptic strength, while LTD responds to diminished signalling to produce changes which decrease synaptic strength (Saal and Malenka, 2005; Hyman, 2006). LTP and LTD are demonstrated in all excitatory synapses within the mammalian brain however, the mechanisms by which they occur differ in different brain regions (Hyman and Malenka, 2001).

LTP as a potential cellular mechanism for learning and memory has been well established within the hippocampus (Mizuno *et al*., 2002; Wolf, 2002). In the CA1 region each excitatory synapse contains two glutamate receptor subtypes, namely α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-aspartate (NMDA) receptors, whose interactions with glutamate to establish LTP and LTD have been implicated in the process of addiction (Figure 1, Nestler, 2001; Mizuno *et al*., 2002; Saal and Malenka, 2005).

When glutamate released from the presynaptic neuron is bound to the AMPA receptor, the receptor channels allow an influx of sodium ions into the post-synaptic neuron, causing the neuron to become depolarized and more likely to fire action potentials (Figure 1, Saal and Malenka, 2005). This depolarization enables the expulsion of a magnesium ion that sits in the pore of the NMDA receptor channel. Interestingly, even with glutamate binding promoting the opening of NMDA receptor, ions will not flow into the neuron until the magnesium is expelled (Figure 1, Bliss and Collingridge, 1993; Saal and Malenka, 2005).

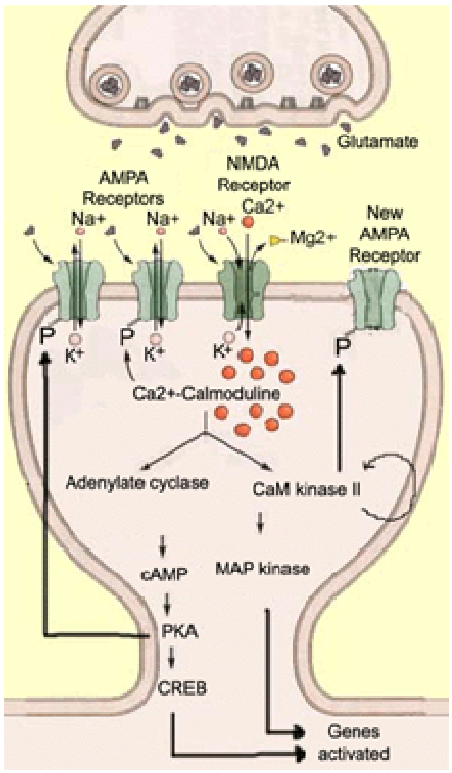


Figure 1: Long-term potentiation signalling pathway

(http://thebrain.mcgill.ca/flash/a/a_07/a_07_m/a_07_m_tra/a_07_m_tra.html)

Thus, NMDA activation is responsible for the induction of LTP, but can only occur due to high levels of synaptic activity and depolarization of the post-synaptic membrane, which is brought about by the activation of AMPA receptors (Bliss and Collingridge, 1993; Hyman and Malenka, 2001; Thomas and Malenka, 2003; Fanselow *et al*., 2005; Saal and Malenka, 2005).

NMDA receptors channels are highly permeable to calcium ions and the influx of calcium into the post-synaptic neuron is the critical trigger for both LTP and LTD (Figure 1, Wolf, 2002; Fanselow *et al*., 2005; Saal and Malenka, 2005). The triggering of LTP or LTD is dependent upon the amount of calcium that enters the post-synaptic cells, with different amounts activating intracellular second messenger pathways which will either increase (in the case of LTP) or decrease (in the case of LTD) synaptic strength of the NMDA containing neuron (Fanselow *et al*., 2005; Saal and Malenka, 2005). LTP results from a large amount of calcium entering the neuron, which subsequently activates protein kinases, with the critical one being Calcium/calmodulin-dependent protein kinases II (CaMKII), while LTD is caused by a small increase in calcium which activates protein phosphatases, including

calcium/calmodulin-dependant protein phosphatase calcineurin (phosphatases 2B) and protein phosphatases 1 (Wolf, 2002; Saal and Malenka, 2005).

LTP induced changes at the glutamate synapses include; an increase in neurotransmitter release per action potential at the presynaptic neuron, the insertion of more AMPA receptors onto the membrane as well as causing the postsynaptic cells more responsive to glutamate, which will aid in mediating the excitatory transmission of glutamate, and an increase in synaptic contacts via the growth of new dendritic spines, while LTD causes the removal of AMPA receptors from the synapse (Figure 1, Wolf, 2002; Fanselow *et al*., 2005; Saal and Malenka, 2005).

As it is well known that LTP and LTD play critical roles in the neuroadaptations that mediate all forms of experience-dependent plasticity, it is likely that they are also the mechanisms by which drugs of abuse induce alterations in neural circuitry (Hyman and Malenka, 2001; Thomas and Malenka, 2003; Saal and Malenka, 2005; Hyman, 2006). In fact, there is ample evidence that LTP/LTD-like changes occur within the mesolimbic system in response to drugs of abuse, and so alter the networks related to motivation and reward (Hyman and Malenka, 2001; Wolf, 2002; Saal and Malenka, 2005).

1.1.5 LTP-associated signaling pathways

LTP and learning within the hippocampus results in the downstream activation of cyclic adenosine monophosphate (cAMP) and cAMP response element-binding protein (CREB) mediated transcription, a pathway whose upregulation is one of the best established adaptations to chronic exposure to drugs of abuse (Figure 1, Nestler, 2001; Kopnisky and Hyman, 2002; Chao *et al*., 2004).

CREB acts as an important transcription factor and regulates gene expression in learning and memory circuits via the cAMP pathway (Nestler, 2001; Mizuno *et al*., 2002; Fanselow *et al*., 2005; Hyman, 2006). CREB-mediated transcription may only occur once CREB has been activated by its phosphorylation at Ser133, which in turn may be induced by a number of transduction cascades, including; the cAMP pathway via protein kinase A (PKA), intracellular calcium via CaMK, the Ras/extracellular signal regulated kinase (ERK) protein kinase pathway, the phosphatidylinositol-3-kinase (PI3K)/Akt kinase pathways and stress induced signaling cascades (Nestler, 2001; Mizuno *et al*., 2002; Chao *et al*., 2004; Hyman,

2006). CREB therefore appears to be a final common denominator of various signaling pathways whose activation may result from a variety of external and/or internal stimuli. CREB regulates the transcription of genes which contain a cAMP response element (CRE) site within their regulatory region, which have been identified in numerous genes found within the nervous system. Such genes include those that encode for neuropeptides, neurotransmitters, signaling proteins and other transcription factors (Nestler, 2001; Hyman, 2006).

A consequence of repeated drug exposure is the altering of the transcription of specific target genes via repeated disruption to intracellular signal transduction pathways, causing altered gene expression and ultimately changes within the neural circuitry that result in behavioural changes (Nestler, 2001). Interestingly, CREB activity and the expression of the genes that are regulated by CREB, are greatly enhanced in response to the repeated exposure to drugs of abuse, with an increase in CREB phosphorylation seen in reward related areas of the brain such as the VTA, the amygdala and the frontal cortex (Hyman and Malenka, 2001; Hyman, 2006). It has been postulated that this phosphorylation of CREB may be one of the pivotal molecular mechanisms behind drug-induced LTP (Hyman, 2006).

CREB therefore plays an important role in the establishment of learning and memory and since the administration of drugs of abuse produces an increase in CREB levels (Hyman, 2005), its function in the development of addictive behaviour justifies further investigation.

1.1.6 Treatment of Addiction

1.1.6.1 Principles of Addiction Treatment

Drug addiction or dependence and its development is a complex process involving both individual vulnerability due to pschychological and biological factors, as well as environmental factors such as sociocultural and economic status (Anglin and Hser, 1990; O' Brien, 2003). There is therefore no simple cure for addiction, which develops as a chronic condition, and its treatment should therefore be seen as a long term venture, in the same vein as those for hypertension and diabetes (Anglin and Hser, 1990; O' Brien, 2003).

In order to develop an effective treatment program, it is important to assess the cause of the drug abuse and impliment the appropriate intervention (Anglin and Hser, 1990; Leshner, 1999). Treatment approaches are varied depending on what its underlying cause is perceived

to be by the treatment provider (Anglin and Hser, 1990). Treatment programs which view addiction as a sign of moral weakness in a person may model their interventions in the form of punishment, incarceration or moral education, while programs viewing addiction as a chronic disease will emphasize the importance of medications and therapeutic and behavioural management of the patient (Anglin and Hser, 1990).

Other factors to which addiction have been attributed are patterns of maladaptive learning of a habit or underlying psychiatric illness such as depression or schizophrenia, where the patient is essentially trying to treat their illness by using addictive drugs (Anglin and Hser, 1990; Leshner, 1999). In both these cases, treatment programs adopt a drug free paradigm and instead focus on treating the underlying cause of the addiction by utilising psychotherapy and cognitive and behavioural management techniques (Anglin and Hser, 1990; Leshner, 1999).

Regardless of modality-specific treatment approaches, all programs consider four main outcome goals in the treatment of addiction:- 1. the reduction or cessation of drug or alcohol use 2. improving the personal health (including medical and psychiatric health) and the quality of life of the patient 3. improving social functioning of the patient in terms of family and social relationships and employment, as part of relapse prevention strategies, and 4. reducing behaviours that may lead to the spread of infectious disease or the perpetration of crime that may be a threat to the public (Anglin and Hser, 1990; McLellan *et al*., 2000).

In the interest of achieving these goals, addiction treatment generally begins with a medical and psychosocial assessment of the patient, and thereafter helping to alleviate the acute physical withdrawal symptoms and detoxify the patient to aid in bringing them to a drug free state (Leshner, 1999; O'Brien, 2003; Yahyavi-Firouz-Abadi and See, 2009). Secondary to the initial detoxification, which alone does not have much therapeutic value, relapse prevention is the next important step in addiction treatment (Anglin and Hser, 1990; Leshner, 1999; Yahyavi-Firouz-Abadi and See, 2009). Relapse prevention strategies may be achieved using traditional psychotherapy and counselling and may also, like detoxification, be achieved using pharmacotherapies (Anglin and Hser, 1990; Yahyavi-Firouz-Abadi and See, 2009).

Relapse prevention strategies aim to identify the psychological and environmental factors that may lead to drug cravings and relapse in patients, and equip patients with the skills to recognise and cope with these situations in a way that will minimize the chances of relapse

occurring (Anglin and Hser, 1990; Carey, 1990; Leshner, 1999). Relapse prevention programs also aim to educate patients that recovery is a long-term process and periodic relapses are to be expected. Relapses should not to be viewed as a failure, but strategies should be offered instead for coping with relapse if it does occur (Carey, 1990; Leshner, 1999; O' Brien, 2003).

1.1.6.2 Pharmacotherapy

The pharmacological treatment of drug addiction targets four main areas of patient management, namely; the pharmacological management of withdrawal symptoms, harm reduction, relapse prevention and pharmacological treatment to prevent or treat medical complications from substance abuse (Anglin and Hser, 1990; Lingford-Hughes *et al.,* 2004; Watson and Lingford-Hughes, 2007).

The management of withdrawal symptoms and harm reduction strategies may broadly be described as detoxification, which aims to reduce the quantity and frequency of substance use and consequently reducing the physical and psychological harms associated with its use (Carey, 1996; Lingford-Hughes *et al.,* 2004).

Detoxification may be achieved using agonist or partial agonist drugs, which act as short-term licit drug substitutes when administered at a controlled dose (Anglin and Hser, 1990; Siegel and Ramos, 2002; Watson and Lingford-Hughes, 2007). During the process of detoxification, it is important that adequate care be given to the patient, not just for the medical symptoms of withdrawal, but also psychological symptoms which may result from drug misuse such as depression or anxiety (Anglin and Hser, 1990; Lingford-Hughes *et al.,* 2004).

Pharmacological intervention that maintain abstinence and prevent relapse typically consist of drugs which reduce the cravings for illicit substances or dampen withdrawal symptoms, which may persist long after detoxification (Siegel and Ramos, 2002; O' Brien, 2003).

Relapse prevention medications may include antipsychotics, antidepressants, anti-anxiety and even anti-obssessive drugs (O' Brien, 2003). Although no medication offers a cure for addiction, in conjunction with psychotherapy they help to bring the patient back to being productive members of society (O' Brien, 2003).

1.1.6.3 Drug types in addiction treatment

The most well established agonist treatment used in both the short-term as part of detoxification, and the long-term as part of a maintenance regime, is the μ-opioid agonist methadone (McLellan *et al.,* 2000; Lingford-Hughes *et al.,* 2004; Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009). Methadone, when administered orally, activates the same receptors as heroin and other opioid drugs, however it has a much lower abuse potential than these illicit opioid drugs. Methadone therefore acts as a licit substitute (Anglin and Hser, 1990; O' Brien, 2003; Watson and Lingford-Hughes, 2007).

The abuse potential of a drug is dependent on its pharmacology, with drugs that illicit rapid onset of euphoria and having a shorter duration of activity having a higher abuse potential than those with a slower onset and a greater period of activity (O' Brien, 2003). These characteristics are a key component in substitution treatment, by utilising drugs that do not produce the alternating "highs" and "lows" that occur with drugs whose effects have a rapid onset but wane just as rapidly, ultimately create an urgent desire for another dose (O' Brien, 2003; Watson and Lingford-Hughes, 2007).

Methadone's effects have a delayed onset as well as long-lasting activity. It has a half-life of ± 24 hours, which decreases its abuse potential in relation to that of heroin (McLellan *et al.,* 2000; O' Brien, 2003; Watson and Lingford-Hughes, 2007). Methadone treatment has proven to be effective in reducing opiate use in addicts, with doses ranging from 80-120mg/day inhibiting the intravenous self-administration of heroin and hydromorphane (Leshner, 1999; McLellan *et al.,* 2000; Vocci *et al.,* 2005).

Substitution treatments have also been used in the treatment of nicotine addiction, with nicotine replacement therapy (NRT) being shown to reduce cravings and withdrawal symptoms in addicts (Watson and Lingford-Hughes, 2007). NRT typically consists of patches, gum and other preparations containing doses of nicotine that activate the nicotinic acetylcholine receptors, and replace some of what the smoker was getting from their cigarettes (Leshner, 1999; Watson and Lingford-Hughes, 2007).

Although no medications have been approved for the treatment of psychostimulant abuse, clinical trials using Baclofen, a drug which has agonist effects on γ-aminobutyric acid B

($GABA_B$) receptors, have been shown to be effective in reducing cocaine self-administration, cravings, as well as the relapse rate in non-opioid dependent, non-treatment seeking cocaine addicts (Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009). However, there are conflicting results about Baclofen's efficacy with heavily dependent cocaine users, with some studies indicating that the higher the level of abuse in patients at the baseline, the more they benefitted from treatment, and others indicating that Baclofen was not effective in initiating abstinence in heavy cocaine dependent patients (Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009).

A new stimulant drug, which has been used to increase alertness in narcoleptic patients but also has been shown to be effective in the treatment of psychostimulant addiction, is Modafinil (Vocci *et al.,* 2005). This drug, which exerts a stimulatory action via non-dopaminergic pathways, enhancing glutamate activity in the brain, has a low abuse potential and has been associated with less craving for amphetamines, as well as less cravings and use of cocaine (Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009). Modafinil works in three ways in attenuating cocaine use; by decreasing symptoms of cocaine withdrawal, by blunting craving as well as subjective response to cocaine, thereby preventing cocaine priming and multiple-use episodes, and lastly, acts to decrease impulsive responding which allows time for cognitive systems and decision making skills to be utilized before drug use occurs (Vocci *et al.,* 2005).

The use of partial agonists in detoxification and maintenance programs is also common, with the dual agonist and antagonist properties of some of these drugs proving to be an effective treatment (Watson and Lingford-Hughes, 2007). The partial μ-opioid agonist buprenorphine has been used as an effective substitute for illicit opiate use, as it is able to occupy opioid rceptors but produce only a partial response (Watson and Lingford-Hughes, 2007). Therefore it activates the same receptors as illicit opiates but produces less euphoria, sedation and positive reinforcement than these opiates or indeed full agonist treatments (Watson and Lingford-Hughes, 2007). Buprenorphine also exerts antagonistic effects on κ-opioid receptors, the activation of which leads to dysphoria, sedation and depersonalization. Acting on these receptors buprenorphine produces less dysphoria in comparison to full agonist drugs (Watson and Lingford-Hughes, 2007). Buprenorphine is often used as an alternate substitute to methadone in both the detoxification and maintenance treatment programs (McLellan *et al.,* 2000; Watson and Lingford-Hughes, 2007; Yahyavi-Firouz-Abadi and See, 2009).

The antidepressant drug bupropion has proven effective in the treatment of nicotine addiction, producing increased dopamine and norepinephrine levels in the mesolimbic system by acting as an uptake inhibitor (Lingford-Hughes and Nutt, 2003). This increase in dopamine levels is sufficient to reduce cravings and aid in the successful cessation of smoking in addicts (Gonzales *et al.,* 2006; Watson and Lingford-Hughes, 2007). In addition to its effects on the mesolimbic dopamine pathway, bupropion has been shown to be a nicotinic antagonist; selectively blocking activation of neuronal acetylcholine nicotinic receptors (Slemmer *et al.,* 2000). This blockade of the effects of nicotine via receptor antagonism, in addition to its dopamine release, suggests bupropion is a partial agonist at nicotinic acetylcholine receptors and this may explain its efficacy in the treatment of nicotine dependence (Slemmer *et al.,* 2000). Due to the prominent role of dopamine in the addictive process, it has been a target of pharmacotherapy, where dopamine 'stabilizers' being either partial agonists or mixed action antagonists are used (Vocci *et al.,* 2005). For example, a partial D3 agonist (BP – 897) has been developed as a possible strategy to block the binding of cocaine to its dopamine binding site (Lingford-Hughes and Nutt, 2003). This partial agonist has been shown to inhibit cocaine seeking behaviour in rodents in response to drug-paired cues. It is thought to do so by stimulating the D3 receptor enough to prevent withdrawal symptoms without causing rewarding effects (Lingford-Hughes and Nutt, 2003).

Naltrexone is an orally administered opioid antagonist which has been shown to be effective as a maintenance treatment, as well as being effective as part of relapse prevention treatment in conjunction with psychosocial interventions (McLellan *et al.,* 2000; Watson and Lingford-Hughes, 2007). Naltrexone has a long lasting activity, with an effective half-life of 96 hours owing to its active metabolite, therefore resulting in long lasting blockade of the effects of heroin and other opiates by preventing them from binding to the μ-opioid receptor (Leshner, 1999; Watson and Lingford-Hughes, 2007; Yahyavi-Firouz-Abadi and See, 2009).

Naltrexone has also been effective in the treatment of alcohol dependence, with a dose of 50mg/day resulting in less craving for alcohol, a reduction in drinking behaviours and a lower rate of relapse in alcohol dependent patients (Leshner, 1999; McLellan *et al.,* 2000; O' Brien, 2003; Adinoff, 2007). Patients also reported less pleasure being felt when they eventually did have a drink (O' Brien, 2003). This may be explained by the mechanism by which alcohol exerts its effects on the opioid system. In both human and animals, alcohol causes the activation of μ-opioid receptors, resulting in the stimulation of endogenous opioids, which

consequently activate the dopamine reward pathway (O' Brien, 2003). Naltrexone has been shown to block the μ-opioid receptors that suppress GABA neurons, and therefore these neurons are able to inhibit ventral tegmental area dopamine neurons (Adinoff, 2007). This may explain the blockade of some of the "highs" associated with alcohol abuse and the lack of euphoria experienced by the alcohol dependent patients (McLellan *et al.,* 2000; O' Brien, 2003).

Naltrexone has also been used in cocaine addiction, where its interaction with relapse-prevention psychotherapy has proven effective in decreasing the prevalence of relapse in abstinent, formerly dependent cocaine-addicted patients (Vocci *et al.,* 2005). Interestingly, this effect was not seen when the drug was paired with any other behavioural therapy type (Vocci *et al.,* 2005).

Disulfiram is another drug which has been reported to have an enhanced effect in conjunction with specific types of psychotherapy (Vocci *et al.,* 2005). An inhibitor of the sulfhydryl-containing enzyme aldehyde dehydrogenase, which plays a key role in the metabolism of alcohol, disulfiram administration results in an accumulation of acetaldehyde after drinking alcohol (Watson and Lingford-Hughes, 2007; Vocci *et al.,* 2005). This accumulated acetaldehyde causes unpleasant feelings such as nausea, vomiting, headache, flushing, palpitations and hypotension, and with a high enough consumption of alcohol may lead to unconsciousness and even death (Watson and Lingford-Hughes, 2007).

The efficacy of this drug in decreasing alcohol intake is therefore due to the patient's fear of these adverse reactions (Watson and Lingford-Hughes, 2007). Consequently, disulfiram has been marketed as a treatment for alcohol addiction, but has also been reported to decrease cocaine use (Watson and Lingford-Hughes, 2007; Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009). This decrease in cocaine use is due to disulfiram's role as a dopamine metabolism inhibitor, and was observed particularly when disulfiram was paired with cognitive behaviour therapy. However this effect was less prevalent when disulfiram treatment was paired with interpersonal behaviour therapy (Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009).

The drug topiramate is also effective in the treatment of both alcohol and cocaine addiction, although marketed as an antiepileptic drug. Topiramate has both GABA enhancing and glutamate inhibiting properties (Vocci *et al.,* 2005; Adinoff, 2007). Topiramate facilitates

GABA functioning through a non-benzodiazapine site on the $GABA_A$ receptor and antagonizes glutamate at AMPA and kianate receptors (Adinoff, 2007). Patients who were abstinent during a two week baseline period and were treated with a 200mg/day dose of topiramate showed a decrease in return to cocaine use as well as an increase in negative urine tests as compared to the placebo paired patients (Vocci *et al.,* 2005; Yahyavi-Firouz-Abadi and See, 2009).

A naturally occurring compound called Ibogaine has been shown to be an effective treatment in drug addiction, and has been shown to decrease the self-administration of cocaine, ethanol, morphine and nicotine in rodents (Adinoff, 2007). This indole alkaloid binds to κ-opioid, N-methyl-D-aspartate (NMDA) glutamate and nicotinic receptors and has been shown to block the release of dopamine in the nucleus accumbens in cocaine sensitive animals (Adinoff, 2007).

Numerous other pharmacotherapeutic treatments exist, acting on various receptors and pathways within the brain to decrease the physical withdrawal symptoms and reduce cravings in addicts. However, just as vital in the treatment of addiction is the management of psychological harms that may be associated with drug misuse (Lingford-Hughes *et al.,* 2004).

Symptoms of psychiatric disorders such as depression, anxiety and psychosis often present in patients who abuse drugs, and the presence of such symptoms may increase subsequent drug use, making treatment especially challenging (Lingford-Hughes *et al.,* 2004). It is therefore vital that for effective harm reduction and relapse prevention, both the addiction as well as the psychiatric disorder be treated concurrently (Lingford-Hughes *et al.,* 2004).

Pharmacotherapy therefore plays a vital role in the treatment of addiction and in conjunction with behavioural therapies and support services, such as vocational rehabilitation and mutual support organizations such as Alcoholics and Narcotics Anonymous, can facilitate the return of drug and alcohol dependent patients to productive functioning (Anglin and Hser, 1990; Carey, 1996; Leshner, 1999).

1.1.7 Animal Models of Addiction

The definition of an animal model is an experimental preparation developed for the purpose of studying a condition found in humans. Animal models operate under the assumption that

homology or analogy can be found in the physiology and behaviours of various animal species and human beings (Markou et al, 1993).

Animal models need to meet certain criteria in order to establish itself as a valid representation of the human condition it is proposed to model (Bakshi and Kalin, 2002). Three types of validity are generally used as a measure of the value of an animal model; face validity, construct validity and predictive validity (Bakshi and Kalin, 2002; Epstein et al, 2006).

Face validity refers to the similarity in appearance between the animal model and the human condition it is trying to represent; construct validity refers to the similarity in the internal mechanism underlying the behaviour or condition that is being modelled; and predictive validity refers to the extent to which the effects induced by an experimental protocol on an animal predicts the effects seen in humans when induced by a similar event (Markou et al, 1993; Bakshi and Kalin, 2002; Epstein et al, 2006).

Though it would be ideal for a model to meet all three of these criteria, it appears that in order for a model to establish itself as being valuable, it need only to meet the requirements of predictive validity and reliability (Markou et al, 1993; Koob, 2000).

The reliability of a model depends upon the consistency and stability of the specific variables that are being observed (Markou et al, 1993; Geyer and Markou, 2000; Hitzemann, 2000). A model should be reproducible under similar conditions, and the effects they produce should be reproducible if the same manipulation is being applied, with minimal variability within individual subjects and between subjects (Markou et al, 1993; Geyer and Markou, 2000; Hitzemann, 2000).

Various animal models exist to mirror the characteristics of drug addiction in humans, and while none seem to cover every aspect of addiction, they provide a valuable tool in the study of this mental disorder.

Self-administration models of addiction study the acute rewarding properties of drugs of abuse, the development of habitual drug seeking and the ultimate development of addictive behaviour (Kalivas *et al.,* 2006). In this model experimental animals will readily self-administer drugs when given free access to it or after being trained to perform a task to gain the drug (Roberts and Koob, 1997; Shippenberg and Koob, 2002; Feltenstein and See, 2008).

Self-administration may be orally (mostly in the case of alcohol), intracranial or intravenously via a chronic indwelling catheter, and generally drugs with a higher abuse potential elicit greater self-administration in the animals (Figure 2, Koob, 2000; Shippenberg and Koob, 2002; Feltenstein and See, 2008). This animal model has been widely used because it has both reliability and good predictive validity (Shippenberg and Koob, 2002).

Intracranial self-stimulation (ICSS) involves the implantation of intracranial electrodes into brain areas involved in motivation and reward, where animals will be able to press a lever in order to receive a short, mild train of electrical stimulation (Figure 2, Roberts and Koob, 1997; Shippenberg and Koob, 2002; Feltenstein and See, 2008). Animals will self-administer this current at high frequency to the areas of the brain that bring about reward, showing that stimulation of such areas is reinforcing (Roberts and Koob, 1997). The administration of drugs of abuse have been shown to decrease the amount of current required to achieve the same level of reward (Shippenberg and Koob, 2002; Feltenstein and See, 2008). Therefore, it can be shown that the greater the abuse potential of the drug, the greater its ability to decrease the ICSS threshold resulting in requiring less stimulation to achieve the same level of reward (Roberts and Koob, 1997; Shippenberg and Koob, 2002; Feltenstein and See, 2008). ICSS is commonly used as it has been shown to have excellent predictive validity for the abuse potential of drugs (Koob, 2000).

Conditioned place preference (CPP) paradigms use the principles of classical conditioning to model addiction in animals (Koob, 2000; Feltenstein and See, 2008). CPP uses the natural reward system of the nucleus accumbens as well as the learning and memory circuit within the dorsal hippocampus to allow for a learnt association to occur between the euphoric effects of the drug of abuse and a particular environment, through repeated pairings of the two (Tropea *et al*., 2008) The animal is exposed to an apparatus comprised of two initially neutral environments differing either in colour, texture, odour or lighting (Feltenstein and See, 2008). The animal is exposed to a drug in one of the compartments and to a vehicle in the other, thereby conditioning it to associate that particular environment with the drug (Roberts and Koob, 1997; Feltenstein and See, 2008). After a number of treatment sessions the animal is given free access to the entire apparatus and its preference is assessed. According to the principles of classical conditioning, if the drug has reinforcing properties the animal should spend a greater amount of time in the drug-paired compartment, indicating seeking behaviour (Figure 2, Roberts and Koob, 1997; Shippenberg and Koob, 2002; Feltenstein and See, 2008).

The CPP paradigm demonstrates both reliability and validity, with drugs producing conditioned preferences for the drug-paired environment also functioning as positive reinforcers within other addiction paradigms (Shippenberg and Koob, 2002). CPP, like ICSS and self-administration models, also provides predictive validity for the abuse potential of drugs (Koob, 2000). CPP has been proven useful as an inexpensive means to assess the rewarding properties of a substance quickly and with minimal training required. Various drugs of abuse, such as opiates, cocaine and nicotine, have been shown to produce CPP, sometimes using a single drug pairing at relatively low doses (Feltenstein and See, 2008).

The reinstatement model of CPP has also demonstrated reliability and predictive validity, with reinstatement in laboratory animals being induced by conditions that have been reported to trigger drug craving and relapse in human addicts, such as acute re-exposure to the drug, drug related cues or stress (Epstein et al, 2006). The reinstatement of extinguished drug-seeking behaviours in response to the priming drug dose or drug associated cues occur in predictable ways and further demonstrates the predictive validity of this model (Shippenberg and Koob, 2002).

However, the use of the CPP model has been shown to have disadvantages and may display a measure of unreliability as compared to self-administration models. CPP models rely on the non-contingent administration of the drug, and animals never experience contingent drug administration, which is the hallmark of addiction (Shippenberg and Koob, 2002; Feltenstein and See, 2008). The neurochemical and behavioural effects of a drug have been shown to be directly influenced by whether the administration of the drug is being controlled by the subject, so a passive exposure to the drug may not always result in the development or appearance of seeking behaviour (Shippenberg and Koob, 2002).

In order to obtain reliable data, factors such as method of administration (i.p or s.c), number of environmental pairings and duration of pairings must be carefully controlled, as they can profoundly affect conditioning behaviour (Shippenberg and Koob, 2002; Feltenstein and See, 2008). The number and duration of the pairings is vital in order to avoid the issue of familiarity and preference bias, which can result in inaccurate behavioural results on the test day (Koob, 2000; Feltenstein and See, 2008). Genotypic differences need also be taken into account, in terms of sensitivity to drugs, as well as the saliency of environmental cues. These differences may result in a decrease in the reinforcing effects of the drug or a failure to

establish the learning and memory processes involved in associative conditioning (Shippenberg and Koob, 2002).

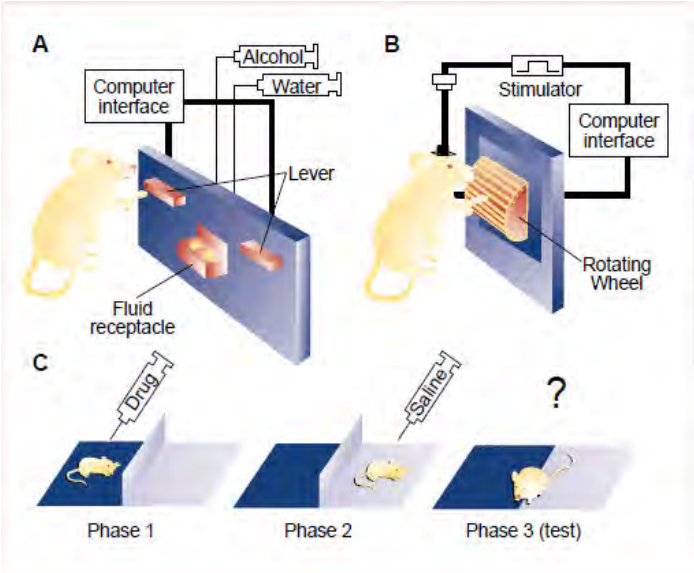


Figure 2: Animal models of drug addiction. A – Self administration
B – Intracranial self stimulation C – Conditioned place preference

(Adapted from Roberts and Koob, 1997)

In summary, addiction presents as a chronically relapsing disorder involving compulsive taking of drugs, and the development of withdrawal symptoms upon its cessation. Many theories have been proposed to explain the mechanism by which addiction is established, with some theories focusing on dysfunctions in learning and memory pathways. Within these learning and memory models, LTP and LTD have been postulated as a possible mechanism by which addiction can develop. At present, treatment options for addicts consists of psychotherapy and pharmacotherapy, and are dependent on the underlying cause of the addictive behaviour. However, due to the high occurance of relapse in addicts, much interest has been given to the study of addiction and its treatment. To that end numerous animal models of addiction have been developed to mirror the addictive state in order to research its pathophysiology and possible treatments.

1.2 Dopamine

1.2.1 Dopamine synthesis

Dopamine is the catecholaminergic neurotransmitter that is primarily associated with the addictive state. It acts within the central nervous system to control a variety of functions via three neural pathways namely the nigrostriatal, tuberoinfundibular and mesolimbic pathways (Pivonello *et al.,* 2007). It is synthesized from the amino acid tyrosine (found in abundance in dietary proteins) which is then converted to L-dopa by the enzyme tyrosine hydroxylase (TH). L-dopa is then converted to dopamine via the enzyme aromatic amino acid decarboxylase (AADC). The resultant dopamine is then sequestered into storage vesicles by vesicular monoamine transporter 2 (VMAT2) (Figure 3, Elsworth *et al.,* 1997; Lawlor *et al.,* 2004).

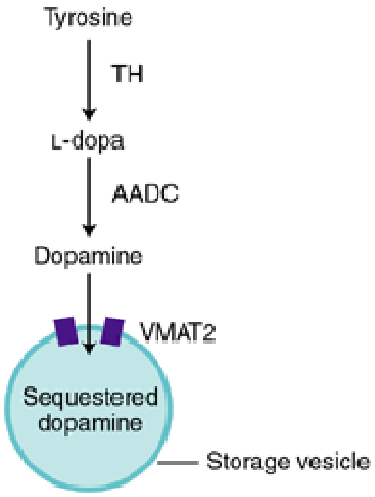


Figure 3: Dopamine synthesis and storage pathway

(Adapted from Lawlor *et al.,* 2004)

1.2.2 Dopaminergic receptors

1.2.2.1 Dopamine receptor structure

Dopamine released into the synaptic cleft binds to dopaminergic receptors of which there are at least 5 different types, namely D_1, D_2, D_3, D_4 and D_5. These receptors share structural characteristics in that they are all G-protein coupled receptors. As such they consist of seven putative membrane spanning helices which form a narrow dihedral hydrophobic cleft,

surrounded by three extracellular and three intracellular loops (Figure 4, Civelli, 2000; Pivonello et al., 2007).

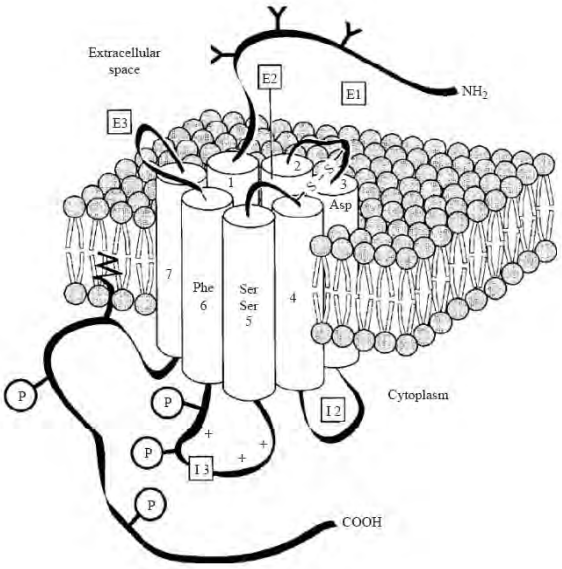


Figure 4: Dopamine receptor structure.
Structural features of D1-like receptors are represented. D2-like receptors are characterized by a shorter COOH-terminal tail and by a bigger third intracellular loop. Residues involved in ligand binding are highlighted in transmembrane domains. Potential phosphorylation sites are represented on the third intracellular loop (I3) and on the COOH terminus. The potential glycosylation sites are represented on the NH2-terminal. E1–E3, extracellular loops; 1–7, transmembrane domains; I2–I3, intracellular loops.

(Pivonello *et al.,* 2007)

1.2.2.2 The Dopamine receptor family

Dopamine and other monoamines do not 'mediate' fast synaptic transmission in the central nervous system, but rather modulate it (Iverson *et al.,* 2007) This dopamine-dependent signal transduction is activated through the interactions with membrane receptors belonging to the 7-trans membrane domain G-protein coupled receptors, of which there are five different types, namely D_1, D_2, D_3, D_4 and D_5 dopaminergic receptors (Samad *et al.,* 1997; Civelli, 2000; Iverson *et al.,* 2007; Janhunen *et al.,* 2007; Pivonello *et al.,* 2007).

Functionally there are two broad groups of dopamine receptors within the dopamine receptor family which are derived from the divergence of two gene families, namely the D1 – like receptors (D_1 and D_5), which are associated with stimulatory functions, and D2 – like

receptors which are associated with inhibitory functions (Civelli, 2000; Pivonello *et al.,* 2007). All the members of the dopamine receptor family are encoded by genes which are localized at different chromosomal loci, yet they display considerable homology in their protein structure and function (Pivonello *et al.,* 2007). D1- like receptors are encoded by genes that lack introns in their proton coding regions and in their transmembrane domains, whereas D_2, D_3 and D_4 genes do possess the introns (Civelli, 2000). D_1 and D_5 share a 79-80% homology and are only 40-45% identical to the D2-like receptors (Civelli, 2000; Pivonello, 2007). D_2 receptors share a 75% homology with D_3 receptors and are between 51-53% identical to the D_4 transmembrane domains. All three of the D2-like receptors are being encoded by genes that are interrupted by introns, with these introns being found in similar positions (Civelli, 2000; Pivonello, 2007). D1-like and D2-like receptors differ in their transmembrane domains by 21 amino acid residues and apparently this feature facilitates selective recognition processes by the two types of receptors (Civelli, 2000).

The dopamine receptors display some heterogeneity in their expression and distribution in various cells, tissues and organs and in this way it is able to stimulate and/or inhibit different functions (Iverson *et al.,* 2007; Pivonello *et al.,* 2007). For instance, the limbic system predominantly shows selectivity of D2-like receptors, with hardly any sign of D1-like receptors. These receptors are located both pre and post synaptically (Iverson *et al.,* 2007; Pivonello *et al.,* 2007), indicative of its role in controlling neurotransmission of especially dopamine (Samad *et al.,* 1997).

1.2.2.3 Mechanism of action

D1-like and D2-like receptors exert their actions and modulate the effects of dopamine and dopaminergic compounds by coupling to and activating different G-protein complexes. Their primary biological activities are the activation and inhibition of adenylyl cyclase respectively, resulting in the stimulation or inhibition of cAMP accumulation (Civelli, 2000; Pivonello *et al.,* 2007). The result of stimulation or inhibition of cAMP is the modulation, either activation or inactivation of protein kinase A, which through either phosphorylation or dephosphorylation regulates the synthesis of various cytoplasmic and nuclear proteins, the functioning of membrane channels, and the sensitization or desensitization of other G-protein couple receptors (Pivonello *et al.,* 2007).

Stimulation of D_1 and D_5 receptors causes interactions with the Gs or Gi stimulating complex to activate adenylyl cyclase and stimulate cAMP accumulation, indicating similar pathways of second-messenger induction for D1-like receptors (Figure 4, Civelli, 2000; Iverson *et al.,* 2007). Dopamine D1-like receptors have a short third intracellular loop, which is typical for receptors that interact with Gs proteins to stimulate cyclic AMP production (Pivonello *et al.,* 2007). Dopamine acting on D_1 receptors activates the formation of cyclic AMP, which activates a cAMP-sensitive protein kinase that causes an increase in the phosphorylation of a substrate known as DARPP-32 (Dopamine and cAMP regulated phosphoprotein, molecular weight 32kDa) and simultaneously inhibiting phosphatase 1 (Figure 5, Iverson *et al.,* 2007). DARPP-32 has a key role in mediating the actions of dopamine by linking these to numerous other neurotransmitter mechanisms, ion channels and transcription factors (Iverson *et al.,* 2007).

Activation of dopamine receptors may also lead to the stimulation of other signal transduction pathways, which include modulating the activity of phospholipase C or releasing arachidonic acid, calcium and potassium channels, as well as sodium/hydrogen exchange and sodium-potassium ATPase (Pivonello *et al.,* 2007).

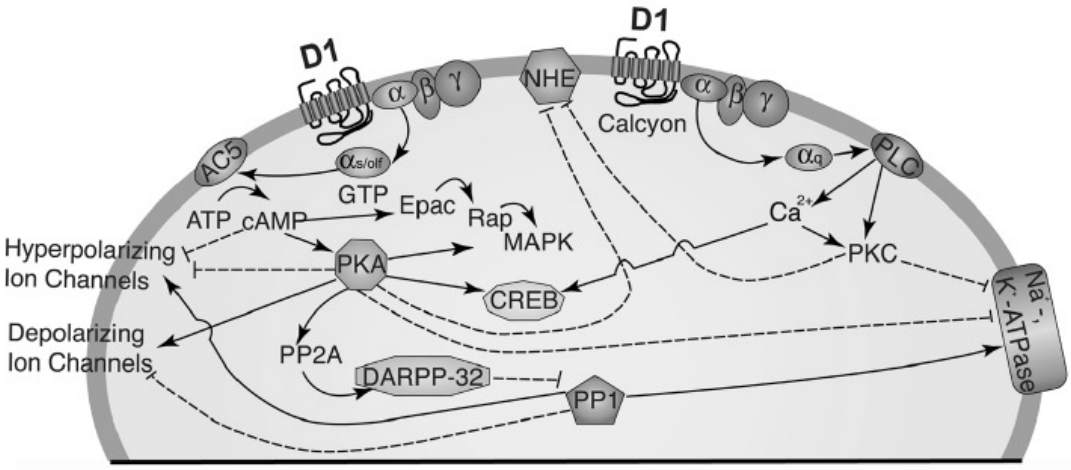


Figure 5: D1-like receptor signaling pathways.

Stimulatory effects are indicated with a solid line ending in an arrowhead, and inhibitory effects with a dashed line ending in a bar. AC5, adenylate cyclase type 5; CREB, cyclic AMP response element binding protein; DARPP-32, dopamine-related phosphoprotein, 32 kDa; MAPK, mitogen-activated protein kinase; NHE, Naþ/Hþ exchanger; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PP1 or PP2A, protein phosphatase 1 or 2A.

(Adapted from Neve et al., 2004)

D2-like receptors, on the other hand, have long third intracellular loops, which are characteristic for receptors which interact with G inhibitory or Gi proteins to inhibit the production of cyclic AMP (Civelli, 2000; Pivonello, 2007). It therefore seems as if it is the third intracellular loop that is responsible for selective control of G-protein coupling and signal transduction (Pivonello *et al.,* 2007). The D2 famly of receptors are further divided into D2S and D2L receptors. The D2S receptor specifically, when activated, induces the stimulation of phospholipase D, which is the enzyme that catalyses the hydrolysis of phosphatidylcholine to phosphatidic acid and choline. (Figure 6, Pivonello *et al.,* 2007). The resultant acid, together with diacylglycerol, are signalling molecules that have been implicated in a number of pathways that regulate cell metabolism, cell growth and differentiation (Pivonello *et al.,* 2007). Dopamine receptors control growth and differentiation via activating mitogen-activated protein kinase (MAPK) and the extracellular signal-regulated kinase (ERK) pathways (through the involvement of Gβγ subunits and protein kinase C). These pathways have also been implicated in the regulation of apoptosis (Pivonello *et al.,* 2007).

The D_4 receptor isoform differs from other dopamine receptors in terms of the length of its third cytoplasmic loop, and also due to the presence of the same insert of a stretch of 16 amino acid residues that occurs one, four, seven or eleven times in their protein structure (Pivonello *et al.,* 2007).

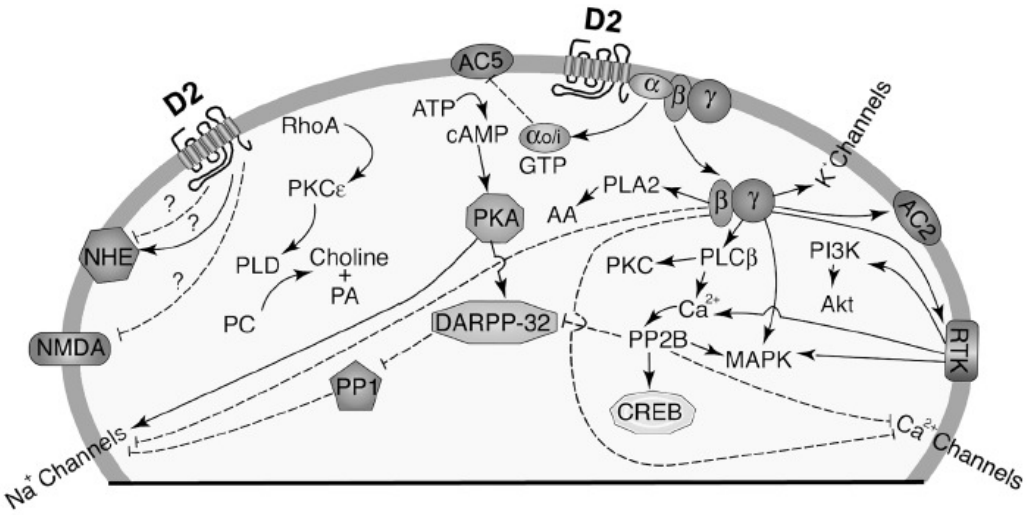


Figure 6: D2-like receptor signaling pathways.

Stimulatory effects are indicated with a solid line ending in an arrowhead, and inhibitory effects with a dashed line ending with a bar. AA, arachidonic acid; AC2 or AC5, adenylate cyclase type 2 or 5; CREB, cyclic AMP response element-binding protein; DARPP-32, dopamine- and cyclic AMP-regulated phosphoprotein, 32 kDa; MAPK, mitogen-activated protein kinase; NHE, Naþ/Hþ exchanger; PA, phosphatidic acid; PC, phosphatidylcholine; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PP1 or PP2A, protein phosphatase 1 or 2A; RTK, receptor tyrosine kinase.

(Adapted from Neve et al., 2004)

1.2.2.4 Distribution of dopamine receptors

Dopamine is widely distributed throughout the central nervous system, in particular the dopaminergic neurons in the substantia nigra, ventral tegmental area and hypothalamus that give rise to the three main dopamine pathways, the nigrostriatal, the mesolimbic and the tubero-infundibular pathways respectively (Figure 7, Janhunen *et al.,* 2007; Pivonello *et al.,* 2007).

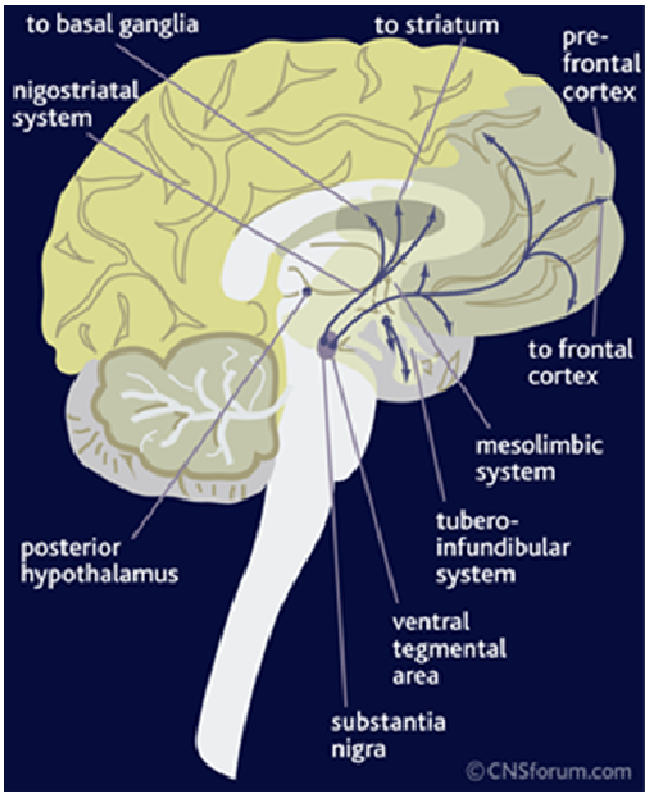


Figure 7: Illustration of the three dopaminergic pathways in the brain

(cnsforum.com)

Dopamine receptors are therefore mainly localized within these pathways, in the striatum, the limbic system, the brain cortex and the infundibulum (Figure 9, Samad *et al.,* 1997; Pivonello *et al.,* 2007). In the mesolimbic and nigrostriatal pathways, both D_1 and D_2 receptors are present (Civelli, 2000). Specifically, there are high levels of D_1 and D_2 mRNAs expressed in the caudate putamen (CPu), nucleus accumbens and olfactory tubercle, with lower levels seen in the septum, hypothalamus and cortex (Civelli, 2000). Within the substantia nigra, hippocampus and the ventral tegmental area, there is high expression of D_2 but not D_1 mRNA, whereas in the amygdala there is expression of D_1 receptors but little, if any D_2 mRNA (Civelli, 2000). The expression of high levels of D_2 dopamine receptors in the pituitary gland mediates the effect of dopamine in the regulation of prolactin gene expression and secretion (Figure 8; Samad *et al.,* 1997; Civelli, 2000; Cheung and Lustig, 2007; Pivonello *et al.,* 2007; Fitzgerald and Dinan, 2008). Dopamine acts via D_2 receptors present on the cell membranes of lactotrophs, the activation of which results in the suppression of prolactin gene expression and the inhibition of prolactin exocytosis (Cheung and Lustig, 2007; Fitzgerald and Dinan, 2008). Gene expression of prolactin is suppressed by the

inhibition of adenylyl cyclase and inositol phosphate metabolism, and exocytosis is inhibited due to modification of potassium and calcium channels (Cheung and Lustig, 2007; Fitzgerald and Dinan, 2008).

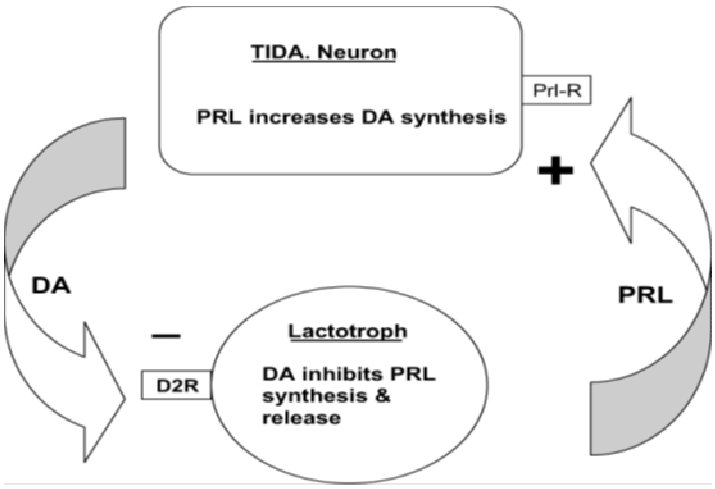


Figure 8: Short-loop feedback mechanism of prolactin regulation.

DA, dopamine; TIDA, tuberoinfundibular dopaminergic neuron; Prl-R, prolactin receptor; D2R, dopamine2 receptor; PRL, prolactin.

(Adapted from Fitzgerald and Dinan, 2008)

The D_3-D_5 receptors are mostly present where D_1 and D_2 mRNA is expressed, but their abundance is lower than the D_1 or D_2 mRNA (Civelli, 2000). Relative to D_1 and D_2, the D_3 and D_4 receptor subtypes are more selectively associated with the limbic brain areas and relatively absent in the nigrostriatal system. The limbic system mostly receives inputs from the ventral tegmental area to influence cognitive, emotional and endocrine functions of the brain and as such, is associated preferentially with the etiology of psychoses rather than locomotor dysfunctions (Civelli, 2000). D_5 receptors in the central nervous system are highly specific, only being found in the hippocampus, the hypothalamus and the parafascicular nucleus of the thalamus (Figure 9). Here the receptors are said to be involved in affective, neuroendocrine or pain-related aspects of dopamine function (Civelli, 2000).

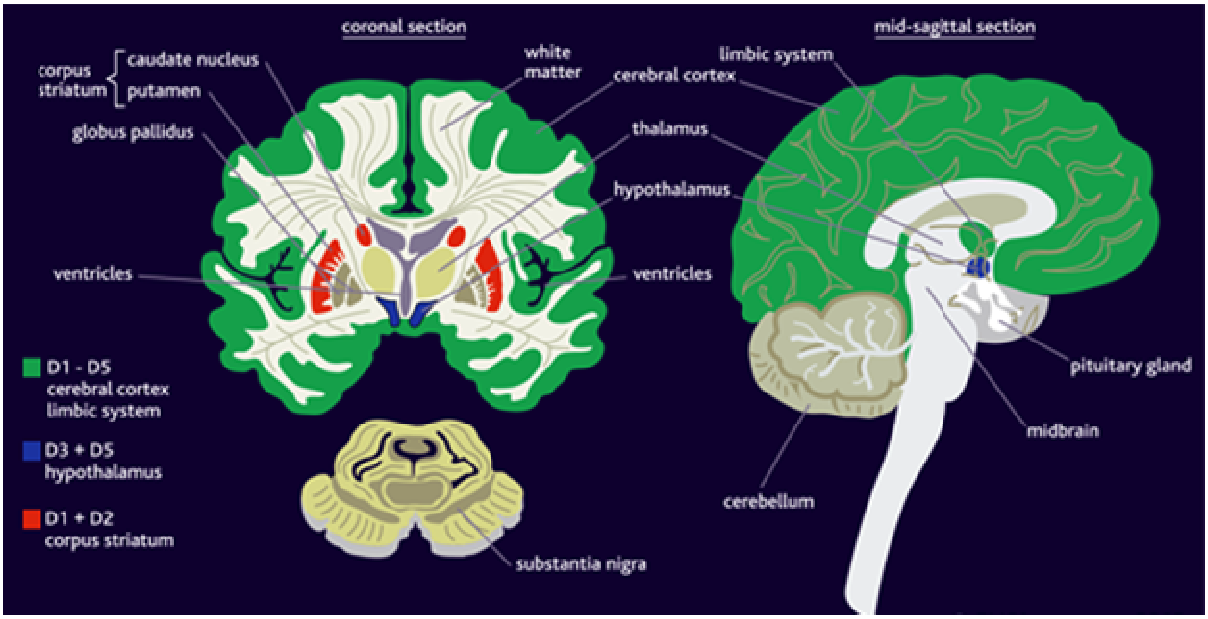


Figure 9: Distribution of dopamine receptors in the brain

(CNSforum.com)

Dopamine receptors are also found widely distributed in the periphery, mainly in the cardiovascular system, the kidneys and the adrenal glands (Pivonello *et al.,* 2007). In the kidney and heart, D1- and D2-like activities have been described with D_4 receptors being seen in the heart and low levels of D_5 activity in the kidneys. In the heart dopamine may increase cardiac output, while in the kidney it seems to stimulate the renin-angiotensin system. The presence of dopamine in the adrenal glands suggests a role for these receptors in the regulation of adrenal hormone synthesis or secretion (Pivonello *et al.,* 2007).

1.2.3 Dopamine pathways

The dopaminergic system plays a pivotal role in the central nervous system and also plays a smaller part in the periphery, particularly in the endocrine, cardiovascular, renal and gastrointestinal systems (Samad et al 1997; Civelli, 2000; Pivonello *et al.,* 2007). Within the brain, the transmission of dopamine is responsible for the control of various physiological functions, including cognition, emotions, hunger, satiety, coordination of movement and the synthesis and secretion of hormones, as well as the regulation of the hypothalamo-pituitary-adrenal axis (Samad *et al.,* 1997; Pivonello *et al.,* 2007).

There are three dopaminergic pathways within the central nervous system, namely; the nigrostriatal pathway, the tubero-infundibular pathway, and the mesocorticolimbic pathway (Figure 7, Behrouz et al 2007; Janhunen *et al.,* 2007).

The nigrostriatal dopamine pathway consists of the axons of neurons whose cell bodies are located in the substantia nigra pars compacta. These neurons project mainly to the dorsal striatum, the CPu. This pathway is a key component of the extrapyramidal motor system and is involved in the control of posture and motor behaviour as well as the learning of motor programs and habits (Figure 7, Civelli, 2000; Behrouz *et al.,* 2007; Janhunen *et al.,* 2007). Degeneration of the nigrostriatal pathway underlies the development of extrapyramidal abnormalities that include Parkinson's disease (Civelli 2000; Janhunen *et al.,* 2007).

The tubero-infundibular dopamine neurons form part of a heterogeneous group of dopamine neurons in the brain which originate in the arcuate nucleus of the mediobasal hypothalamus and project ventrally into the median eminence to terminate adjacent to the hypophyseal portal vessels (Figure 7, Behrouz *et al.,* 2007). The dopamine that is released into the portal circulation is transported to the anterior pituitary where it tonically inhibits the secretion of prolactin (Behrouz *et al.,* 2007). The re-uptake of dopamine is mediated by the action of high volume–low affinity dopamine transporters and low volume-high affinity dopamine transporters (Behrouz *et al.,* 2007). Unlike other dopamine neurons, the neurons of the tubero-infundibular pathway are not affected in Parkinson's disease (Behrouz *et al.,* 2007).

The mesolimbic dopamine projections originate in the midbrain, specifically the ventral tegmental area and have three major trajectories namely the dorsal striatum, ventral striatum (including the nucleus accumbens) and the prefrontal cortex and other limbic structures (Figure 7, Civelli, 2000; Shephard *et al.,* 2006; Behrouz *et al.,* 2007; Iverson, 2007; Janhunen *et al.,* 2007). These long midbrain dopaminergic projecting axons form classic synapses with their target neurons. Usually dopaminergic synaptic activity is regulated by a dopamine autoreceptor-mediated mechanism (Behrouz *et al.,* 2007). Apart from projecting into the nucleus accumbens, the ventral tegmental area has dense connections that terminate in the medial pre-frontal cortex, temporal cortices, ventral pallidum and basal forebrain structures such as the amygdala, bed nucleus of the stria terminalis and the lateral septum (Dobbs *et al.,* 2008, Herrold *et al.,* 2008).

The mesocorticolimbic pathway plays a critical role in diverse motivational processes including procuring and consuming food, engaging in sexual behaviour as well as in drug reward and addiction-like behaviour (Dobbs *et al.,* 2008; Herrold *et al.,* 2008). Each of the structures within this pathway carries out different yet important functions that cumulatively have been implicated in reward e.g. the ventral tegmental area, the nucleus accumbens and the ventral pallidum have all been shown to be critically involved in psychostimulant (such as methamphetamine) drug-induced behaviour in rats (Herrold *et al.,* 2008). The cortical and limbic areas of the brain which receive projections from the ventral tegmental area are important for the expression of emotions, reactivity to conditioned cues, planning and judgement and have therefore been implicated in reward (Tomkins and Sellers, 2001).

As well as having a well-established role in the development of drug dependence and reward behaviour, the mesocorticolimbic system is the principle dopaminergic pathway involved in the etiology of psychosis (Civelli, 2000; Janhunen *et al.,* 2007). This leads to difficulties with regard to dopamine-related treatments as blockade of the dopamine system may reduce psychoses and drug dependence but lead to side–effects that include extrapyramidal dysfunctions (Civelli 2000).

The terms mesolimbic and mesocortical systems are sometimes used instead of the combined mesocorticolimbic system due to the difference in their functional properties. (Janhunen *et al.,* 2007). It is the mesolimbic pathway that is responsible for the control of motor behaviour and motivation, emotions and rewards whereas the mesocortical system is involved in higher cognitive functions such as working memory, as well as learning and reward (Janhunen *et al.,* 2007).

1.2.4 Dopamine in motivation and reward

Animals are able to learn goal-directed behaviour based on their knowledge of the potential outcome of their actions and the value of these outcomes, in a learning paradigm known as ‘the cognitive value system’ (Iverson *et al.,* 2007). There is also a second value system that operates when we experience reinforcement and when we determine how much we ‘like’ the outcomes of our actions or behaviours- in this case the term ‘pleasure’ is often used (Iverson *et al.,* 2007).

Dopamine plays a significant role in this reward and reinforcement paradigm and is at the centre of a complex neural circuitry that each contributes to the component processes that make up reinforcement, motivation and learning (Iverson *et al.,* 2007). The intimate relation of the nucleus accumbens to areas of the limbic system that are innervated by dopaminergic neurons has led to the proposal that the mesolimbic dopamine pathway is central to these behaviours.

The nucleus accumbens can be divided into core and shell subregions and these regions differ in terms of neuronal organization and efferent targets, with the shell including projections from the substantia nigra (A9) mesencephalic neurons (Iverson *et al.,* 2007). This pattern organization puts the nucleus accumbens shell in control of vast areas of the striatum and enables it to strengthen behaviours that are otherwise coordinated by the nucleus accumbens core and the dorsal striatum (Iverson *et al.,* 2007). The core of the nucleus accumbens transmits information about the importance or the salience of the stimuli that we are exposed to on a daily basis, and this includes our sensitivity to rewarding or aversive stimuli, unexpected stimuli, condition reinforcers, as well as to their predictability or novelty (Iverson *et al.,* 2007; Volkow *et al.,* 2007; Dobbs *et al.,* 2008).

The nucleus accumbens core is particularly functional in the learning of an association between two stimuli or classical conditioning (referred to as S-S learning), unlike the dorsal striatum which is mostly involved in the learning of an association between a stimulus and a response (referred to as S-R learning) (Messinger *et al.,* 2001; Iverson *et al.,* 2007; Everitt *et al*., 2008). During S-S associative learning an initial neutral stimulus precedes a second stimulus, the latter of which elicits a particular response (Clark *et al.,* 1998; Messinger *et al.,* 2001; Mok *et al.,* 2010). Repeated exposure to these two stimuli results in long-term associative memories being formed. This is facilitated by the strengthening of the connections between the neurons representing the two stimuli (Messinger *et al.,* 2001). The final outcome of this arrangement is that initally a neuron that would respond to only one of the associated stimuli will now respond to both (Clark *et al.,* 1998; Messinger *et al.,* 2001; Mok *et al.,* 2010). This results in the original neutral stimulus now being able to elicit a response even in the absence of the second stimulus.

Therefore individual neurons respond to sensory stimuli that become associated with a response or a reward (S-R associative learning) (Messinger *et al.,* 2001; Mok *et al.,* 2010).

The stimulus elicits a response and for every correct response given, a reward is received which strengthens the association between the stimulus and the action or response (Messinger *et al.,* 2001; Mok *et al.,* 2010). This type of associative learning allows the learning of an action through the consequences of response outcomes and reiterates that behaviours that are reinforced positively are more likely to reoccur (Mok *et al.,* 2010).

The dopamine inputs to both the nucleus accumbens and the striatum ensure a coordinated mechanism of modulation of goal-directed and procedural learning. This arrangement allows the balance between these two paradigms and facilitates adaptation to the environment as the animal experiences changes in stimuli and events (Iverson *et al.,* 2007).

This diversity of dopamine effects is likely translated by the specific brain regions it modulates, i.e. limbic, cortical and striatal (Volkow *et al.,* 2007).

Phasic fast-burst firings of dopamine neurons, (firing at a frequency of > 30Hz) which occur when there are large transient increases in the release of synaptic dopamine, are functionally relevant signals at post-synaptic sites that encode reward predictions or incentive salience. These signals also facilitate learning, and are involved in the reinforcing effects of drugs (Iverson *et al.,* 2007; Volkow *et al.,* 2007). This phasic burst firing contrasts to normal tonic dopamine cell firing which is slow at approximately 5Hz and is responsible for the maintenance of baseline, steady state dopamine. This slow firing is believed to set the overall responsiveness of the dopamine system.

The mesolimbic dopamine system projects beyond the nucleus accumbens and the pathways that project to the amygdala are vital for the associative Pavlovian learning that underlies the responses of animals to salient or rewarding events (Iverson *et al.,* 2007). Due to the important role of dopamine transmission in numerous behavioural responses, a disruption of these associative processes can lead to devastating effects, causing complex central nervous system disorders such as the inappropriate effects in schizophrenia, heightened distractibility in attention deficit hyperactivity disorder, as well as the development of drug addiction (Iverson *et al.,* 2007; Janhunen *et al.,* 2007).

The reinforcing effects of drugs of abuse are not only dependent on increases in striatal dopamine but also on the rate of that increase, with a faster increase leading to more intense reinforcing effect (Volkow *et al.,* 2007). An increase in the level of dopamine in the dorsal

striatum is involved in the motivation to procure a drug when the addicted subject is exposed to a stimulus which they associate with the drug (conditioned stimuli) (Volkow *et al.,* 2007). Long-term use of drugs has been shown to decrease dopamine function with corresponding reductions in D2 receptor expression and dopamine release in the striatum in addicted subjects (Volkow *et al.,* 2007). Reductions in D2 receptors in the striatum are associated with a reduction in the activity of the orbitofrontal cortex, which is the area of the brain responsible for salience attribution and motivation and with compulsive behaviours. Similarly reduced activity of the cingulated gyrus, the region that is involved in inhibitory control and impulsivity, is part of the repertoire of frontal regions of the brain implicated in the loss of control and compulsive drug intake by which addiction is characterized (Volkow *et al.,* 2007).

1.2.5 Dopamine in methamphetamine addiction

Brain imaging studies conducted on chronic methamphetamine abusers showed a decrease in dopamine transporter levels, an effect that can persist for months after cessation of drug use (Shephard *et al.,* 2006). Chronic methamphetamine use also reduced the tissue levels of dopamine as evidenced by post-mortem studies (Shephard *et al.,* 2006).

Methamphetamine increases dopamine levels by releasing it from the terminals via the dopamine transporters (Volkow *et al.,* 2007). Intravenous administration of methamphetamine increased extracellular dopamine concentrations in the striatum and this increase was associated with an increase in self-reports of highs and euphoria (Volkow *et al.,* 2007). The speed at which the drug enters the brain also affects their reinforcing effects and therefore intravenous administration enters the brain faster and induces faster dopamine changes when compared to oral administration, which results in a slow increase of dopamine levels (Volkow *et al.,* 2007). Interestingly in methamphetamine use, it is the fast uptake of the drug that is associated with the development of the high, and not the actual brain concentration (Volkow *et al.,* 2007).

In summary, dopamine is a catecholaminergic neurotransmitter involved in numerous physiological functions, including the addictive state. Dopamine acts via three pathways in accordance with its function, namely the nigrostriatal, tuberoinfundibular and mesolimbic pathways. Within these pathways, dopamine exerts its effects via 5 different receptors, i.e. D_1-D_5, which exerts either a stimulatory or inhibitory effect. Within the mesolimbic system,

dopamine plays a role in reward and reinforcement, as well as the motivation and learning processes involved in addiction. These roles are mediated by the D_1 and D_2 receptors present in these neurocircuits. The administration of methamphetamine has been shown to increase dopamine transmission in this pathway, and this has been linked with the drugs reinforcing and addictive properties.

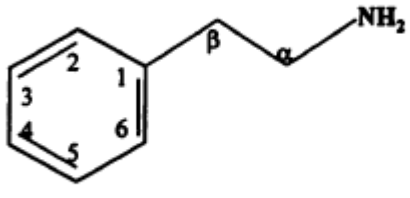
1.3. Methamphetamine

1.3.1 The methamphetamine problem

Methamphetamine is a highly addictive stimulant drug whose illicit use and resultant addiction has become an alarming global phenomenon (Herrold *et al.,* 2008). In 2000, the National Household Survey on Drug Abuse showed that approximately 8.8 million people have tried methamphetamine at least once. This potent central nervous system stimulant drug has become one of the world's most widely abused illicit substances (National Institute of Drug Abuse, 2008). In South Africa, the abuse of this drug has increased dramatically with a report from the United Nations Office on Drugs and Crime (UNODC) on 23 July 2008 stating that, "while dagga is the drug most abused in South Africa, amphetamine-style stimulants like tik (methamphetamine) pose the greatest threat on the drugs market."

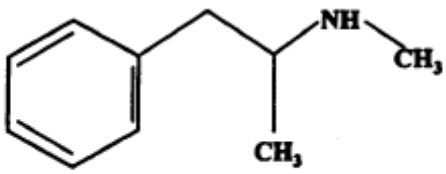
1.3.2 Chemistry

Methamphetamine is a chemical compound belonging to the amphetamine family and is the most widely abused of all amphetamine-like drugs (Albertson *et al.,* 1999; Phillips *et al.,* 2008). All amphetamines share a common phenylethylamine skeleton, which can be altered at its α-ring, β-ring or its phenyl ring, in order to bring about various pharmacological actions (Figure 10, Albertson *et al.,* 1999). Methamphetamine is distinctive from other amphetamines by the presence of two methyl groups located on the α-side chain and the amine group of this skeleton (Figure 11, Albertson *et al.,* 1999). Therefore the systemic name for methamphetamine is N, α–dimethylphenethylamine with a chemical formula of $C_{10}H_{15}N$ (Logan, 2002). Methamphetamine is the most hyperstimulating of the amphetamine analogues with the impact of its psychological and behavioural functions being due to its effects on the neurochemistry of the central nervous system (Halikitis *et al.,* 2001).



Phenylethylamine

Figure 10: Basic Phenylethylamine structure



Methamphetamine

Figure 11: Methamphetamine structure

(Albertson *et al.,* 1999)

Methamphetamine exists in two isomeric forms with the two enantiomers being, dextrorotatory (D) methamphetamine (the more active enantiomer) and levorotatory (L) methamphetamine (the less active enantiomer) (Fowler *et al.,* 2007; Mendleson *et al.,* 2008). The configuration at the chiral centre dictates the central nervous system activity of the compound, with the d-isomer of methamphetamine having stimulant effects on the central nervous system up to 3-4 times greater than the L-isomer (Logan, 2002).

The d-methamphetamine isomer differs from its L-isomer in both physiologic and pharmacologic potency, being more potent and having a more intense central psychostimulant effect (Shoblock *et al.,* 2002; Fowler *et al.,* 2008). For this reason D-methamphetamine has a higher abuse liability than its L-isomer and is a prescription drug (DEA schedule II), while L-methamphetamine is an easily available over-the-counter drug (Fowler *et al.,* 2007; Mendleson *et al.,* 2008; Dufka *et al.,* 2008). L-methamphetamine acts as a nasal decongestant and is the active ingredient in the Vicks® Vapor Inhaler produced in America (Logan, 2002; Fowler *et al.,* 2007; Mendleson *et al.,* 2008; Dufka *et al.,* 2008).

1.3.3 Synthesis

D-methamphetamine is also relatively easy to synthesize and this leads to its illicit production in clandestine laboratories (Albertson *et al.,* 1999; Barr *et al.,* 2006; Fowler *et al.,* 2007). There are two common methods of illicit methamphetamine production, both by the reduction of ephedrine or its cousin pseudoephedrine (Figure 12, Derlet *et al.,* 1990; Logan, 2002, Fowler *et al.,* 2007).

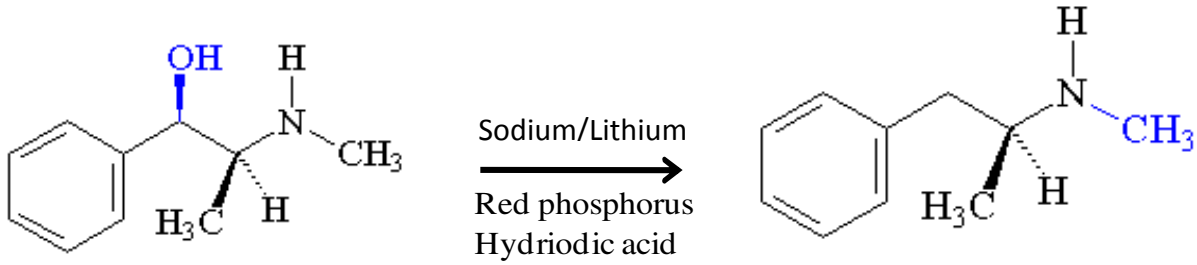
The first method uses hydriodic acid and red phosphorus to bring about the reduction of L-ephedrine or D-pseudoephedrine (Figure 12, Derlet *et al.,* 1990; Logan, 2002, Fowler *et al.,* 2007). The resultant product with either precursor is pure D-methamphetamine, with yields of 54-82% (Logan, 2002). Red phosphorus is easily obtained from the striker plates of matchboxes or road flares, while hydroiodic acid is relatively easy to synthesize from iodine (Logan, 2002).

The second method of methamphetamine synthesis uses either sodium or lithium metal in condensed liquid ammonia to reduce either of the two precursor compounds (Figure 12, Logan, 2002). The lithium required for this method can be obtained from lithium batteries,

while the sodium is made from the electrolytic reduction of molten sodium hydroxide and liquid ammonia (Logan, 2002). The enantiospecific product from this method is also D-methamphetamine (Logan, 2002).

In either method of production, the substitution of phenylpropanolamine as the precursor will result in the synthesis of amphetamine (Logan, 2002).

D-methamphetamine is a lipid soluble, pure base form of methamphetamine that is highly volatile and evaporates when exposed to air (Derlet *et al.,* 1990). Therefore the form of methamphetamine that is commonly sold under the names "speed", "tik" or "crank" is methamphetamine hydrochloride, which is D-methamphetamine which has been converted to a water-soluble crystalline form by using hydrochloride (Derlet *et al.*. 1990).



Ephedrine Methamphetamine

Figure 12: Synthesis of d-methamphetamine from ephedrine

(Adapted from www.chm.bris.ac.uk/motm/methamphetamine/methh.htm)

1.3.4 Medical uses

While D-methamphetamine is a potent addictive drug abused worldwide, it is noteworthy that it is currently marketed as Desoxyn® (Abbott Laboratories) for the treatment of Attention Deficit Disorder and Attention Deficit Hyperactivity Disorder (an alternative to the more commonly used methylphenidate (Ritalin®), which is also a member of the amphetamine family), narcolepsy and exogenous obesity (Halkitis *et al.,* 2001; Greenhill *et al.,* 2002; Fowler *et al.,* 2007; Kish 2008). The L-isomer of methamphetamine is also used as a decongestant as previously mentioned (Fowler *et al.,* 2007; Mendleson *et al.,* 2008; Dufka *et al.,* 2008).

1.3.5 Routes of administration

Illicit methamphetamine occurs in various forms; powder, tablets and translucent crystals resembling rock candy or salt (Halkitis *et al.,* 2001). There are numerous routes of methamphetamine administration, including intravenous injection, inhalation, smoking and ingestion (Halikitis *et al.,* 2001). The crystalline form of methamphetamine or "crystal meth" is the more commonly abused form of the drug and is responsible for a more rapid intense stimulant effect when it is smoked (Kish, 2008).

The smoking and intravenous injection of methamphetamine produces the fastest rates of absorption, with a lag time of approximately 7-10 seconds when smoked and 15-30 seconds when injected (Halikitis, 2001). Inhalation and intramuscular injection produce slower absorption rates with lag times approximating 3-5 minutes when inhaled and 5 minutes when administered intramuscularly (Halikitis, 2001). The oral ingestion of methamphetamine in its tablet form produces the slowest rate of absorption, with a lag time of approximately 20-30 minutes (Halikitis, 2001).

The effects of the drug can last in the region of 8 – 24 hours after administration depending on the amount used and the route of administration (Halikitis, 2001).

1.3.6 Pattern of use and drug effects

It is the immediate psychological effects of methamphetamine that gives it its tremendous potential for abuse due to the "rush" given to the user upon administration (Logan, 2002; Lineberry *et al.,* 2006) Methamphetamine, even in small amounts, leads to potent autonomic and central nervous system effects (Halikitis *et al.,* 2001). Methamphetamine has become the psychostimulant of choice due to its significantly higher elimination half-life as opposed to other stimulants such as cocaine and ecstasy, leading to these positive behavioural and psychological effects lasting substantially longer than with other drugs (Tominaga *et al.,* 2004; Barr, 2006). The effects can last 6 – 12 hours longer than cocaine, depending on the dose and the pH of the urine (Tominaga *et al.,* 2004). Methamphetamine also differs from other stimulants in that it has relatively high lipid solubility, allowing for more rapid transfer of the drug across the blood brain barrier, thereby enhancing the drugs' central effects (Barr *et al.,* 2006).

The incentives for the initial use of methamphetamine vary widely, from shift workers wanting to combat fatigue, students to aid in increase concentration and studying, teenagers and others who use it as a dietary aid to accelerate weight loss, to the purely hedonistic purpose and recreational use of the drug (Logan, 2002). It is this purpose for use that will ultimately dictate the pattern of use. Regardless of the initial motivation, habituation to the central nervous system effects of the drug develops quickly, and if use is continued the user generally deteriorates into binge using (Logan, 2002).

Although not a common pattern of abuse, the administration method that receives the greatest amount of research is that of the single therapeutic dose of methamphetamine (5-10mg) (Logan, 2002). It is at this dose that the user experiences the alerting and anorectic effects, feelings of euphoria and well-being, increased energy, elimination of fatigue and drowsiness, increased libido, a general increase in psychomotor activity, an alteration to self-esteem and self-confidence, as well as intensifying emotions and suppressing the appetite (Derlet *et al.,* 1990; Halikitis *et al.,* 2001; Logan, 2002; Tominaga *et al.,* 2004; Barr *et al.,* 2006; Lineberry *et al.,* 2006; Schifano *et al.,* 2007; Buxton and Dove, 2008; Phillips *et al.,* 2008; Shoptaw *et al.,* 2008). In men, ejaculation is delayed and the intensity of the orgasm is enhanced which, coupled with the increase in libido associated with methamphetamine use, gives it its popular reputation as the "sex drug" (Logan, 2002; Buxton and Dove, 2008; Shoptaw *et al.,* 2008). As well as alerting symptoms, due to the stimulating effects on the central nervous system, the user may also experience restlessness, increased irritability, insomnia, aggressiveness, paranoia, anxiety, dizziness, overstimulation, mild confusion and in rare instances panic or psychotic states (generally in individuals predisposed to schizophrenia) (Halikitis *et al.,* 2001; Logan, 2002; Tominaga *et al.,* 2004; Buxton and Dove, 2008; Shoptaw *et al.,* 2008).

1.3.7 Metabolism and clearance of methamphetamine

Methamphetamine undergoes phase I metabolism by N-demethylation to amphetamine via the cytochrome P4502D6 isoenzyme system, which also plays a role in the aromatic hydroxylation of methamphetamine (Lin et al 1997; Logan, 2002; Dostalek *et al.,* 2007). The amphetamine is then extensively metabolized to a variety of metabolites including norephedrine and p-hydroxyamphetamine, both of which are pharmacologically active, and may be glucuronidated prior to excretion (Logan, 2002). Methamphetamine undergoes four different metabolic processes in both humans and rats, namely, aromatic or p-hydroxylation,

aliphatic or β-hydroxylation, N-demethylation and deamination (Figures 13 and 14. Caldwell *et al.,* 1972; Musshoff 2000; Kanimori *et al.,* 2005; Dostalek *et al.,* 2007). In vivo studies in humans show that the main primary metabolic reactions are aromatic hyroxylation and N-demethylation and these processes account for the production of the p-hydroxy derivative, p-hydroxymethamphetamine, and amphetamine respectively, which make up almost 50% of all the metabolites that are excreted (Caldwell *et al.,* 1972; Musshoff 2000).

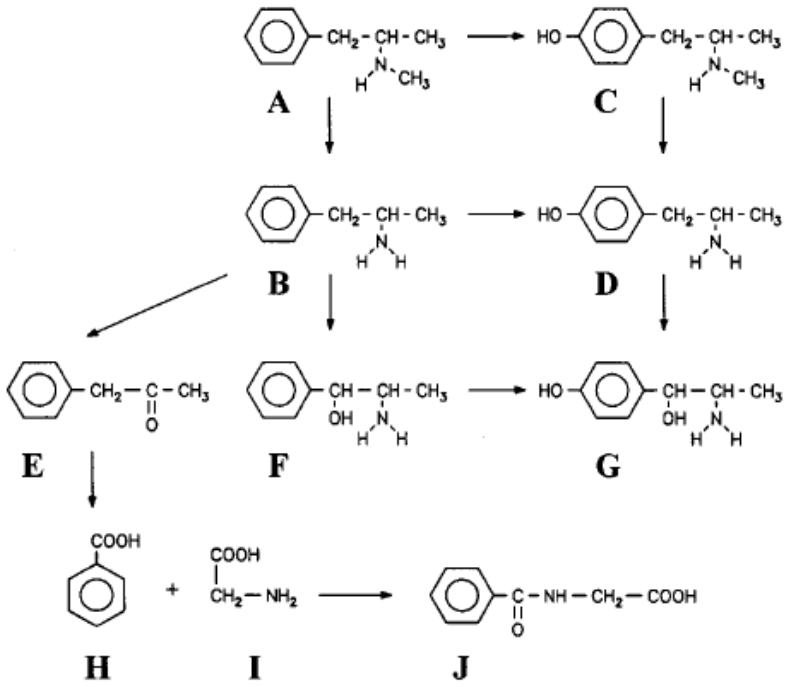


Figure 13: Main metabolic pathways of methamphetamine and amphetamine in man:

(A) methamphetamine;
(B) amphetamine; (C) 4-hydroxymethamphetamine; (D) 4-hydroxyamphetamine;
(E) phenylacetone(phenyl-2-propanone); (F) norephedrine; (G) 4-hydroxynorephedrine;
(H) benzoic acid; (I) glycine; (J) hippuric acid.

(Musshoff *et al.,* 2000)

There is a distinct difference between methamphetamine metabolism in man and rats, with the primary metabolic reaction in rats being aromatic hydroxylation (Caldwell et al 1972). In a study by Caldwell, the urine of 3 rats and 2 men were analysed and it was shown that the main metabolite that is found in the urine of man is unchanged methamphetamine whereas in rats unchanged drug only accounts for approximately 1/10 of dose excreted, with 3% of urine metabolites appearing as amphetamine in both man and rat. Both man and rat were shown to

excrete norephedrine derivatives, d-hydroxyephedrine in the rat and norephedrine in man (Caldwell *et al.,* 1972; Musshoff 2000).

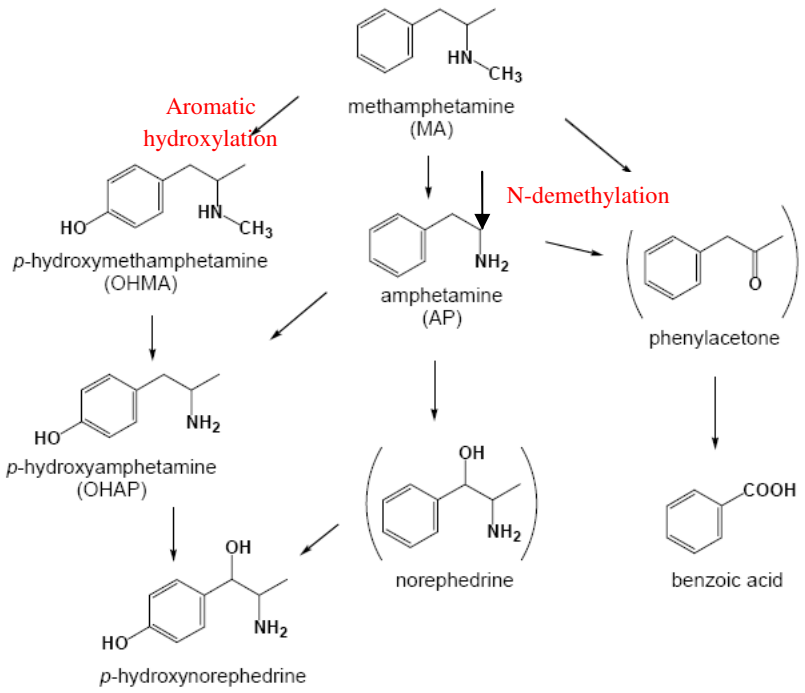


Figure 14: Summary of the metabolic pathway of methamphetamine in the rat

(Kanimori *et al.,* 2005)

1.3.8 Peripheral effects of methamphetamine

The peripheral effects of methamphetamine are mainly a result of its actions as an α-, β1- and β2 adrenergic agonist (Logan, 2002). The effects of methamphetamine on epinephrine and norepinephrine release by the adrenal glands are characteristic to those of a fight or flight response (Barr *et al.,* 2006; Schifano *et al.,* 2007). The effects mediated by the α-receptors include mydriasis (pupil dilation), vasoconstriction, bronchial muscle dilation, bladder contraction and urinary retention, stomach cramps, hyperthermia, muscle and skin tremors and coronary dilation (Halkitis *et al.,* 2001; Logan, 2002; Barr, 2006; Schifano *et al.,* 2007). Also typical to an adrenaline response, methamphetamine causes acceleration in heart rate, hyperglycaemia, hypertension, specifically an increase in venous blood pressure due to the constriction of the peripheral vasculature (Albertson *et al.,* 1999; Halkitis *et al.,* 2001; Logan,

2002; Tominaga *et al.,* 2004; Barr, 2006). Dry mouth, perspiration or chills are also sometimes experienced by the user (Halkitis *et al.,* 2001).

The toxic effects of methamphetamine is related to multiple factors which include among others, oxidative stress with the production of oxygen and nitrogen reactive species, aberrant catecholaminergic transmission, excitotoxicity, mitochondrial dysfunction, and apoptosis (Williams *et al.,* 2003; Darke *et al.,* 2008; Sharma *et al.,* 2008). The neurotoxic effects of methamphetamine involves the degeneration of the nerve terminals of dopamine and serotonin that are found in the frontostriatal region, resulting in the long lasting depletion of these monoamines and in the alteration in the regulation of these systems (Darke *et al.,* 2008). Due to the oxidative stress mechanism of methamphetamine neurotoxicity, the use of selective antioxidants may prove to be neuroprotective (Darke *et al.,* 2008).

Methamphetamine causes intoxication by potentiating presynaptic nerve terminals to release catecholamine neurotransmitters norephinephrine and dopamine, causing the stimulation of post-synaptic receptor and inhibiting their reuptake, increasing their levels in the synaptic space (Tungtananuwat *et al.,* 2009).

Chronic use of methamphetamine severely affects the cardiovascular system with premature and accelerated development of artherosclerosis leading to an increased risk of myocardial infarction (Buxton and Dove, 2008; Darke *et al.,* 2008; Shoptaw *et al.,* 2008). Chronic use also leads to ventricular hypertrophy and this can predispose the user to methamphetamine-induced myocardial ischemia and/or arrhythmias (Buxton and Dove, 2008; Darke *et al.,* 2008; Shoptaw *et al.,* 2008).

The physical manifestations of methamphetamine abuse include severe weight loss, dental decay and dry mouth known as “meth mouth” and scabbed skin, due to psychosis at high doses of the drug whereby the user has the persistent feeling of insects crawling on them and constantly scratching and picking at their skin (Schifano *et al.,* 2007; Buxton and Dove, 2008).

1.3.9 Central effects of methamphetamine

The effect profile of methamphetamine is very complex, with acute low dose administration exerting stimulant, alerting effects, with progressively more disorienting effects on cognition, reasoning and psychomotor ability occurring with increasing dose and duration of drug use

(Logan, 2002; Tominaga *et al.,* 2004). During withdrawal from methamphetamine, a depressant-like profile occurs, including anhedonia and decreased motivation, which is often compounded by delusions and psychotic episode, especially after high-dose or chronic use (Logan, 2002; Barr *et al.,* 2006).

The primary mechanism by which amphetamines' exert their effects is by increasing the levels of extracellular monoamine neurotransmitters within the central nervous system (Barr *et al.,* 2006; Kish, 2008). The sympathetic neurotransmitters particularly targeted are serotonin, the catecholamines norepinephrine and epinephrine, with the principle monoamine being dopamine. The structural similarities to methamphetamine and these neurotransmitters are clear (Figure 15, Halikitis *et al.,* 2001; Logan, 2002; Barr *et al.,* 2006; Kish, 2008).

Studies have shown that with a single injection of methamphetamine there is a marked increase in dopamine, serotonin and norepinephrine in the dopamine-rich subdivisions of the striatum such as the caudate, putamen, ventral striatum (the nucleus accumbens is included in this brain area) as well as in the pre-frontal cortex (Kish, 2008; Herrold *et al.,* 2008). It is the enhanced release of norepinephrine from their central neurons that is responsible for the alerting and anorectic effects of amphetamines, including methamphetamine, and together with the release of dopamine from dopaminergic nerve terminals that is responsible for the locomotor stimulating effects of the drug (Logan, 2002). The stereotyped repetitive behaviour that is associated with high dose methamphetamine use is also a feature of dopamine release, particularly in the neostriatum (Logan, 2002).

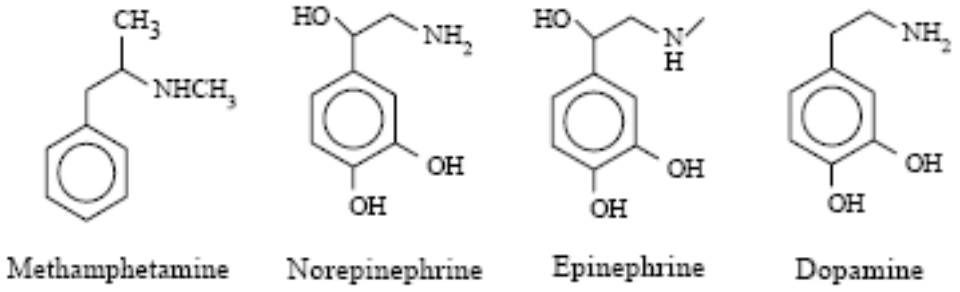


Figure 15: Structures of the sympathomimetic methamphetamine and the neurotransmitters whose release they promote.

(Adapted from Logan 2002)

The amphetamines' potent stimulating effects appear to result by promoting the release of these biogenic amines via various mechanisms including; promoting their release from stores in the nerve terminals, the reverse transport of the neurotransmitter through the plasma membrane transporters and the redistribution of catecholamines from synaptic vesicles to the cytosol (Logan, 2002; Nagai *et al.,* 2005; Barr *et al.,* 2006). Additionally, amphetamines block the activity of monoamine transporters and decrease the expression of dopamine transporters at the cell surface (Figure 16; Barr *et al.,* 2006). Evidence also shows that amphetamines increase cytosolic levels of monoamines by the inhibition of monoamine oxidase (MAO) and by stimulating the activity and expression of tyrosine hydroxylase (TH) (Figure 16; Barr *et al.,* 2006).

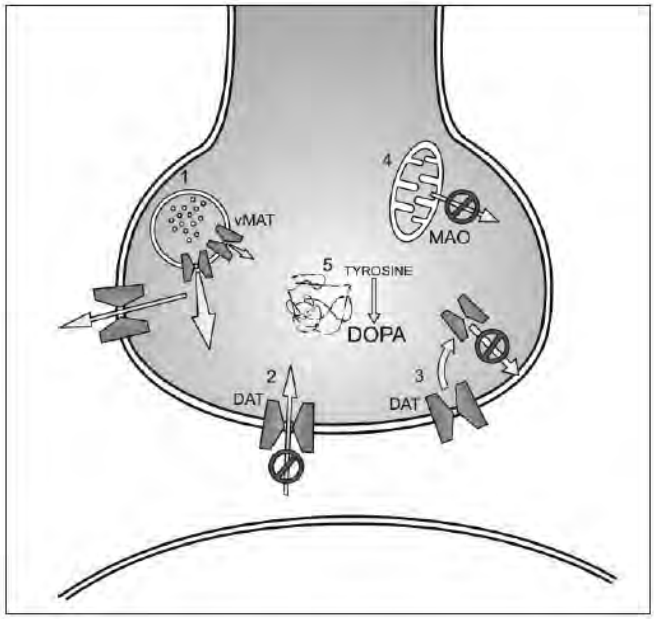


Figure 16: Physiological mechanisms by which methamphetamine increase synaptic levels of monoamines, principally dopamine (DOPA): (1) redistribution of catecholamines from synaptic vesicles to the cytosol; (2) reversing transport of neurotransmitter through plasma membrane transporters; (3)blocking the activity of monoamine transporters , (3) decreasing expression of dopamine transporters at the cell surface (4) increasing cytosolic levels of monoamines by inhibiting the activity of monoamine oxidase (MAO) (5) increasing activity and expression of the tyrosine hydroxylase

DAT = dopamine transporter; vMAT = vesicular monoamine transporter.

(Adapted from Barr *et al.,* 2006)

As previously stated, methamphetamine increases the concentration of extracellular dopamine in part by interfering with the dopamine transporter as well as increasing the amount of tyrosine hydroxylase in the mesolimbic dopamine system (Nagai *et al.,* 2005; Rocha *et al.,* 2008). Tyrosine hydroxylase is the rate limiting enzyme in dopamine synthesis and it is responsible for the conversion of L-Dopa to dopamine (Serretti *et al.,* 1998, Figure 17). Therefore by potentiating its increase; methamphetamine increases dopamine levels within the mesocorticolimbic system (Rocha *et al.,* 2008, Figure 17). Within the nucleus accumbens methamphetamine acts as a potent substrate for dopamine transporters and cause increases in extracellular dopamine levels by reverse transport (Dobbs *et al.,* 2008).

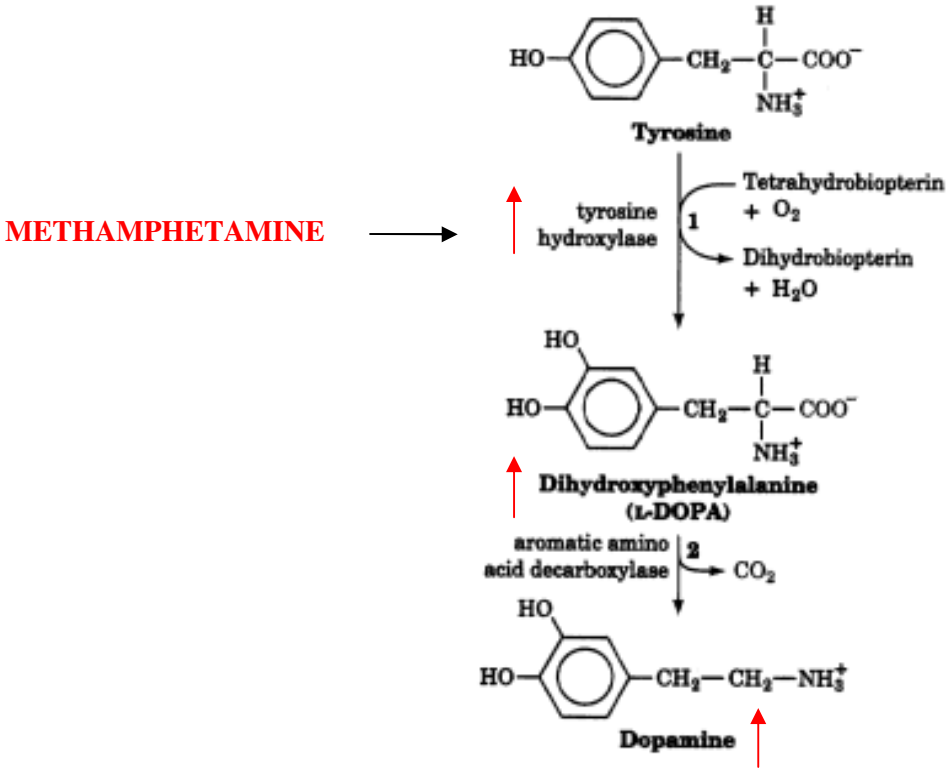


Figure 17: Illustration of the dopamine biosynthetic pathway. The effect of methamphetamine on this pathway is shown in red

(Adapted from ww2.coastal.edu/.../molecules/molecules.html)

Under normal conditions vesicular monoamine transporter-2 (VMAT2) transports cytoplasmic dopamine into reserpine-sensitive tubulovesicular organelles within the dendrites (Dobbs *et al.,* 2008). This transport results in a decrease in cytoplasmic dopamine concentration (Dobbs *et al.,* 2008). As there is an increase in the concentration of

methamphetamine, its lipophilicity facilitates its diffusion into the dendrite (Dobbs *et al.,* 2008). This causes a disruption of VMAT2 therefore preventing the restoration of dopamine into vesicles (Dobbs *et al.,* 2008). The resultant build-up of cytoplasmic dopamine causes a reversal of dopamine transport on the dendrites and induces somatodendritic release of dopamine into the synapse (Dobbs *et al.,* 2008).

In summary, methamphetamine is a stimulant drug belonging to the amphetamine family of drugs, whose global abuse has become an alarming problem. This easily synthesized drug can be administered in various ways, such as inhalation/snorting, injection, ingestion and smoking. The administration of methamphetamine results in a potent "rush", which contributes to its reinforcing properties and increased potential for abuse. Peripheral effects of methamphetamine mirror those of a sympathetic "flight or fright' response, due to its agonist effects on β1 and β2 adrenergic receptors. Centrally, acute low dose administration produce stimulatory and alerting effects, while progressively causing disorienting effects on cognition and reasoning with chronic use at higher doses. Methamphetamine administration also exerts effects within the mesolimbic dopaminergic system, increasing extracellular dopamine concentration within this circuit. This effect is brought about by methamphetamine's ability to reverse VMAT, and increase the concentration of TH in the mesolimbic dopaminergic neurons. This increase in dopamine transmission is thought to play a role in the reinforcing effects of methamphetamine.

1.4. Vasopressin and Oxytocin

Tolerance, dependence and addiction to drugs such as methamphetamine may involve mechanisms of neuroadaptation that relates to learning and memory, at both a cellular as well as a system level (Gimpl *et al.,* 2001). Within the paradigm of learning and memory, some interest has been shown in the involvement of neurohypophyseal hormones vasopressin and oxytocin in this area of neuroscience (Engelmann *et al.,* 1996).

1.4.1 Biosynthesis

Vasopressin and oxytocin are nonapeptide hormones that are synthesized primarily within magnocellular neurons. Their cell bodies are located in the paraventricular and supraoptic nuclei in the hypothalamus (Alescio-lautier *et al.,* 2000; Dyatkin *et al.,* 2002). They are made up structurally of nine amino acids with a disulphide bridge between two cytseine residues at the 1 and 6 positions. (Gimpl *et al.,* 2001; Holmes *et al.,* 2003; Caldwell et al; 2008) Oxytocin and vasopressin share an 80% homology, differing in two amino acids at positions 3 and 8. Vasopressin contains phenylalanine at position 3 and arginine at position 8, and oxytocin has isoleucine at position 3 and leucine at position 8 (Figure 18, Gimpl *et al.*, 2001; Holmes *et al.,* 2003; Caldwell *et al*., 2008). The neuropeptides are synthesized from large precursor molecules which are processed to biologically active peptides via post translational processing. This occurs within the neurosecretory vesicles into which it is packaged (Gimpl *et al*., 2001; Heinrichs *et al.,* 2004; Landgraf *et al.,* 2004).

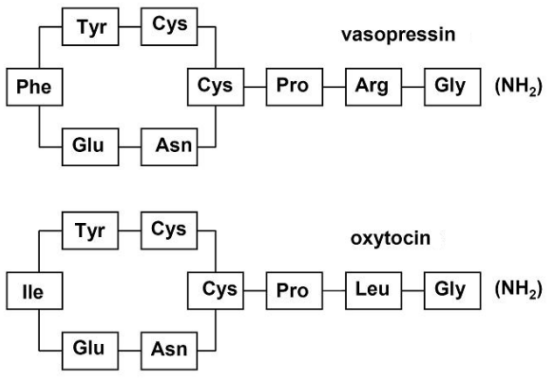


Figure 18: Amino-acid structure of Vasopressin and Oxytocin

(Adapted from Jellema *et al.,* 2009)

1.4.2 Vasopressin receptors and peripheral function

Vasopressin, or anti-diuretic hormone as it is also known, exerts both peripheral and central function via its 3 receptor subtypes, namely V1a, V1b and V2 receptors (Lolait *et al.,* 1995; Alescio-lautier *et al.,* 2000; René *et al.,* 2000; Holmes *et al.,* 2003; Egashira *et al.,* 2005; Caldwell *et al.,* 2008). The endocrine effects of vasopressin are mediated through its interaction with these receptors on target organs (Alescio-lautier *et al.,* 2000).

All three vasopressin receptor subtypes belong to the family of guanine nucleotide – binding protein (G-protein) coupled receptors, which are characterized by 7 hydrophobic transmembrane domain ά helices joined by alternating intracellular and extracellular loops, an extracellular amino-terminal and a cytoplasmic carboxy terminal domain (De Wied, 1997; René *et al.,* 2000; Holmes *et al.,* 2003; Caldwell *et al.,* 2008).

V1a and V1b receptors act via $G_{q/11}$ coupling, which results in the stimulation of phospholipase C, which in turn generates the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG) (Howl *et al.,* 1995; Landgraf *et al.,* 1995; Lolait *et al.,* 1995; Alescio-lautier *et al.,* 2000; Holmes *et al.,* 2003; Caldwell *et al.,* 2008). V1a receptor activity is involved predominantly within the cardiovascular systems, but also plays a role in modulating endocrine systems and carbohydrate metabolism (Howl *et al.,* 1995; Lolait *et al.,* 1995; Alescio-lautier *et al.,* 2000; René *et al.,* 2000; Caldwell *et al.,* 2008).

V2 receptors stimulate adenylate cyclase through coupling with Gs proteins, thereby increasing cyclic adenosine monophosphate (cAMP), which connect to various cellular mechanisms, including ion channels, transcription factors and metabolic enzymes (Lolait *et al.,* 1995; Alescio-lautier *et al.,* 2000; René *et al.,* 2000; Holmes et al; 2003; Caldwell *et al.,* 2008). V2 activity plays an important role in the regulation of plasma osmolality and volume, working in the collecting ducts of the kidney to promote water reabsorption (Hirasawa *et al.,* 1994; Ishunina *et al.,* 1999; Dyatkin *et al.,* 2002).

1.4.3 Peripheral effects of oxytocin

Oxytocin that is released as part of the hypothalamo-neurohypophyseal system, functions primarily in the peripheral reproductive tissues, and plays a role in the processes of lactation,

parturition and sexual behaviour, (Ishunina *et al.,* 1999; Ivell *et al.,* 1999; Gimpl *et al.,* 2001; Holmes *et al.,* 2003; Heinrichs *et al.,* 2004; Ross *et al.,* 2009).

Oxytocin exerts its effects via interactions with its receptor, which, like vasopressin, belongs to the G-protein coupled receptor family (René *et al.,* 2000; Gimpl *et al.,* 2001; Marazziti *et al.,* 2006). It acts in the same manner as vasopressin V1 receptors, i.e. coupling via Gq/11 class of proteins and activating phospholipase C to result in the formation of IP_3 and DAG (Gimpl *et al.,* 2001).

1.4.4 Central distribution of vasopressin and oxytocin receptors and fibres

In addition to the magnocellular projections making up the hypothalamo-neurohypophyseal pathway, oxytocin and vasopressin are released centrally into the brain by parvocellular neuron projections, to the median eminence as part of the hypothalamo-pituitary adrenal system as well as to other discrete brain areas (Engelmann *et al.,* 1997; Kovács *et al.,* 1998; Ishunina *et al.,* 1999; Alescio-lautier *et al.,* 2000). This indicates both peripheral and central functions for these neuropeptides (Kovács *et al.,* 1998; Ishunina *et al.,* 1999; Alescio-lautier *et al.,* 2000; Klimkiewicz, 2001).

Due to the wide distribution of oxytocin and vasopressin within the brain, they have been classified as serving both an endocrine and a neurotransmitter or neuromodulator function (Howl *et al.,* 1995; Lolait *et al.,* 1995; Engelmann *et al.,* 1996; Boccia *et al.,* 1998; Kovács *et al.,* 1998; Ishunina *et al.,* 1999; Heinrichs *et al.,* 2004; Pittman *et al.,* 2005). Unlike classical neurotransmitters that can only be released at the synaptic cleft, oxytocin and vasopressin can be released from the axon terminals as well as the dendrites and soma of hypothalamic neurons (Engelmann *et al.,* 1997; Ross *et al.,* 2009). They can therefore diffuse through to the extracellular space and achieve widespread distribution due to their long half–life (Landraf *et al.,* 2004; Ross *et al.,* 2009). The central effects of vasopressin and oxytocin may include various autonomic, endocrine and behavioural effects, and may be mediated by fragments and derivatives of the neuropeptide as well as the whole, as opposed to peripheral effects, which require the entire molecule (Van Heuven-Nolsen *et al.,* 1984; Engelmann *et al.,* 1996; Pittman *et al.,* 2005).

Extrahypothalamic vasopressin is seen in the highest concentrations within the supraoptic and the suprachiasmatic nuclei, with substantial levels also detected in the septum and the locus coeruleus (Caldwell *et al.,* 2008; Koob, 2008). Vasopressinergic fibres have also been localized in various cerebral regions, from the olfactory bulb to the spinal cord, the septal region, the bed nucleus of the stria terminalis and the medial amygdaloid nucleus (Alescio-lautier *et al.,* 2000; Caldwell *et al.,* 2008; Koob, 2008). Vasopressin V1 receptor binding sites generally correspond with vasopressinergic projection sites, but are also highly expressed in the extended amygdala of the rat, with high concentrations in the lateral and supracapsular bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala and the shell of the nucleus accumbens (Koob, 2008). More specifically, V1b receptors are highly expressed in areas such as the hypothalamus, hippocampus and cerebral cortex (Egashira *et al.,* 2005). The V1b receptor subtype is also in the anterior pituitary gland, where it mediates the role of extrahypothalamic vasopressin in the potentiation of corticotrophin releasing hormone, and the subsequent stimulation of the release of adrenocorticotrophin hormone (Lolait *et al.,* 1995; Alescio-lautier *et al.,* 2000; René *et al.,* 2000; Caldwell *et al.,* 2008).

Within the brain vasopressin carries out numerous functions such as thermoregulation, regulation of blood pressure, brain development and circadian rhythm, cardiovascular homeostasis, regulation of aggression, certain aspects of pair bonding, social recognition, behavioural regulation, but in particular it is involved in learning and memory processes (Walter *et al.,*1978; Kovács *et al.,*1979; Biegon *et al.,*1984; Van Heuven-Nolsen *et al.,*1984; Lolait *et al.,*1995; Engelmann *et al.,*1996; Boccia *et al.,*1998; Ishunina *et al.,*1999; Alescio-lautier *et al.,*2000; Pittman *et al.,*2005; Caldwell *et al.,*2008).

Vasopressin has been shown to exert a long-term effect on the maintenance of learnt responses by facilitating consolidation, storage and retrieval of memory (Kovács *et al.,* 1979; De Wied, 1997; Alescio-lautier *et al.,* 2000; Heinrichs *et al.,* 2004). This long-term facilitatory effect of vasopressin on memory was elucidated in tests of active and passive avoidance, with an increase in latency time observed upon both intraventricular and subcutaneous administration of vasopressin (Van Ree *et al.,* 1985; De Wied *et al.,* 1991; De Wied *et al.,* 1997; Boccia *et al.,* 1998; Engelmann *et al.,* 1998; Klimkiewicz, 2001).

The brain structures involved in the effects of vasopressin on learnt behaviours are the areas innervated by extrahypothalamic vasopressinergic pathways, specifically those within the limbic structures such as the septum, hippocampus and the amygdala, with V1a receptors found in the hippocampus (Biegon *et al.,* 1984; De Wied, 1997; Alescio-lautier *et al.,* 2000). The hippocampus and its related temporal-lobe areas play a pivotal role in memory formation and is one of the primary central targets for the memory enhancing effects of vasopressin, whether administered subcutaneously or intracerebroventricularly (Alescio-lautier *et al.,* 2000; Klimkiewicz, 2001).

This effect within the limbic system may have a correlation with the enhancing effect that vasopressin has on catecholamine turnover in the hippocampal dendate gyrus, the dorsolateral septum and the nucleus of the tractus solitarius (Kovács *et al.,* 1979; Biegon *et al.,* 1984; Van Heuven-Nolsen *et al.,* 1984; Landgraf *et al.,* 1995). It is suggested that vasopressin acts more specifically on the utilization of noradrenaline in terminal areas of the coeruleotelencephalic noradrenaline system (Kovács *et al.,* 1979; Biegon *et al.,* 1984; Van Heuven-Nolsen *et al.,* 1984).

The effect of vasopressin on memory may also be explained by its excitation of hippocampal and septal neurons, where vasopressin enhances the response of neurons to glutamate. This action of the hormone is proposed to serve as a neuromodulator for excitation in the limbic midbrain (De Wied, 1997). An increase in the slope and amplitude of field EPSPs in the dendate gyrus was seen in the presence of calcium, and this was induced by nanomolar concentrations of vasopressin, indicating a potential role of vasopressin in long-term potentiation (De Wied, 1997).

Oxytocin fibres and nerve endings have been identified in numerous parts of the brain including the dorsomedial hypothalamic nucleus, thalamic nuclei, dorsal and ventral hippocampus, subiculum, entorhinal cortex, medial and lateral septal nuclei, amygdala, olfactory bulbs, mesencephalic central grey nucleus, substantia nigra, locus coeruleus, raphe nucleus and nucleus of the tractus solitarius (Gimpl *et al.,* 2001).

It is apparent from the wide distribution of oxytocin fibres in the brain that it has many central functions, including thermoregulation, social interaction, analgesia, gastric motility,

osmoregulation, inhibition of the stress response, with the primary functions being social attachment and the facilitation of the maternal behaviour required for the survival of offspring (Ishunina *et al.,* 1999; Gimpl *et al.,* 2001; Egashira *et al.,* 2005; Heinrichs *et al.,* 2004; Ross *et al.,* 2009). Rodent studies have shown that oxytocin is important in facilitating the onset of maternal behaviour, however is not required for its maintenance as administration of an oxytocin antagonist did not inhibit maternal behaviour that has already been established (Gimpl *et al.,*2001; Ross *et al.,* 2009).

In learning and memory, the general understanding is that oxytocin has an effect opposite to that of vasopressin, in that it attenuates the consolidation and retrieval of memory and having an overall amnesic effect (Kovács *et al.,* 1979; De Wied, 1997; Ishunina *et al.,* 1999; Heinrichs *et al.,* 2004). The sites of action for these learning and memory effects are within the limbic areas of the brain, the amygdala, septum, some thalamic areas and the hippocampus, where oxytocin receptors have been identified (De Wied, 1997; Heinrichs *et al.,* 2004).

It has been suggested that the general theory with regards to vasopressin and oxytocin in learning and memory is subject to change dependent on specific behavioural tests, the specific administration or endogenous stimulation of the neuropeptide as well as the specific brain areas involved (Kovács *et al.,* 1979; Sahgal *et al.,* 1984; Van Ree *et al.,* 1985; Engelmann *et al.,* 1996; De Wied, 1997; Boccia *et al.,* 1998; Heinrichs *et al.,* 2004).

In summary, vasopressin and oxytocin are neurohormones synthesized within magnocellular neurons, the cell bodies of which are found in the hypothalamus. They exert peripheral effects, and have a wide distribution within the brain, leading them to be classified as both endocrine hormones and neurotransmitters. Both vasopressin and oxytocin have been shown to play a role in learning and memory processes, with vasopressin having a facilitatory effect and oxytocin having an inhibitory effect. These roles in learning and memory have prompted interest in their possible roles in the addictive state, which has been linked to neuroadaptations linked to learning and memory.

1.5 Aim of the present study

Maladaptive processes in learning and memory have been implicated as a possible mechanism behind the development of addiction (Kelly, 2004; Saal and Malenka, 2005). To this end we developed an interest into a possible role for vasopressin and oxytocin in the drug addiction process. As such the present study wished to address the following questions:

1. Does vasopressin antagonism block the development of drug seeking behaviour during addiction?

2. Does oxytocin administration block the development of drug seeking behaviour during addiction?

3. If yes, what are the mechanisms by which both these hormones would exert their effects?

Our experimental approach to answer these questions briefly entailed:

1. The establishment of a rat model displaying properties of drug addiction. Here we treated male Sprague-Dawey rats with methamphetamine and assessed their addictive behaviour using a place preference paradigm.

2. The role of vaspressin antagonism was determined by treating "addicted" rats during a drug seeking period with vasopressin V1b antagonist, SSR 149415.

3. In parallel but independent experiments "addicted" rats were similarly treated with oxytocin.

4. Dopamine levels in the striata and the expression of CREB in the hippocami of the same animals were subsequently measured in our investigation into possible molecular mechanisms by which our treatment strategies could mediate their effects.

Chapter 2

Cassandra O. Subiah, Musa V. Mabandla, Alisa Phulukdaree, Anil A. Chuturgoon and Willie M. U. Daniels (2012) The effects of vasopressin and oxytocin on methamphetamine–induced place preference behaviour in rats. Metabolic Brain Disease doi: 10.1007/s11011-012-9297-7 Published online 25 March 2012

Abstract

Methamphetamine is a highly addictive stimulant drug whose illicit use and resultant addiction has become an alarming global phenomenon. The mesolimbic dopaminergic pathway has been shown to be fundamental to the establishment of addictive behaviour. This pathway, as part of the reward system of the brain, has also been shown to be important in classical conditioning, which is a learnt response. Within the modulation of learning and memory, the neurohypophyseal hormones vasopressin and oxytocin have been reported to play a vital role, with vasopressin exerting a long- term facilitatory effect and oxytocin exerting an inhibitory effect. Therefore we adopted a conditioned place preference model to investigate whether vasopressin V1b receptor antagonist SSR 149415 or oxytocin treatment would cause a decrease in the seeking behaviour in a reinstatement paradigm. Behavioural findings indicated that methamphetamine induced a change in the place preference in the majority of our animals. This change in place preference was not seen when vasopressin was administered during the extinction phase. On the other hand the methamphetamine-induced change in place preference was enhanced during the reinstatement phase in the animals that were treated with oxytocin. Striatal dopamine levels were determined, as methamphetamine is known to increase dopamine transmission in this area. Significant changes in dopamine levels were observed in some of our animals. Rats that received both methamphetamine and oxytocin had significantly higher striatal dopamine than those that received oxytocin alone. Western blot analysis for hippocampal cyclic AMP response element binding protein (CREB) was also conducted as a possible indicator of glutamatergic NMDA receptor activity, a pathway that is important for learning and memory. The Western blot analysis showed no changes in hippocampal pCREB expression. Overall our data led us to conclude that methamphetamine treatment can change place preference behaviour in rats and that this change may be partially restored by vasopressin antagonism, but exaggerated by oxytocin.

Introduction

Drug addiction remains one of the biggest health and social problems worldwide (Buxton and Dove, 2008). South Africa is not excluded from this statistic with continuing escalation in the use of illicit drugs especially between the ages of 15 – 25 years being reported by the South African Community Epidemiology Network on Drug Use (SACENDU Report June 2011). Addiction is said to be a chronically relapsing disorder that is characterized by a compulsion to take a drug, a lack of control in limiting this intake and the withdrawal of the drug resulting in the development of a negative emotional state associated with severe signs of discomfort (Koob, 2000; Shippenberg et al. 2007; Koob and Volkow, 2010).

Once addiction is established, it tends to follow a chronic course consisting of periods of abstinence that are usually followed by relapse (Hyman and Malenka, 2001). Repeated exposure to drugs of abuse may also produce tolerance in users, whereby molecular adaptations result in a diminished effect of the drug despite a constant dose, requiring the user to increase their dose to achieve the same effect (Roberts and Koob, 1997; Hyman and Malenka, 2001). This increase in dosage may lead to the exacerbation of the molecular changes that lead to addiction (Hyman and Malenka, 2001).

It is now commonly accepted that the mesocorticolimbic pathway plays a critical role in diverse motivational processes including drug reward and addiction-like behaviour (Dobbs et al. 2008; Herrold et al. 2008). Each of the structures within this pathway in particular the ventral tegmental area, the nucleus accumbens and the ventral pallidum has all been shown to be critically involved in psychostimulant (such as methamphetamine) drug-induced behaviour in rats (Herrold et al. 2008). The cortical and limbic areas of the brain, which subsequently receive projections from these areas, are important for the expression of emotions, reactivity to conditioned cues, planning and judgement, and have therefore been implicated in reward (Tomkins and Sellers 2001).

In general addictive behaviour is characterised by drug-induced dopamine release leading to compulsive drug use followed by a period of drug absence leading to the development of drug seeking behaviour. It has been postulated that cellular changes at the level of the synapse may underlie the behavioural changes associated with addiction (Hyman, 2005). Long term potentiation and depression are two phenomena that have been associated with synaptic plasticity (Calabresi et al. 2007; Kauer et al. 2007) and may therefore be suitable

candidates to mediate such synaptic alterations. LTP and learning within the hippocampus, via glutamate receptor activation, results in the downstream activation of cyclic adenosine monophosphate (cAMP) and cAMP response element-binding protein (CREB) mediated transcription, a pathway whose upregulation is one of the best established adaptations to chronic exposure to drugs of abuse (Nestler, 2001; Kopnisky and Hyman, 2002; Chao et al. 2004). CREB acts as an important transcription factor and regulates gene expression in learning and memory circuits via the cAMP pathway (Nestler, 2001; Mizuno et al. 2002; Fanselow et al. 2005; Hyman, 2006).

Since LTP and CREB activation are important in learning and memory, and have been shown to be activated in response to drugs of abuse, it has been postulated that addictive behaviour may have elements of learning and memory underlying its development (Nestler, 2002; Kelley, 2004).

Within the paradigm of learning and memory, much interest has been shown in the involvement of neurohypophyseal hormones vasopressin and oxytocin (Engelmann et al. 1996). The general theory with regards to learning and memory is that vasopressin has a facilitatory and oxytocin an inhibitory effect on learning and memory. However this theory remains controversial and is subject to change dependent on specific behaviours as well as the specific brain areas being studied (Kovács et al. 1979; Sahgal et al. 1984; Van Ree et al. 1985; Engelmann et al. 1996; de Wied, 1997; Boccia et al. 1998; Heinrichs et al. 2004). For instance the long term facilitatory effects of vasopressin on memory was elucidated in tests of active and passive avoidance, with an increase in latency time observed upon both intraventricular and subcutaneous administration of vasopressin (Van Ree et al. 1985; de Wied et al. 1991; de Wied et al. 1997; Engelmann et al. 1998; Klimkiewicz, 2001). Notably this effect was not seen in other memory tests such as water maze and hole-board tests (Klimkiewicz, 2001). Other studies have shown that oxytocin may reduce the abuse potential of many drugs, with administration of oxytocin resulting in the attenuation of tolerance development to morphine and heroin as well as decreasing the intravenous self-administration of heroin in rats (Kovács et al. 1987; Gimpl et al. 2001; Marazziti et al. 2006; Qi et al. 2009; Carson et al. 2010).

The aim of the present study was therefore to investigate whether the administration of oxytocin and the vasopressin antagonist SSR 149415, could reverse methamphetamine-

induced addictive behaviour in a conditioned place preference rat model. In the study dopamine levels in the striatum were also determined as a molecular indicator of the effect of methamphetamine administration. In addition the levels of cyclic AMP response element binding protein (CREB) in the hippocampus were assessed as an indirect indicator of the involvement of glutamatergic receptors in learning and memory processes associated with addictive behaviour.

Materials and methods

Animals

Forty-eight male Sprague-Dawley rats ranging in weight from 200-250g were used. Animals were housed in the Biomedical Resource Centre at the University of KwaZulu-Natal, Westville campus under standard laboratory conditions of 23±2°C room temperature, 70% humidity and a 12hour day-night cycle with lights-on at 06h00. Food and water was made available *ad libitum*. Chemicals used in our protocol were SSR 149 415 (a gift from Sanofi-Aventis), (+)-methamphetamine hydrochloride and oxytocin lyophilized powder, ~50 IU/mg solid, both of which were purchased from Sigma Aldrich, USA.

Animals were randomly divided into 6 groups (n=8) and received the following treatment; Group 1 – saline; Group 2 – saline followed by SSR 149415 (the vasopressin V1b antagonist); Group 3 - saline followed by oxytocin; Group 4 – methamphetamine followed by saline; Group 5 - methamphetamine followed by SSR 149415; Group 6 - Methamphetamine followed by oxytocin. The Animal Ethics Subcommittee of the University of KwaZulu-Natal approved all procedures, which were all in accordance to the guidelines of the National Institutes of Health (Ethics clearance number 044/08/Animal).

Apparatus

A place preference box was used to condition the rats to methamphetamine. The box had dimensions of 45x45x45cm and was divided into two equally sized compartments by a sliding partition. Each compartment had distinct features. For instance the walls of one compartment had a black and white checked appearance while the walls of the other compartment remained plain black. Also, a wire mesh covered the floor of the checked compartment, while the other compartment floor remained smooth. The behaviour of the

animal in the two-compartment box was recorded and the time spent in each compartment was assessed at a later stage.

Place Preference Procedure

The place conditioning procedure used in this experiment included the (i) acquisition of Conditioned Place Preference -CPP- (consisting of three phases namely pre-exposure, conditioning phase and CPP test), (ii) extinction training and (iii) reinstatement (Mueller and Stewart, 2000; Zhou and Zhu, 2006; Qi et al. 2009, see Figure 1 for experimental outline).

Acquisition

Phase 1 - Each test animal received three pre-exposure tests before the experiment, in which they were allowed access to the entire apparatus for 15 min. The amount of time spent in each chamber was monitored and an average time taken from the three tests to determine the naturally preferred chamber for the animal.

Phase 2 - On days 1, 3, 5, and 7, the rats in groups 2, 3 and 5 were given a dose of methamphetamine (2.5mg/kg, i.p.), and then placed into the less preferred chamber for 50 min. Our dose was established from a dose dependent study conducted by DeMarco et al. (2009) where doses of 1, 2.5, 5 and 10mg/kg did not produce a dose-dependent change in place preference. As higher doses did not result in greater place preference changes, we decided to use the moderate dose of 2.5mg/kg. In groups 1, 4 and 6 saline was injected on these days. On days 2, 4, 6, and 8, rats received an equivalent volume of 0.9% saline (1ml, i.p.) before being confined to the preferred compartment, for 50 min. This strategy was adopted to ensure that the association with the non-preferred chamber was indeed driven by methamphetamine.

Phase 3 - On day 9, the sliding partition separating the two chambers was removed and the rats allowed free access to the entire apparatus for 15 min without any restriction. The amount of time spent in each chamber was recorded.

Extinction

After conditioning and the initial CPP test, from days 10 to 17, saline was alternate paired four times with each of the chambers, once per day, over 8 days.

The extinction phase of this model therefore allows for exposure to the place preference box during a period of abstinence. During this time of 'sobriety', we administered our treatments to determine whether established seeking behaviour could be attenuated, and relapse prevented.

In groups 3 and 5 oxytocin (1mg/kg, s.c.) and SSR149415 (1mg/kg, s.c.) respectively was administered instead of saline during this extinction phase. The doses of oxytocin and SSR 149 415 were established from studies by Cui et al (2001) and Feifel et al (2011), where subcutaneous dose of 1mg/kg oxytocin was seen to exert central effects. Most studies using SSR 149 415 administered doses i.p. so we used the same dose as for oxytocin. On day 18, a second CPP test was performed.

Reinstatement

On the next day following the second CPP test (day19), the test animals were given a priming injection of methamphetamine (1mg/kg, i.p.) and 10 min later, the rats were allowed free access to the entire apparatus for 15 min. The priming dose used is in accordance with priming i.p. doses from previous studies (Rogers et al. 2008; Holtz et al. 2012). The amount of time spent in each chamber was once again recorded. Our protocol is a modified version of Zhou and Zhu (2006), where there was a 10 min interval after morphine injection before CPP testing. Research using methamphetamine tested for CPP immediately after priming injection; however the methamphetamine was administered i.c.v. (Carson et al, 2010). We therefore took into account that i.p. administration results in methamphetamine taking longer to reach the brain and exert its effects, and allowed for a 10 min interval so that maximal effect would occur within our 15 minute test period.

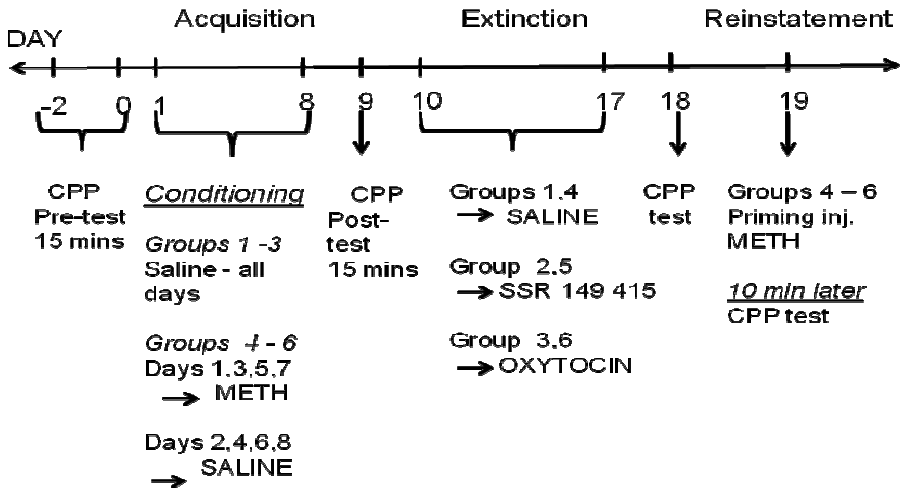


Figure 1: Outline of the 3 phase conditioned place preference protocol

Tissue Collection

On day 20, animals were sacrificed by decapitation, and their striatal and hippocampal tissue were harvested,snap frozen in liquid nitrogen, then stored in a biofreezer at -75°C. The tissue was later used for the analysis of dopamine and phosphorylated CREB (pCREB) levels respectively.

Dopamine assay

A commercial Dopamine ELISA kit (IBL International GMBH, Hamburg, Germany) was used to quantify dopamine concentration. Striatal tissue was thawed on ice and then 500µl of EDTA (1M)-HCl (0.1N) buffer was added. Samples were sonicated while on ice, for thirty seconds, using the Misonix Sonicator model XL2000-010 (USA). Sonicated samples then underwent centrifugation at 13500 rpm for fifteen minutes at 4ºC. The dopamine content of the supernatants was then determined by ELISA following the manufacturer's protocol.

Western blot

Total hippocampus protein was extracted from each sample using Cytobuster™ (Calbiochem, UK) reagent, supplemented with protease inhibitors, as per manufacturer guidelines. Protein concentration was determined by the bicinchoninic acid assay (Sigma, Germany) and standardized to 1 mg/ml. Samples containing 40 µg of protein were boiled in Laemmli buffer for 5 minutes and then subjected to electrophoresis in 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Separated proteins were then electro-transfered to polyvinylidene

difluoride membranes (PVDF). After blocking with Tris-buffered saline (TBS) containing 5% bovine serum albumin and 0.1% Tween 20, the membrane was immuno-probed with rabbit anti-p-CREB Ser133 (1: 500; Cell Signalling Technology, USA) overnight at 4°C. The PVDF membrane was then subjected to 5 washes (10 minutes each) with TBS containing 0.1% Tween 20. The membrane was then exposed to secondary antibody (anti-rabbit-horse-radish-peroxidase (HRP)-conjugate; 1:10, 000; Bio-Rad, USA) for 1 hour at RT. Anti-β-actin-HRP (Sigma, Germany) was used for internal loading controls. After further washing, antigen-antibody complexes were detected by chemiluminescence using the Immune-star™ HRP substrate kit (Bio-Rad, USA). Chemiluminescent signals were detected with the UViTech Alliance 2.7 gel documentation system. Images were acquired and analyzed with UViTech Analysis™ image analysis software (UViTech, USA). Following densitometric analysis on sample bands and corresponding loading standards (B-Actin), mean relative band density was divided by mean relative band density of B-actin to obtain a normalised relative stimulation/inhibition of treated samples.

Statistical Analysis

Data was analysed using GraphPad Prism (version 5, San Diego, California, USA). All the data was tested for normality using the Kolmogorov-Smirnov test and some found to be not normally distributed. Subsequently all data was analysed using the non-parametric tests. For the behavioural data Friedman' test (similar to parametric repeated measures ANOVA) was used to determine the presence of any significant differences between the test times. This was followed by the Dunn's multiple comparison test. In the case of the dopamine levels and CREB expression levels the Kruskal-Wallis test was used to assess the significance of differences between groups. For the dopamine levels, this was then followed by Mann-Whitney U test. Differences were considered significant when p values were < 0.05. In graphs the data is represented as mean ± SEM with medians reported in the figure legends.

Results

Behavioural tests

Behavioural data is represented as a measure of time spent in the preferred side of the place preference box. Place preference behaviour in the control group administered with saline showed no significant difference in time spent in preferred side throughout the 3 phases

(Figure 2a). Similar results were seen in the groups receiving oxytocin and the vasopressin antagonist in the extinction phase (Figure 2b and 2c).

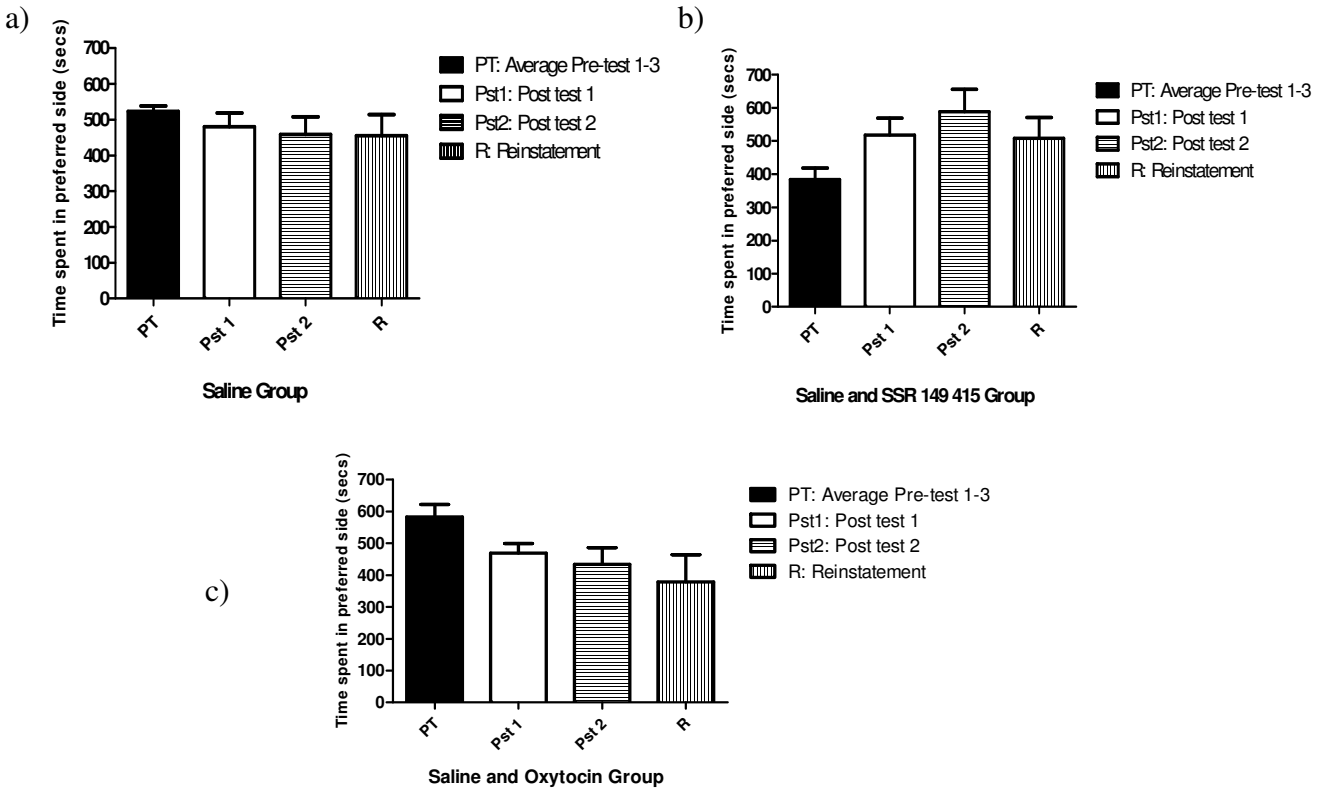


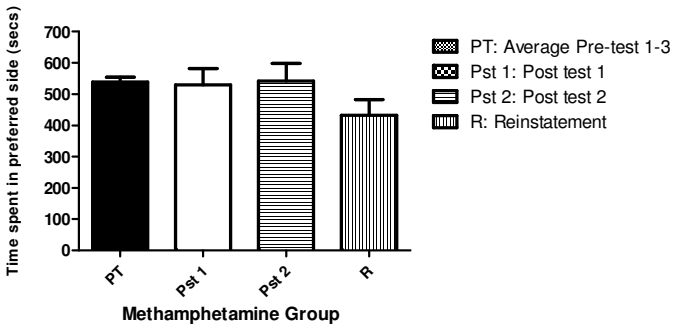
Figure 2: Conditioned place preference behaviour in groups receiving saline in the acquisition phase and (a) saline, (b) the vasopressin antagonist SSR149415 or (c) oxytocin during the extinction phase. All animals received saline during the reinstatement phase. PT – pre test; Pst1 – post test 1; Pst 2 – post test 2; R - reinstatement

Graph is presented as mean ± SEM (n=8) and was analysed using Friedman's test. Respective medians are: Saline (PT – 515.8; Pst1 – 510.0; Pst2 – 501.5; R – 463.5) SSR 149415 (PT - 376.7; Pst1 – 548.5; Pst2 – 563.0; R – 528.5) Oxytocin (PT – 588.2; Pst1 – 467.0; Pst2 – 420.5; R – 266.5)

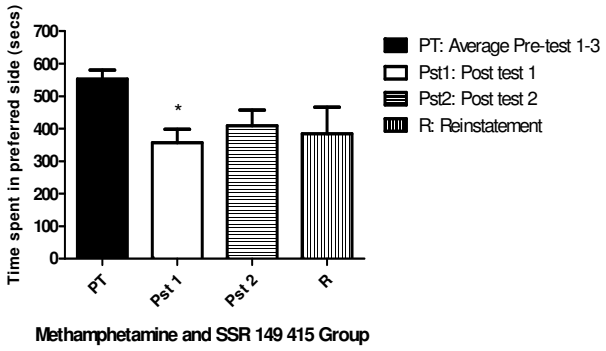
Animals treated with methamphetamine and saline showed no significant differences in their pre-test, post-test and reinstatement times (Figure 3a). However in a separate experiment methamphetamine independently caused a significant reduction in time spent in the preferred side, i.e. a comparison between pre-test and post-test 1 (Figure 3b; $p < 0.05$) and this difference was partially blocked by subsequent treatment with SSR 149 415 during the extinction phase. These animals also did not show a change in their place preference during the reinstatement phase (Figure 3b). In a third experiment methamphetamine once again

significantly reduced the time spent by the animals in the preferred side (Figure 3c; $p < 0.05$). This significant reduction was not evident when animals were treated with oxytocin during the extinction phase, but was re-established during the reinstatement phase (Figure 3c).

a)



b)



c)

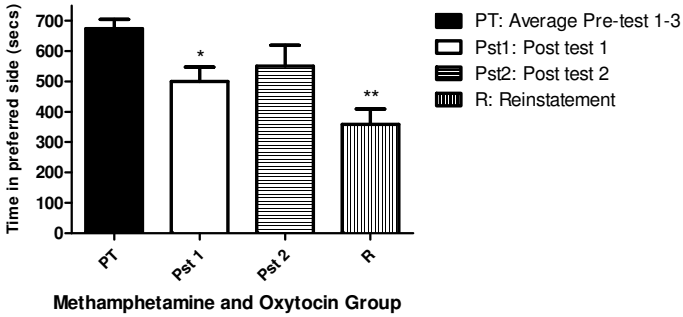


Figure 3: Conditioned place preference behaviour in groups receiving methamphetamine in the acquisition phase and (a) saline, (b) the vasopressin antagonist SSR149415 or (c) oxytocin during the extinction phase. All animals received methamphetamine during the reinstatement phase. PT – pre test; Pst1 – post test 1; Pst 2 – post test 2; R - reinstatement

Graph is presented as mean ± SEM (n=8) and was analysed using Friedman's test followed by Dunn's multiple comparison test with $p < 0.05$ considered significant. Respective medians are: Saline (PT – 540.8; Pst1 – 530.5; Pst2 – 551.5; R – 407.5) SSR 149415 (PT – 551.7; Pst1 – 327.0; Pst2 – 356.5; R – 344.0) Oxytocin (PT – 674.2; Pst1 – 497.0; Pst2 – 629.0; R – 312.5)

Figure 3a: no significance

Figure 3b: *PT significantly different from Pst1

Figure 3c: *PT significantly different from Pst1; ** PT significantly different from R

Striatal dopamine levels

Five animals from the non-drug treated groups were randomly selected for ELISA analysis, while the five animals that best displayed seeking behaviour were chosen from the drug treated groups. Significant differences in striatal dopamine levels were observed between some of the oxytocin and methamphetamine treated groups (Figure 4). Saline treated animals that received either the vasopressin antagonist SSR 149415 or oxytocin showed no significant effect on striatal dopamine levels. However there was a significant difference in dopamine levels between the animals that received saline and oxytocin when compared to the methamphetamine-oxytocin treated group significant (Figure 4; $p<0.01$).

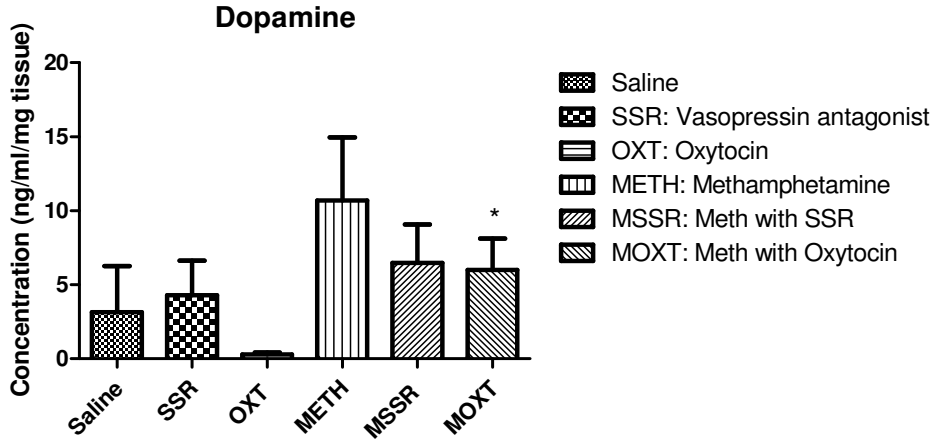


Figure 4: Striatal dopamine concentrations per mg of tissue. Data is presented as mean ± SEM (n=5) and was analysed using Kruskal-Wallis test followed by the Mann-Whitney U test. Respective medians are: Saline – 0.036; SSR – 1.747; OXT – 0.284; Meth – 6.809; MSSR – 4.366; MOXT – 4.537

*OXT significantly different from MOXT, p = 0.0079.

Saline – control group receiving saline

SSR – control group receiving SSR 149 415

OXT – control group receiving oxytocin

METH – methamphetamine treated group receiving saline in extinction phase

MSSR – methamphetamine treated group receiving SSR 149 415 in extinction phase MOXT – methamphetamine treated group receiving oxytocin in extinction phase

Hippocampal pCREB levels

Western blot analysis of pCREB levels in the hippocampus revealed no significant differences in band intensity between all groups.

Discussion

Addiction is a major problem in the world today, impacting people on a physical, mental and in a social manner (Leshner, 1997). While there are many theories on the development of addiction, there exists a great amount of evidence linking addiction to the process of learning and memory (Hyman, 2005). To that end, we were interested in investigating the role of two neurohormones involved in learning and memory, vasopressin and oxytocin, within the addictive process. Our drug of interest was methamphetamine, a psychostimulant drug widely abused globally, and showing increased abuse here in South Africa in recent years (Salo et al. 2008; Plüddemann et al. 2010). We adopted a place preference model of addiction consisting of 3 phases; acquisition to model the initial drug taking experience and subsequent addiction; extinction, to model a period of sobriety; and reinstatement, to model relapse in human addicts.

Behavioural findings in our three control groups receiving saline in the acquisition phase showed no place preference changes in this phase, indicating the non-addictive properties of our vehicle. Similarly the administration of the oxytocin and the vasopressin antagonist SSR149415 during the extinction phase also did not result in significant changes in place preference behaviour, indicating that administration of oxytocin and the vasopressin antagonism had no effect on the determination of preference in non-addicted animals.

Behavioural findings of the drug-treated groups showed mixed positive place preference behaviour. In the first set of experiments we were unable to show methamphetamine-induced changes in place preference behaviour. This was mainly due to the variability in drug-responses with 3 out of the 8 animals showing a marked preference change post drug

treatment and 1 animal spending nearly equal time in each compartment. These variances in behaviour resulted in non-significant differences and masked any indication of addictive tendencies in this group. However the data from this experiment did indicate that animal handling or the injection itself did not determine or influence behaviour. Animals in subsequent experiments consistently showed significant decreases in time spent in preferred compartments in post-test 1 as compared to the initial preference in the pre-tests. The variation in the animal responses to methamphetamine observed in the initial experiments is therefore difficult to explain.

Those animals that exhibited a change in place preference to the drug-paired compartment indicated addictive behaviour and seeking tendencies. This finding is in agreement with that of others (Herrold et al. 2008). In the methamphetamine group treated with the vasopressin antagonist during the extinction phase, preference in post-test 2 showed no significant difference when compared to the initial preference of the animals. This was also seen in the reinstatement phase, where no significant decrease in place preference behaviour was noted in response to the priming dose of methamphetamine. This may indicate that administering a vasopressin antagonist may partially alter the seeking behaviour of the animals, as well as prevent reinstatement of drug seeking when given a priming dose of the drug. This is in accordance with a study by Zhou et al. (2008), where SSR149415 was used during the extinction phase of heroin addiction, and showed that V1b antagonism significantly reduced the reinstatement of drug taking in animals.

Administering oxytocin during the extinction phase also attenuated the methamphetamine-induced place preference change in animals. This suggested that oxytocin may have the potential to reverse drug-seeking behaviour, as was shown in a comparable study by Qi et al. (2009). Their findings indicated that intracerebrovascular administration of oxytocin facilitated the extinction of methamphetamine-induced place preference, and this was linked to its role as an amnesic neuropeptide. Interestingly our behavioural findings at reinstatement showed a significant decrease in time spent in preferred side, when compared to the pre-test times. This significant increase in time spent in the drug-paired compartment show that oxytocin was not effective in inhibiting the reinforcing effects of the priming dose of

methamphetamine. However, this result is in accordance with previous findings (Qi et al. 2009), where the priming injection of methamphetamine reinstated the extinguished place preference behaviour.

Dopamine levels in striatal tissue were evaluated between the various treatment groups. A significant difference in dopamine concentration was seen between the two oxytocin-treated groups that were pre-treated with saline and methamphetamine respectively. An increase in striatal dopamine content agrees with previous reports on the neurochemical effects of methamphetamine (Larsen et al. 2002; Pierce et al. 2006). Our data appears to be in line with findings suggesting a role for dopamine in addictive behaviour. The vasopressin antagonist SSR149415 did not alter dopamine levels significantly, indicating that vasopressin antagonism possibly does not act via dopamine in its partial blocking of methamphetamine-induced addictive processes. A possible mechanism by which SSR149415 could have exerted its effects may involve suppression of the hypothalamo-pituitary-adrenal (HPA) axis. Vasopressin 1b receptors have been identified within the anterior pituitary gland, where it works synergistically with corticotropin-releasing hormone (CRH) to stimulate the release of adrenocorticotropic hormone (Tanoue et al. 2004; Dempster et al. 2007). CRH has been shown to play a role in cue- and methamphetamine- induced reinstatement (Moffett et al. 2007). It is therefore possible that the antagonistic effect of SSR149415 on vasopressin V1b receptors may decrease the activity of CRH and the HPA axis, thereby decreasing methamphetamine-induced relapse behaviour.

Following our behaviour results, where oxytocin increased place preference behaviour at reinstatement, we expected oxytocin to increase dopamine levels in the striatum. Although it has long been accepted that oxytocin cannot cross the blood brain barrier, it has been hypothesized that when administered subcutaneously, neuropeptides may pass in small amounts through the blood brain barrier and exert some central effects (Kovács et al. 1998; Cui et al. 2001). However, our observations show that oxytocin did not affect dopamine levels, with no significant difference when compared to the saline control group. Not surprisingly, oxytocin did not have an effect in decreasing dopamine levels in the methamphetamine treated group.

Studies investigating the interaction between oxytocin and dopamine in relation to addiction have been limited, since the majority of experiments focused mainly on reproductive physiology (Cle´ment et al. 2008; Frye and Walf, 2010; Hedges et al. 2010) and maternal/social behaviour (Shahrokh et al. 2010; Robinson et al. 2011; Lenz and Sengelaub, 2010). However there are reports implicating oxytocin in the reinforcing and long-term adverse effects of drug use (McGregor et al. 2008). Despite of the numerous studies implicating the involvement of oxytocin in dopamine-mediated addictive behaviour, its exact role and mechanism of action remains unclear. However a possible mechanism by which oxytocin increases the reinstatement of methamphetamine could be via its role in nitric oxide production. Oxytocin promotes the production of nitric oxide by protein kinase C- and calcium- induced activation of nitric oxide synthase (Zingg and Laporte, 2003). Nitric oxide is a highly diffusible neurotransmitter due to its gaseous nature and can readily act on a widespread area (Orsini et al. 2002), and has been implicated in cocaine-induce CPP as well as the development of methamphetamine reinstatement behaviour following priming injection (Li et al. 2002). The increase in nitric oxide levels due to oxytocin may result in the increase in reinstatement behaviours observed in our animals, although this remains for further investigation.

We also investigated the effects of methamphetamine, SSR149415 and oxytocin on CREB expression levels in the hippocampus. Glutamate activation of N-methyl-D-aspartate (NMDA) receptors in the hippocampus triggers long-term potentiation and memory formation (Sarantis et al. 2009). Glutamate, binding to NMDA receptors, results in the downstream activation of CREB by promoting its phosphorylation at Ser133 (West et al. 2002; Balazs, 2006). This activated CREB is known to play a critical role in the synaptic plasticity associated with learning and memory, as well as addiction (Walters et al. 2003; Balazs, 2006). To that end, we conducted Western blot analysis to determine if the CREB pathway was activated and phosphorylated CREB was present within the hippocampus. Results showed no significant differences in pCREB levels in all groups, indicating that this signalling pathway was not stimulated in our model.

In conclusion, we found some evidence that methamphetamine did induce seeking behaviour within a place preference model. Our findings suggest that vasopressin antagonism may play a role in decreasing reinstatement of methamphetamine behaviour, while oxytocin seems to exacerbate seeking tendencies and increase relapse behaviour. While we have observed that CREB activation in the hippocampus does not play a role in the effects of the vasopressin antagonist and oxytocin, neither do they alter dopamine levels in the striatum, and the exact mechanism by which they exerts their behavioural effects is unclear.

Acknowledgements

The authors wish to thank the staff of the Biomedical Resource Unit of the University of KwaZulu-Natal for technical assistance and the Medical Research Council and the National Research Foundation of South Africa for financial support. This manuscript is part of the Masters degree of one of the authors (C. Subiah).

References

Balazs R (2006) Trophic Effect of Glutamate. Current Topics in Medicinal Chemistry 6:961-968. doi:10.2174/156802606777323700

Boccia MM, Kopf SR, Baratti CM (1998) Effects of a Single Administration of Oxytocin or Vasopressin and Their Interactions with Two Selective Receptor Antagonists on Memory Storage in Mice. Neurobiology of Learning and Memory 69:136–146. doi: http://dx.doi.org/10.1006/nlme.1997.3817

Buxton JA and Dove NA (2008) The burden and management of crystal meth use. Canadian Medical Association Journal 178: 1537-1539. doi:10.1503/cmaj.071234

Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. TRENDS in Neurosciences 30:211-219. doi:10.1016/j.tins.2007.03.001

Carson DS, Cornish JL, Guastella AJ, Hunt GE, McGregor IS (2010) Oxytocin decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats. Neuropharmacology 58:38-43. doi:10.1016/j.neuropharm.2009.06.018

Chao J and Nestler EJ (2004) Molecular Neurobiology of Drug Addiction. Annual Review of Medicine 55:113–32. doi: 10.1146/Annurev.Med.55.091902.103730

Cle´ment P, Peeters M, Bernabe J, Denys P, Alexandre L, Giuliano F (2008) Brain oxytocin receptors mediate ejaculation elicited by 7-hydroxy-2-(di-N-propylamino) tetralin (7-OH-DPAT) in anaesthetized rats. British Journal of Pharmacology 154:1150–1159. doi:10.1038/bjp.2008.176

Cui S, Bowen RC, Gu G, Hannesson DK, Yu PH, Zhang X (2001) Prevention of Cannabinoid Withdrawal Syndrome by Lithium: Involvement of Oxytocinergic Neuronal Activation. The Journal of Neuroscience 21:9867–9876

de Wied D (1997) Neuropeptides in learning and memory processes. Behavioural brain research 83:83-90. doi: 10.1016/S0166-4328

de Wied D, Elands J, Kovács G (1991) Interactive effects of neurohypophyseal neuropeptides with receptor antagonists on passive avoidance behavior: Mediation by a cerebral neurohypophyseal hormone receptor? Proceedings of the National Academy of Sciences of the United States of America 88: 1494-1498. doi: 10.1073/pnas.88.4.1494

DeMarco A, Dalal RM, Pai J, Aquilina SD, Mullapudi U, Hammel C, Kothari SK, Kahanda M, Liebling CNB, Patel V, Schiffer WK, Brodie JD and Dewey SL (2009) Racemic Gamma Vinyl-GABA (R,S-GVG) Blocks Methamphetamine-Triggered Reinstatement of Conditioned Place Preference. Synapse 63:87–94. doi:10.1002/syn.20582

Dempster EL, Burcescu I, Wigg K, Kiss E, Baji I, Gadoros J, Tamás Z, Kennedy JL, Vetró A, Kovacs M, Barr CL (2007) Evidence of an Association Between the Vasopressin V1b Receptor Gene (AVPR1B) and Childhood-Onset Mood Disorders. Archives of General Psychiatry 64:1189-1195. doi: 10.1001/archpsyc.64.10.1189

Dobbs LK and Mark GP (2008) Comparison of systemic and local methamphetamine treatment on acetylcholine and dopamine levels in the ventral tegmental area in the mouse. Neuroscience 156: 700-711. doi:10.1016/j.neuroscience.2008.07.052

Engelmann M, Wotjak CT, Neumann I, Ludwig M, Landgraf R (1996) Behavioural Consequences of Intracerebral Vasopressin and Oxytocin: Focus on Learning and Memory.

Neuroscience and Biobehavioural Reviews 20:341-58. doi: http://dx.doi.org/10.1016/0149-7634(95)00059-3

Fanselow MS, Poulos AM. (2005) The neuroscience of mammalian associative learning. Annual Review of Psychology 56:207-34. doi: 10.1146/annurev.psych.56.091103.070213

Feifel D, Shilling PD, Belcher AM (2011) The effects of oxytocin and its analog, carbetocin, on genetic deficits in sensorimotor gating European Neuropsychopharmacology doi:10.1016/j.euroneuro.2011.09.004

Frye CA and Walf AA (2010) Oxytocin and/or steroid hormone binding globulin infused into the ventral tegmental area modulates progestogen-mediated lordosis. Neuropharmacology 58:44–49. doi: 10.1016/j.neuropharm.2009.07.006

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiological reviews 81:629-83

Hedges VL, Staffend NA, Meisel RL (2010) Neural mechanisms of reproduction in females as a predisposing factor for drug addiction. Frontiers in Neuroendocrinology 31:217–231. doi:10.1016/j.yfrne.2010.02.003

Heinrichs M, Meinlschmidt G, Wippich W, Ehlert U, Hellhammer DH (2004) Selective amnesic effects of oxytocin on human memory. Physiology & behaviour 83:31-8. doi:10.1016/j.physbeh.2004.07.020

Herrold A.A Shen F, Graham MP, Harper LK, Specio SE, Tedford CE, Napier TC (2008) Mirtazapine treatment after conditioning with methamphetamine alters subsequent expression of place preference. Drug and Alcohol Dependence 99:231-9. doi:10.1016/j.drugalcdep.2008.08.005

Holtz NA, Lozama A, Prisinzano TE, Carroll ME (2012) Reinstatement of methamphetamine seeking in male and female rats treated with modafinil and allopregnanolone. Drug and Alcohol Dependence 120:233–237 doi:10.1016/j.drugalcdep.2011.07.010

Hyman SE (2005) Addiction: A Disease of Learning and Memory. The American Journal of Psychiatry 162:1414-1422 doi: 10.1176/appi.ajp.162.8.1414

Hyman SE and Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nature Reviews, Neuroscience. 2:695-703. doi:10.1038/35094560

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annual Review of Neuroscience 29:565-98. doi: 10.1146/annurev.neuro.29.051605.113009

Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nature 8:844-858 doi:10.1038/nrn2234. doi:10.1038/nrn2234

Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161-79. doi: 10.1016/j.neuron.2004.09.016

Klimkiewicz T (2001) Memory effects of arginine vasopressin (AVP) and [7-9] fragment of its peptide chain in rats. Acta neurobiologiae experimentalis 61: 267-276

Koob (2000) Neurobiology of Addiction: Toward the Development of New Therapies. Annals of the New York Academy of Sciences 909: 170–185. doi: 10.1111/j.1749-6632.2000.tb06682.x

Koob GF and Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35: 217–238. doi:10.1038/npp.2009.110

Kopnisky KL and Hyman SE (2002) Molecular and Cellular Biology of Addiction. Neuropsychopharmacology: The Fifth Generation of Progress 1368 – 1379

Kova´cs GL, Sarnyai Z, Szabo G (1998) Oxytocin and Addiction: A Review. Psychoneuroendocrinology 23:945–962 doi:http://dx.doi.org/10.1016/S0306-4530(98)00064-X

Kovács GL, Bohus B, Versteeg DH, de Kloet ER, de Wied D (1979) Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic-midbrain structures. Brain Research 175:303-14. doi: 10.1016/0006-8993(79)91009-6

Kovács GL, Laczi F, Vecsernyés M, Hódi K, Telegdy G, László FA (1987) Limbic oxytocin and arginine 8-vasopressin in morphine tolerance and dependence. Experimental Brain Research 65:307-11. doi: 10.1007/BF00236302

Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D (2002) Methamphetamine-Induced Degeneration of Dopaminergic Neurons Involves Autophagy and Upregulation of Dopamine Synthesis. The Journal of Neuroscience 22:8951–8960

Lenz KM and Sengelaub DR (2010) Maternal care effects on the development of a sexually dimorphic motor system: The role of spinal oxytocin. Hormones and Behavior 58:575–581. doi:10.1016/j.yhbeh.2010.07.010

Leshner AI (1997) Addiction Is a Brain Disease, and It Matters. Science 278:45-47. doi: 10.1126/science.278.5335.45

Li S, Ren Y, Zheng J (2002) Effect of 7-nitroindazole on drug-priming reinstatement of D-methamphetamine-induced conditioned place preference. European Journal of Pharmacology 443: 205–206. doi: http://dx.doi.org/10.1016/S0014-2999(02)01580-7

Marazziti D, Bani A, Casamassima F, Catena M, Consoli G, Gesi C, Iovieno N, Massei GJ, Muti M, Ravani L, Romano A, Roncaglia I, Scarpellini P (2006) Oxytocin: An old hormone for new avenues. Clinical Neuropsychiatry 3:302-321

McGregor IS, Callaghan PD, Hunt GE (2008) From ultrasocial to antisocial: a role for oxytocin in the acute reinforcing effects and long-term adverse consequences of drug use? British Journal of Pharmacology 154:358–368. doi:10.1038/bjp.2008.132

Mizuno M, Yamada K, Maekawa N, Saito K, Seishima M, Nabeshima T (2002) CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. Behavioural Brain Research 133:135-41. doi: http://dx.doi.org/10.1016/S0166-4328(01)00470-3

Moffett MC and Goeders NE (2007) CP-154,526, a CRF type-1 receptor antagonist, attenuates the cue-and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior in rats. Psychopharmacology 190:171–180. doi 10.1007/s00213-006-0625-7

Mueller D, Stewart J (2000) Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behavioural Brain Research 115: 39–47. doi: 10.1016/S0166-4328(00)00239-4

Nestler EJ (2001) Molecular Basis Of Long-Term Plasticity Underlying Addiction. Neuroscience 2:119-128. doi:10.1038/35053570

Nestler EJ (2002) Common molecular and cellular substrates of addiction and memory. Neurobiology of Learning and Memory 78: 637-647. doi: 10.1006/nlme.2002.4084

Orsini C, Izzo E, Koob GF, Pulvirenti L (2002) Blockade of nitric oxide synthesis reduces responding for cocaine self-administration during extinction and reinstatement. Brain Research 925:133–140. doi: http://dx.doi.org/10.1016/S0006-8993(01)03267-X

Pierce RC, Kumaresana V (2006) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neuroscience and Biobehavioral Reviews 30:215–238. doi:10.1016/j.neubiorev.2005.04.016

Plüddemann A, Flisher AJ, McKetind R, Parrye C, Lombardg C (2010) Methamphetamine use, aggressive behavior and other mental health issues among high-school students in Cape Town, South Africa. Drug and Alcohol Dependence 109: 14–19. doi:10.1016/j.drugalcdep.2009.11.021

Qi J, Yang J, Wang F, Zhao Y, Song M, Wu C (2009) Effects of oxytocin on methamphetamine-induced conditioned place preference and the possible role of glutamatergic neurotransmission in the medial prefrontal cortex of mice in reinstatement. Neuropharmacology 56:856–865. doi:10.1016/j.neuropharm.2009.01.010

Roberts AJ and Koob GF (1997) The Neurobiology of Addiction An Overview. Alcohol Health & Research World 21:101-106

Robinson DL, Zitzman DL, Williams SK (2011) Mesolimbic dopamine transients in motivated behaviors: focus on maternal behavior. Frontiers in Child and Neurodevelopmental Psychiatry 2:23. doi: 10.3389/fpsyt.2011.00023

Rogers JL, De Santis S and See RE (2008) Extended methamphetamine self-administration enhances reinstatement of drug seeking and impairs novel object recognition in rats. Psychopharmacology 199:615–624. doi: 10.1007/s00213-008-1187-7

Sahgal A (1984) A critique of the vasopressin-memory hypothesis. Psychopharmacology 83 : 215 – 228. doi: 10.1007/BF00464785

Salo R, Nordahl TE, Leamon MH, Natsuakib Y, Moore CD, Waters C, Carter CS (2008) Preliminary evidence of behavioral predictors of recurrent drug-induced psychosis in methamphetamine abuse. Psychiatry Research 157:273–277. doi:10.1016/j.psychres.2007.04.018

Sarantis K, Matsokis N, Angelatou F (2009) Synergistic interactions of dopamine D1 and glutamate NMDA receptors in rat hippocampus and prefrontal cortex: involvement of ERK1/2 signaling. Neuroscience 163:1135–1145. doi:10.1016/j.neuroscience.2009.07.056

Shahrokh DK , Zhang T, Diorio J, Gratton A, Meaney MJ (2010) Oxytocin-Dopamine Interactions Mediate Variations in Maternal Behavior in the Rat. Endocrinology 151:2276–2286. doi: 10.1210/en.2009-1271

Shippenberg TS, Zapata A, Chefer VI (2007) Dynorphin and the Pathophysiology of Drug Addiction. Pharmacology and therapeutics 116:306–321. doi: 10.1016/j.pharmthera.2007.06.011

Tanoue A, Ito S, Honda K, Oshikawa S, Kitagawa Y, Koshimizu T, Mori T, Tsujimoto G (2004) The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. The Journal of Clinical Investigation 113:302–309. doi:10.1172/JCI200419656.

Tomkins DM and Sellers EM (2001) Addiction and the brain: the role of neurotransmitters in the cause and treatment of drug dependence. Canadian Medical Association Journal 164: 817-821

Van Ree JM, Hijman R, Jolles J, de Wied D (1985) Vasopressin and related peptides: Animal and human studies. Progress in Neuro-Psychopharmacology and Biological Psychiatry 9:551–559. doi: http://dx.doi.org/10.1016/0278-5846(85)90016-8

Walters CL, Kuo Y, Blendy JA (2003) Differential distribution of CREB in the mesolimbic dopamine reward pathway. Journal of Neurochemistry 87: 1237–1244. doi: 10.1046/j.1471-4159.2003.02090.x

West AE, Griffith EC, Greenberg ME (2002) Regulation of transcription factors by neuronal activity. Neuroscience 3:921-931. doi:10.1046/j.1471-4159.2003.02090.x

Zhou L and Zhu Y (2006) Changes of CREB in rat hippocampus, prefrontal cortex and nucleus accumbens during three phases of morphine induced conditioned place preference in rats. Journal of Zhejiang University SCIENCE B 7:107-113. doi:10.1631/jzus.2006.B0107

Zhou Y, Leri F, Cummins E, Hoeschele E, Kreek MJ (2008) Involvement of Arginine Vasopressin and V1b Receptor in Heroin Withdrawal and Heroin Seeking Precipitated by Stress and by Heroin. Neuropsychopharmacology 33: 226–236. doi:10.1038/sj.npp.1301419

Zingg HH and Laporte SA (2003) The oxytocin receptor. TRENDS in Endocrinology and Metabolism 14:222–227. doi: http://dx.doi.org/10.1016/S1043-2760(03)00080-8

Chapter 3

Drug addiction is a complex multi-factorial disease that occurs as a consequence of repeated pharmacological exposure to psychostimulant drugs causing pathological changes within the brain (Chao *et al.,* 2004; Kalivas and O'Brien, 2008). Synaptic plasticity within learning and memory, and reward circuits have been implicated as part of the neuroadaptations that occur in addiction, with circuits usually linked with associative learning being recruited in response to the drug (Kopnisky and Hyman, 2002; Thomas and Malenka, 2003; Hyman, 2006).

Neuropeptides oxytocin and vasopressin are involved in the process of learning and memory with vasopressin being facilitatory and oxytocin being inhibitory. These hormones have also been shown to play a role in addiction, with oxytocin showing a tendency to reduce the reinforcing properties of various drugs (Qi *et al*., 2009; Carson *et al*., 2010).

However, few studies have been done to elucidate the molecular mechanisms by which these neuropeptides exert their effects. In addition, to the best of our knowledge there are no reports whether these hormones have any role in methamphetamine addiction. To this end we were interested in investigating whether vasopressin antagonism and oxytocin treatment would have any beneficial effects in a conditioned place preference (CPP) model of methamphetamine addiction. CPP uses the principles of associative learning to model seeking behaviour in animals, and therefore offers the ideal model to investigate the learning and memory aspects of addiction. We hypothesized that using a vasopressin V1b antagonist (SSR 149415) and oxytocin as our treatments during the extinction phase of our 3 phase reinstatement model, the associative learnt neuroadaptations and behaviours associated with methamphetamine administration would be inhibited, resulting in a decreased tendency to relapse when faced with a priming dose of the drug.

Our behavioural results in the acquisition phase showed that methamphetamine did induce place preference behaviour in some of our animals and an increase in the time spent in the drug-paired compartment when tested after the acquisition phase indicated seeking behaviour in the drug treated animals. Although CPP does display validity as an animal model of addiction, it has been found to show some unreliability due to various factors as outlined in

chapter 1. Possibly due to genotypic differences in rodents, there was a poor appearance of place preference in the methamphetamine group treated with saline, with only 3 out of 8 animals showing a marked positive place preference and 1 spending nearly equal time in each compartment.

Whilst this result does seem to reflect a failure of our addiction model in this group, it can be seen that each group receiving methamphetamine underwent the same procedure during the acquisition phase, and as such their results are comparable. Therefore, the significant positive CPP in the other two groups serves to support the validity of our model, and allows for the conclusion that variances among animals may occur in this model.

Treatment with the vasopressin antagonist resulted in a reduction in seeking behaviour and the inhibition of primer-induced reinstatement in methamphetamine addicted animals. This reduction in seeking behaviour was evident from our behavioural data, where the significant decrease in time spent in the initial preferred side in response to methamphetamine treatment was lost, indicating an attenuation of drug seeking behaviour. The effect of vasopressin antagonism on the reinstatement of seeking behaviours was also evident from our behavioural data, with no increase in time spent in the drug-paired compartment being observed.

Oxytocin treatment also seemed to show a tendency towards attenuating drug seeking behaviour, with time spent in the drug-paired compartment showing a definite, though not significant decrease. Interestingly, reinstatement times in the drug-paired compartment showed a marked increase when compared with post test 1, showing that oxytocin may tend to attenuate seeking behaviour, but was not effective in inhibiting reinstatement of seeking behaviour when presented with a priming dose of the drug.

As an increase in dopamine release in response to methamphetamine is a known neurochemical effect, we evaluated the striatal levels of dopamine to determine if the neurobiological marker of addiction was present, and if oxytocin and the vasopressin antagonist had any effect on this. Our data showed an increase in striatal dopamine content following methamphetamine administration; however no changes in dopamine levels were seen in response to oxytocin or SSR 149415, indicating that their attenuating affects did not result via dopaminergic pathways.

Within the learning and memory processes associated with addiction, CREB has been seen to play a vital role in the signalling cascades for long-term potentiation and memory formation, as well as being upregulated in response to repeated drug exposure (Mizuno *et al*., 2002; Chao *et al*., 2004). To examine the mechanism by which these addictive processes occurred, we evaluated CREB levels in the hippocampus of our animals using western blot analysis. However no significant differences in CREB levels were found between any of our experimental groups.

In conclusion, it is well established that addiction shares many neural mechanisms with learning and memory, and that the development of addiction could be viewed as a case of inappropriate associative learning (Kopnisky and Hyman, 2002). Neuropeptides vasopressin and oxytocin have been implicated in the processes of learning and memory, although their exact roles in this regard are widely debated (Engelmann *et al*., 1996). Studies using these neuropeptides in the context of addiction have produced varying results, with no clear mechanism of action indicated as to how they affect the neural circuits implicated in addiction.

Our study showed potential in indicating SSR 149 415 as a possible treatment in methamphetamine addicts as it both attenuated methamphetamine seeking in the extinction phase, as well as inhibited the reinstatement of drug seeking in response to a primer. We have also identified oxytocin as a possible treatment to aid in the extinction of drug seeking behaviour; however oxytocin fell short as it was not sufficient to overcome the reinforcing effects of a drug primer. This short fall could be, in part, due to the dose of oxytocin that was given. It has been hypothesized that neuropeptides can enter the brain in very small amounts, passing through the blood brain barrier when given subcutaneously (Kovács *et al*., 1998). At a dose of 1mg/kg, the amount of oxytocin actually reaching its brain target could therefore have been negligible. However, even with potentially minimal neural penetration, oxytocin did influence drug seeking behaviours after its administration during the period of ‘sobriety’.

A recommendation for future studies would therefore be to administer the vasopressin antagonist and oxytocin in a dose-dependent manner, to establish whether higher doses may improve its efficay in attenuating drug seeking behaviour. It would also be useful to know

whether these treatment options are equally efficacious to the various drugs of abuse. A further refinement of the experimental 3 phase model of addiction would be to use only the animals that show strong positive place preference behaviour in phase 1, to minimise behavioural and neurochemical variability. In the present study data from all animals were used in the statistical analyses and this approach could have masked some of our obtained findings.

Additional References

1. Adinoff B (2004) Neurobiologic Processes in Drug Reward and Addiction. Harvard Review of Psychiatry 12(6): 305–320
2. Albertson TE, Derlet RW, B E Van Hoozen BE (1999) Methamphetamine and the expanding complications of amphetamines. Western Journal of Medicine 170(4): 214–219
3. Alescio-Lautier B, Paban V, Soumireu-Mourat B (2000) Neuromodulation of memory in the hippocampus by vasopressin. European Journal of Pharmacology 405: 63-72
4. Anglin MD and Hser Y (1990) Treatment of Drug Abuse. Crime and Justice 13:393-460
5. Bakshi VP, Kalin NH (2002) Animal models and endophenotypes of anxiety and stress disorders. Neuropsychopharmacology: The Fifth Generation of Progress 883–900
6. Barr AM, Panenka WJ, MacEwan GW, Thornton AE, Lang DJ, Honer WG, Lecomte T (2006) The need for speed: an update on methamphetamine addiction. Journal of psychiatry and neuroscience 31(5):301-13
7. Behrouz B, Drolet RE, Sayed ZA, Lookingland KJ, Goudreau JL (2007) Unique responses to mitochondrial complex I inhibition in tuberoinfundibular dopamine neurons may impart resistance to toxic insult. Neuroscience 147(3):592-8
8. Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25(3):515-32
9. Biegon A, Terlou M, Voorhuis T.D, De Kloet ER (1984) Arginine-vasopressin binding sites in rat brain: A quantitative autoradiographic study. Neuroscience Letters 44(3):229–234
10. Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361(6407):31-9
11. Boccia MM, Kopf SR, Baratti CM (1998) Effects of a Single Administration of Oxytocin or Vasopressin and Their Interactions with Two Selective Receptor

Antagonists on Memory Storage in Mice. Neurobiology of Learning and Memory 69(2):136–146

12. Buxton JA and Dove NA (2008) The burden and management of crystal meth use. Canadian Medical Association Journal 178: 1537-1539

13. Caldwell HK, Lee H, Macbeth AH, Young WS, III (2008) Vasopressin: Behavioral Roles of an "Original" Neuropeptide. Progress in Neurobiology 84(1):1-24

14. Caldwell J, Dring LG, Williams RT (1972) Metabolism of [IC] Methamphetamine in Man, the Guinea Pig and the Rat. The biochemical journal 129: 11-22

15. Carey, KB (1996) Substance Use Reduction in the Context of Outpatient Psychiatric Treatment: A Collaborative, Motivational, Harm Reduction Approach. Community Mental Health Journal 32(3):291-306

16. Carson DS, Cornish JL, Guastella AJ, Hunt GE, McGregor IS (2010) Oxytocin decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats. Neuropharmacology 58(1):38-43

17. Chao J and Nestler EJ (2004) Molecular neurobiology of drug addiction. Annual Review of Medicine 55:113-32

18. Cheung CC and Lustig RH (2007) Pituitary development and physiology. Pituitary 10:335–350

19. Civelli (2000) Molecular Biology of the Dopamine Receptor Subtypes. Psychopharmacology: 4th Generation of Progress

20. Clark RE and Squire LR (1998) Classical Conditioning and Brain Systems: The Role of Awareness. Science 280: 77-81

21. Darke S, Kaye S, Mcketin R, Duflou, J. (2008) Major physical and psychological harms of methamphetamine use. Drug and Alcohol Review 27: 253–262

22. de Wied D (1997) Neuropeptides in learning and memory processes. Behavioural brain research 83(1-2):83-90

23. de Wied D, Elands J, Kovács G (1991) Interactive effects of neurohypophyseal neuropeptides with receptor antagonists on passive avoidance behaviour: Mediation by a cerebral neurohypophyseal hormone receptor? Proceedings of the National Academy of Sciences of the United States of America 88(4): 1494-1498

24. Derlet RW and Heischober B (1990) Methamphetamine. Stimulant of the 1990s? Western Journal of Medicine 153(6): 625–628

25. Dobbs LK and Mark GP (2008) Comparison of systemic and local methamphetamine treatment on acetylcholine and dopamine levels in the ventral tegmental area in the mouse. Neuroscience 156(3):700-11

26. Dostalek M, Jurica J, Pistovcakova J, Hanesova M, Tomandl J, Linhart I, Sulcova A (2007) Effect of methamphetamine on cytochrome P450 activity. Xenobiotica 37(12):1355-66

27. Dufka F, Galloway G, Baggott M, Mendelson J (2009) The effects of inhaled L-methamphetamine on athletic performance while riding a stationary bike: a randomised placebo-controlled trial. British Journal of Sports Medicine 43(11):832-5

28. Dyatkin AB, Hoekstra WJ, Hlasta DJ, Andrade-Gordon P, de Garavilla L, Demarest KT, Gunnet JW, Hageman W, Look R and Maryanoff BE (2002) Bridged Bicyclic Vasopressin Receptor Antagonists with V2-Selective or Dual V1a/V2 Activity. Bioorganic & Medicinal Chemistry Letters 12:3081–3084

29. Egashira N, Tanoue A, Matsuda T, Koushi E, Harada S, Takano Y, Tsujimoto G, Mishima K, Iwasaki K, Fujiwara M (2007) Impaired social interaction and reduced anxiety-related behaviour in vasopressin V1a receptor knockout mice. Behavioural Brain Research 178(1): 123-127

30. Elsworth JD and Roth RH (1997) Dopamine Synthesis, Uptake, Metabolism, and Receptors: Relevance to Gene Therapy of Parkinson's Disease. Experimental Neurology 144(1):4–9

31. Engelmann M, Wotjak CT, Neumann I, Ludwig M, Landgraf R (1996) Behavioural Consequences of Intracerebral Vasopressin and Oxytocin: Focus on Learning and Memory. Neuroscience and Biobehavioural Reviews 20(3):341-58

32. Epstein DH, Preston KL, Stewart J, Shaham Y (2006) Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology 189:1–16

33. Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW and Robbins TW (2008) Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philosophical transactions of the Royal Society B: Biological Sciences 363:3125-3135

34. Fanselow MS, Poulos AM. (2005) The neuroscience of mammalian associative learning. Annual Review of Psychology 56:207-34.

35. Feltenstein MW and See RE (2008) The neurocircuitry of addiction: an overview. British Journal of Pharmacology 154(2):261-74

36. Fitzgerald P and Dinan TG (2008) Prolactin and dopamine: What is the connection? A Review Article. Journal of Psychopharmacology 22: 12-19

37. Fowler JS, Kroll C, Ferrieri R, Alexoff D, Logan J, Dewey SL, Schiffer W, Schlyer D, Carter P, King P, Shea C, Xu Y, Muench L, Benveniste H, Vaska P, Volkow ND (2007) PET studies of d-methamphetamine pharmacokinetics in primates: comparison with l-methamphetamine and (--)-cocaine. Journal of nuclear medicine: official publication, Society of Nuclear Medicine 48(10):1724-32

38. Geyer MA and Markou A (2000) Animal Models of Psychiatric Disorders. Psychopharmacology - The Fourth Generation of Progress

39. Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiological reviews 81(2):629-83

40. Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, Watsky EJ, Gong J, Williams KE, Reeves KR (2006) Varenicline, an α4β2 Nicotinic Acetylcholine Receptor Partial Agonist, vs Sustained-Release Bupropion and Placebo

for Smoking Cessation A Randomized Controlled Trial. JAMA: the Journal of the American Medical Association 296(1):47–55

41. Greenhill L, Beyer DH, Finkleson J, Shaffer D, Biederman J, Conners CK, Gillberg C, Huss M, Jensen P, Kennedy JL, Klein R, Rapoport J, Sagvolden T, Spencer T, Swanson JM, Volkow N (2002) Guidelines and algorithms for the use of methylphenidate in children with Attention-Deficit/ Hyperactivity Disorder. Journal of Attention Disorders 6 (1):89-100
42. Halkitis PN, Parsons JT, Stirratt MJ (2001) A double epidemic: crystal methamphetamine drug use in relation to HIV transmission among gay men. Journal of Homosexuality 41(2):17-35
43. Heather N (1998) A conceptual framework for explaining drug addiction. Journal of Psychopharmacology 12(1) 3-7
44. Heinrichs M, Meinlschmidt G, Wippich W, Ehlert U, Hellhammer DH (2004) Selective amnesic effects of oxytocin on human memory. Physiology & behaviour 83(1):31-8
45. Herrold A.A Shen F, Graham MP, Harper LK, Specio SE, Tedford CE, Napier TC (2008) Mirtazapine treatment after conditioning with methamphetamine alters subsequent expression of place preference. Drug and Alcohol Dependence 99(1-3):231-239
46. Hirasawa A, Hashimoto K, Tsujimoto G (1994) Distribution and developmental change of vasopressin V1A and V2 receptor mRNA in rats. European Journal of Pharmacology 267(1):71-75
47. Hitzemann R (2000) Animal Models of Psychiatric Disorders and their Relevance to Alcoholism. Alcohol Research & Health 24(3):149-158
48. Holmes CL, Landry DW, Granton JT (2003) Science Review: Vasopressin and the cardiovascular system part 1 – receptor physiology. Critical Care. 7(6): 427–434
49. Howl J, Wheatley M. (1995) Molecular pharmacology of V1a vasopressin receptors. General Pharmacology 26(6):1143-52

50. Ishunina TA and Swaab DF (1999) Vasopressin and Oxytocin Neurons of the Human Supraoptic and Paraventricular Nucleus; Size Changes in Relation to Age and Sex. The Journal of Clinical Endocrinology & Metabolism 84(12):4637-4644

51. Ivell R, Walther N (1999) The role of sex steroids in the oxytocin hormone system. Molecular and Cellular Endocrinology 151(1-2):95-101

52. Iversen SD, Iversen LL (2007) Dopamine: 50 years in perspective. Trends in Neurosciences 30(5):188-93

53. Janhunen S, Ahtee L (2007) Differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways: implications for drug development. Neuroscience and Biobehavioural Reviews 31(3):287-314

54. Jellema J, Balt J, Broeze K, Scheele F and Weijmer M (2009) Hyponatraemia during pregnancy The Internet Journal of Gynecology and Obstetrics. 12(1)

55. Kalivas PW and O'Brien C (2008) Drug Addiction as a Pathology of Staged Neuroplasticity. Neuropsychopharmacology 33: 166–180

56. Kalivas PW, Peters J, Knackstedt L (2006) Animal models and brain circuits in drug addiction. Molecular Interventions 6(6):339-44

57. Kanamori T, Tsujikawa K, Ohmae Y, Iwata YT, Inoue H, Kishi T, Nakahama T, Inouye Y (2005) A study of the metabolism of methamphetamine and 4-bromo-2,5 dimethoxyphenethylamine (2C-B) in isolated rat hepatocytes. Forensic Science International 148(2-3):131-137

58. Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44(1):161-79

59. Kelley AE and Berridge KC (2002) The Neuroscience of Natural Rewards: Relevance to Addictive Drugs. The Journal of Neuroscience 22(9): 3306-3311

60. Kish S.J. (2008) Pharmacologic mechanisms of crystal meth. Canadian Medical Association Journal 178(13): 1679-82

61. Klimkiewicz T (2001) Memory effects of arginine vasopressin (AVP) and [7-9] fragment of its peptide chain in rats. Acta neurobiologiae experimentalis 61(4): 267-276

62. Koob (2000) Neurobiology of Addiction: Toward the Development of New Therapies. Annals of the New York Academy of Sciences 909: 170–185

63. Koob GF (2000) Animal Models of Drug Addiction. Psychopharmacology: The Fourth Generation of Progress

64. Koob GF (2008) A Role for Brain Stress Systems in Addiction. Neuron 59(1): 11–34

65. Koob GF and Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35(1):217-38.

66. Kopnisky KL and Hyman SE (2002) Molecular and Cellular Biology of Addiction. Neuropsychopharmacology: The Fifth Generation of Progress 1367-1379

67. Kova´cs GL, Sarnyai Z and Szabo G (1998) Oxytocin and Addiction: A Review Psychoneuroendocrinology 23(8):945–962

68. Kovács GL, Bohus B, Versteeg DH, de Kloet ER, de Wied D (1979) Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic-midbrain structures. Brain Research 175(2):303-314

69. Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F and Engelmann M (1995) V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behaviour in rats. Journal of Neuroscience 15(6):4250-8

70. Lawlor PA and During MJ (2004) Gene therapy for Parkinson's disease. Expert Reviews in Molecular Medicine 6:1-18

71. Leff P, EM Mora, Calva, JC, Valdés A, Acevedo R, Morales A, Medécigo M,. Antón B (2000) Neurobiology of Addiction, Neuroanatomical, Neurochemical, Molecular and Genetic aspects of morphine and cocaine addiction. Part 1. Salud Mental 23(3): 46-51

72. Leshner AI (1999) Science-based views of drug addiction and its treatment. JAMA: the Journal of the American Medical Association 282(14):1314-1316

73. Lin LY, Di Stefano EW, Schmitz DA, Hsu L, Ellis SW, Lennard MS, Tucker GT and Cho AK (1997) Oxidation of Methamphetamine and

Methylenedioxymethamphetamine by CYP2D6. Drug Metabolism and Disposition 25(9):1059-1064

74. Lineberry TW and Bostwick JM (2006) Methamphetamine Abuse: A Perfect Storm of Complications. Mayo Clinic Proceedings 81(1):77-84

75. Lingford-Hughes and Nutt (2003) Neurobiology of addiction and implications for treatment. British Journal of Psychiatry 182: 97-100

76. Lingford-Hughes AR, Welch S, Nutt DJ (2004) Evidence-based guidelines for the pharmacological management of substance misuse, addiction and comorbidity: recommendations from the British Association for Psychopharmacology. Journal of Psychopharmacology 18(3):293–335

77. Logan BK (2002) Methamphetamine – Effects on human performance and behaviour. Forensic Science Reviews 14: 133-151

78. Lolait SJ, O'Carroll AM, Mahan LC, Felder CC, Button DC, Young WS 3rd, Mezey E, and Brownstein MJ (1995) Extrapituitary expression of the rat V1b vasopressin receptor gene. (G protein-coupled receptor/neurohypophyseal hormones/*in situ* hybridisation/rat brain) Proceedings of the National Academy of Sciences of the United States of America 92: 6783-6787

79. Marazziti D, Bani A, Casamassima F, Catena M, Consoli G, Gesi C, Iovieno N, Massei GJ, Muti M, Ravani L, Romano A, Roncaglia I, Scarpellini P (2006) Oxytocin: An old hormone for new avenues. Clinical Neuropsychiatry 3(5) 302-321

80. Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993) Animal models of drug craving. Psychopharmacology 112:163-182

81. McLellan TA, Lewis DC, O'Brien CP, Kleber HD (2000) Drug Dependence, a Chronic Medical Illness: Implications for Treatment, Insurance, and Outcomes Evaluation. JAMA: the Journal of the American Medical Association 284(13):1689-1695

82. Mendelson JE, McGlothlin D, Harris DS, Foster E, Everhart T, Jacob P 3rd, Jones RT (2008) The clinical pharmacology of intranasal l-methamphetamine. BMC Clinical Pharmacology 8:4

83. Messinger A, Squire LR, Zola SM, and Albright TD (2001) Neuronal representations of stimulus associations develop in the temporal lobe during learning. Proceedings of the National Academy of Sciences of the United States of America 98(21):12239–12244

84. Mizuno M, Yamada K, Maekawa N, Saito K, Seishima M, Nabeshima T (2002) CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. Behavioural Brain Research 133(2):135-41

85. Mok LW, Estevez AF, Overmier JB (2010) Unique Outcome Expectations as a Training and Pedagogical Tool. The Psychological Record 60: 227–248

86. Musshoff (2000) Illegal or legitimate use? Precursor compounds to amphetamine and methamphetamine, Drug Metabolism Reviews 32(1) 15–44

87. Nagai T, Noda Y, Ishikawa K, Miyamoto Y, Yoshimura M, Ito M, Takayanagi M, Takuma K, Yamada K, Nabeshima T (2005) The role of tissue plasminogen activator in methamphetamine-related reward and sensitization. Journal of Neurochemistry 92(3):660-7

88. Nestler EJ. (2001) Molecular basis of long-term plasticity underlying addiction. Nature Reviews, Neuroscience 2(2):119-28

89. Neve KA, Seamans JK, Trantham-Davidson H (2004) Dopamine Receptor Signaling. Journal of receptors and signal transduction 24 (3):165–205

90. O'Brien, CP (2003) Research Advances in the Understanding and Treatment of Addiction. The American Journal on Addictions 12:36-47

91. Phillips TJ, Kamens HM, Wheeler JM (2008) Behavioral genetic contributions to the study of addiction-related amphetamine effects. Neuroscience and Biobehavioral Reviews 32(4):707-59

92. Pittman QJ and Spencer SJ (2005) Neurohypophysial peptides: gatekeepers in the amygdala. Trends in Endocrinology and Metabolism 16(8):343-4

93. Pivonello R, Ferone D, Lombardi G, Colao A, Lamberts SW, Hofland LJ (2007) Novel insights in dopamine receptor physiology. European Journal of Endocrinology 156 (1):13-21

94. Qi J, Yang J, Wang F, Zhao Y, Song M, Wu C (2009) Effects of oxytocin on methamphetamine-induced conditioned place preference and the possible role of glutamatergic neurotransmission in the medial prefrontal cortex of mice in reinstatement. Neuropharmacology 56:856–865

95. René P, Lenne F, Ventura M, Bertagna X, de Keyzer Y (2000) Nucleotide sequence and structural organization of the human vasopressin pituitary receptor (V3) gene. Gene 241 (1):57–64

96. Ring R (2005) The Central Vasopressinergic System: Examining the Opportunities for Psychiatric Drug Development. Current Pharmaceutical Design 11(2): 206-219

97. Roberts AJ and Koob GF (1997) The Neurobiology of Addiction An Overview. Alcohol Health & Research World 21(2):101-106

98. Rocha A, Valles R, Hart N, Bratton GR, Nation JR (2008) Developmental lead exposure attenuates methamphetamine dose-effect self-administration performance and progressive ratio responding in the male rat. Pharmacology, Biochemistry and Behaviour 89(4):508-14

99. Ross HE, Young LJ (2009) Oxytocin and the neural mechanisms regulating social cognition and affiliative behaviour. Frontiers in Neuroendocrinology 30(4):534-47

100. Saah T (2005) The evolutionary origins and significance of drug addiction. Harm Reduction Journal 2: 8

101. Saal D and Malenka RC (2005) The role of synaptic plasticity in addiction. Clinical Neuroscience Research 5(2–4):141–146

102. Sahgal A (1984) A critique of the vasopressin-memory hypothesis. Psychopharmacology 83 : 215 – 228

103. Samad TA, Krezel W, Chambon P, Borrelli E (1997) Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proceedings of the National Academy of Sciences of the United States of America 94(26):14349-54

104. Schifano F, Corkery JM and Cuffolo G (2007) Smokable (“ice”, “crystal meth”) and non smokable amphetamine-type stimulants: clinical pharmacological and epidemiological issues, with special reference to the UK. Annali dell'Istituto Superiore di Sanita 43(1): 110-115

105. Serretti A, Macciardi F, Verga M, Cusin C, Pedrini S, Smeraldi E (1998) Tyrosine hydroxylase gene associated with depressive symptomatology in mood disorder. American Journal of Medicinal Genetics 81(2):127-30

106. Sharma, H.S., Kiyatkin, E.A. (2008) Rapid morphological brain abnormalities during acute methamphetamine intoxication in the rat: An experimental study using light microscopy. Journal of Chemical Neuroanatomy 37(1):18-32

107. Shephard JD, Chuang DT, Shaham Y, Morales M (2006) Effect of methamphetamine self-administration on tyrosine hydroxylase and dopamine transporter levels in mesolimbic and nigrostriatal dopamine pathways of the rat. Psychopharmacology 185(4):505-13

108. Shippenberg TS and Koob GF (2002) Recent advances in animal models of drug addiction. Neuropsychopharmacology: The Fifth Generation of Progress 1381-1397

109. Shippenberg TS, Zapata A, Chefer VI (2007) Dynorphin and the Pathophysiology of Drug Addiction. Pharmacology & Therapeutics 116(2): 306–321

110. Shoblock JR, Sullivan EB, Maisonneuve IM, Glick SD (2003) Neurochemical and behavioral differences between d-methamphetamine and d-amphetamine in rats. Psychopharmacology (Berl). 165(4):359-69

111. Shoptaw S, Heinzerling KG, Rotheram-Fuller E, Steward T, Wang J, Swanson AN, De La Garza R, Newton T, Ling W (2008) Randomized, placebo-controlled trial of bupropion for the treatment of methamphetamine dependence. Drug and Alcohol Dependence 96(3):222-32

112. Siegel S and Ramos BMC (2002) Applying Laboratory Research: Drug Anticipation and the Treatment of Drug Addiction. Experimental and Clinical Psychopharmacology 10(3):162–183

113. Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and Memory. Annual Reviews of Neuroscience 21:127–48

114. Slemmer JE, Martin BR, Damaj MI (2009) Bupropion Is a Nicotinic Antagonist[1]. The Journal of Pharmacology and Experimental Therapeutics 295(1):321- 327

115. Thomas MJ and Malenka RC (2003) Synaptic plasticity in the mesolimbic dopamine system. Philosophical Transactions of the Royal Society B: Biological Sciences 358(1432): 815–819

116. Tominaga GT, Garcia G, Dzierba A, Wong J (2004) Toll of methamphetamine on the trauma system. Arch Surg. 139(8):844-7

117. Tomkins DM and Sellers EM (2001) Addiction and the brain: the role of neurotransmitters in the cause and treatment of drug dependence. Canadian Medical American Journal 164(6): 817-821

118. Tropea TF, Kosofsky BE, Rajadhyaksha AM (2008) Enhanced CREB and DARPP-32 phosphorylation in the nucleus accumbens and CREB, ERK, and GluR1 phosphorylation in the dorsal hippocampus is associated with cocaine-conditioned place preference behaviour . Journal of Neurochemistry 10:1780–1790

119. Tungtananuwat W and Choenkhwuanma S (2009) Risk of methamphetamine abuse promoted cerebro-cardiovascular defects in Thai cadavers which sent to autopsy at Institute of Forensic Medicine, Thailand. Journal of Health Research 23(3): 147-151

120. van Heuven-Nolsen D, De Kloet E.R, De Wied D, Versteeg D.H. (1984) Microinjection of vasopressin and two related peptides into the amygdala: enhancing effect on local dopamine neurotransmission Brain Research 293(10):191-195

121. Van Ree JM, Hijman R, Jolles J, de Wied D (1985) Vasopressin and related peptides: Animal and human studies. Progress in Neuro-Psychopharmacology and Biological Psychiatry 9(5–6):551–559

122. Vanderschuren LJ and Everitt BJ (2005) Behavioral and neural mechanisms of compulsive drug seeking. European Journal of Pharmacology 526(1-3):77-88

123. Vocci FJ, Acri J, Elkashef A (2005) Medication development for addictive disorders: the state of the science. American Journal of Psychiatry. 162(8):1432-40

124. Volkow ND, Fowler JS, Wang GJ (2003) The addicted human brain: insights from imaging studies. The Journal of Clinical Investigation 111: 1444–1451

125. Volkow ND, Fowler JS, Wang GJ, Swanson JM, Telang F (2007) Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. Archives of Neurology 64(11):1575-9

126. Volkow ND, Wang GJ, Fowler JS, Telang F (2008) Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philosophical Transactions of the Royal Society B: Biological Sciences 363(1507):3191-200.

127. Walter R, Van Ree JM, De Wied D (1978) Modification of conditioned behaviour of rats by neurohypophyseal hormones and analogues. Proceedings of the National Academy of Sciences of the United States of America 75(5): 2493–2496.

128. Watson B and Lingford-Hughes A (2007) Pharmacological treatment of addiction. Psychiatry 6(7):309-312

129. Williams MT, Blankenmeyer TL, Schaefer TL, Brown CA, Gudelsky GA, Vorhees CV (2003) Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. Brain Research, Developmental Brain Research 147(1-2):163-75

130. Winger G, Woods JH, Galuska CM, Wade-Galuska T (2005) Behavioral Perspectives on the Neuroscience of Drug Addiction. Journal of the Experimental Analysis of behaviour 84(3): 667–681

131. Wolf ME (2002) Addiction: Making the Connection Between Behavioral Changes and Neuronal Plasticity in Specific Pathways. Molecular interventions 2 (3): 146 – 157

132. Yahyavi-Firouz-Abadi N and See RE (2009) Anti-relapse medications: Preclinical models for drug addiction treatment. Pharmacology & Therapeutics 124:235–247