Neural Substrates of Amphetamine Induced Impulsive Behaviour

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Impulsivity is a pathological feature of drug addiction. Amphetamine is a highly addictive drug that is amongst the most harmful recreational drugs abused within the UK (Nutt, King, & Phillips, 2010). Interestingly, however, amphetamine has a paradoxical relationship with impulsivity and can both alleviate and induce impulsive behaviour depending on pre-baseline levels of impulsivity and the dimension of impulsivity that is being measured. The current thesis sought to investigate the relationship between different patterns of amphetamine administration and impulsivity in the form of behavioural inhibition, and the neural substrates of amphetamine induced behavioural disinhibition, using the symmetrically reinforced Go/No-go task in rats (Harrison, Everitt, & Robbins, 1999). To assess the effects of different patterns of amphetamine administration on behavioural inhibition, separate groups of rats were treated with subchronic (4-day) and chronic (11-day) amphetamine and were tested on the Go/No-go task during drug treatment and drug withdrawal. Following two weeks of drug withdrawal, sensitivity to the acute effects of amphetamine in rats was tested with acute drug challenges. To assess the role of nucleus accumbens core D_2 and $GABA_A$ receptors in the mediation of behavioural inhibition and amphetamine-induced behavioural disinhibition, separate groups of rats were also treated with intra-nucleus accumbens core infusions of the D_2 antagonist eticlopride and $GABA_A$ agonist muscimol. Results revealed that short duration and high frequency binge-like amphetamine administration produced longer term increases in behavioural disinhibition than longer term and less frequent but overall higher dosing of amphetamine in rats. However, neither the binge-like (4-day) or longer term amphetamine regimes (11-day) caused any enduring changes in sensitivity to the acute disinhibitory effects of amphetamine in rats. Infusions of either eticlopride or muscimol into the NAcb core had no effect on behavioural inhibition assessed under baseline conditions, however, eticlopride infusions produced full behavioural reversal of amphetamine induced behavioural disinhibition and muscimol infusions produced partial reversal of amphetamine induced behavioural disinhibition. Taken together, these results demonstrate that different patterns of amphetamine administration produce different effects on the duration of behavioural disinhibition in rats, and further, that amphetamine induced activation of the D_2 receptors within the nucleus accumbens core mediates amphetamine induced behavioural disinhibition on the symmetrically reinforced Go/No-go task. Results additionally support the possibility of dopamine-GABA interactions in the mediation of amphetamine induced behavioural disinhibition on the symmetrically reinforced Go/No-go task in rats.

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Abbreviations

%	Percentage
°C	Degrees Centigrade
µg	Micrograms
5-HIAA	5-hydroxyindoleactic acid
5CSRTT	Five Choice Serial Reaction Time Task
5HT	5-Hydroxytryptamine (Serotonin)
ACC	Anterior Cingulate Cortex
ADHD	Attention Deficit Hyperactivity Disorder
AMPT	Alpha-Methyl-Para-Tyrosine
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATS	Amphetamine Type Stimulant
BIS	Barrett Impulsivity Scale
BLA	Basolateral Amygdala
BSS	Behavioural Satiety Sequence
CNS	Central Nervous System
CPT	Continuous Performance Task
CRF	Continuous Reinforcement Schedule
CS	Conditioned Stimulus
cs	Centiseconds
DA	Dopamine
DAT	Dopamine Transporter
df	Degrees of Freedom
DLPFC	Dorsolateral Prefrontal Cortex
dmSNR	Dorsomedial Substantia Nigra Pars Reticular
dmSTN	Dorsomedial Subthalamic Nucleus
DRL	Differential Rates of Low Reinforcement
DRT	Delayed Reward Task
dSNR	Dorsal Substantia Nigra Pars Reticular
DTI	Diffusion Tract Imaging
dVP	Dorsal Ventral Pallidum
DWI	Diffusion Weight Imagine
FA	Fractional Anisotropy

fMRI	Functional Magnetic Resonance Imaging
FR	Fixed Ratio
GABA	Gamma-Aminobutyric Acid
HDAC1	Histone Deacetylase 1
Hipp	Hippocampus
HPA	Hypothalamic-Pituitary Axis
Hrs	Hours
i.c.	Intracerebral
i.p.	Intraperitoneal
I_7	Eysenck's Impulsivity Questionnaire
ICD	International Classification of Diseases
ICSS	Intracranial Self-Stimulation
IFG	Inferior Frontal Gyrus
iGP	Internal Segment of the Globus Pallidus
IGT	Iowa Gambling Task
IHYP	Later Hypothalamus
IL	Infralimbic Cortex
ILN	Intralaminar thalamic nuclei
IMT/DMT	Immediate Memory Task/ Delayed Memory Task
IOFC	Lateral Orbitofrontal Cortex
IPC	Inferior Parietal Cortex
IRT	Inter Response Time
IST	Information Sampling Task
ITI	Inter Trial Interval
Kg	Kilograms
MA	Methamphetamine
mA	Milliamps
MAD	Mean Adjusted Delay
MAO	Monoamine Oxidase
MDA	3,4-Methylenedioxyamphetamine
MDMA	3,4-Methylenedioxy-*N*-Methylamphetamine
MFFT	Matching Familiar Figures Task
mg	Milligrams
ml	Millilitres
MPH	Methylphenidate

mRNA	Messenger Ribose Nucleic Acid
NA	Noradrenaline
NAcb	Nucleus Accumbens
NaCl	Sodium Chloride
NARI	Noradrenaline Reuptake Inhibitor
NAT	Noradrenaline Transporter
ng	Nanogram
nM	Nanomole
NS	Non-significant
OFC	Orbitofrontal Cortex
PET	Positron Emission Topography
PIT	Pavlovian Instrumental Transfer
PL	Prelimbic Cortex
pmol	Picomole
PPC	Posterior Parietal Cortex
preSMA	Pre Supplementary Motor Area
RT	Reaction Time
SEM	Standard Error of the Mean
SERT	Serotonin Transporter
SPSS	Statistical Package for the Social Sciences
SSD	Stop Signal Delay
SSRI	Selective Serotonin Reuptake Inhibitor
SST	Stop Signal Task
STN	Subthalamic Nucleus
UPPS	Urgency Premeditation Perseverance Sensation-Seeking
V	Volts
VLPFC	Ventromedial Prefrontal Cortex
VMAT 1	Vesicular Monoamine Transporter 1
VMAT 2	Vesicular Monoamine Transporter 2
VTA	Ventral Tegmental Area

Chapter 1 General Introduction

1.1 The global problem of Drug Addiction

Drug addiction is a wide-spread global health pandemic. Approximately 230 million people world-wide (accounting for 5% of the global population) have been estimated to use illicit drugs whilst approximately 12% of this figure, equating to 27 million, are dependent on illegal drugs (World Drug Report (WDR), 2012). The cost associated with treating this level of drug use and dependency amongst users has been placed at between £128-160 billion (WDR, 2012) and within the UK the social economic cost of addiction has been estimated at 15.4 billion (National Treatment Agency for Substance Misuse (NTA), 2012). More specifically, the costs associated with treatment of drug dependence within the NHS are estimated at £488 million per year, whilst every drug user not gaining treatment costs the tax payer roughly £26,074 per year in crimes (NTA, 2012).

The health consequences of drug use and dependence span a range of severe mental and physical health problems including depression, anxiety, psychosis, infectious diseases and cancer. Co-morbidity of mental health problems and drug use is extremely high, with 70% of patients in drug treatment services, and 86% of patients in alcohol treatment services, diagnosed with an additional mental health problem in the UK (NTA, 2012). Drug addiction also feeds mortality rates associated with leading global health burdens including cardiovascular disease, pulmonary disease, cancers and blood borne infections (HIV/AIDS, Hepatitis B/C, Tuberculosis (TB), Ineffective endocarditis) (Global Burden of Disease Study (GBD), 2010). The impact of drug use on mortality is demonstrated by figures reporting an increase in drug use globally of approximately 50 million alongside an increase in global drug related deaths by 191.2% between 1990-2010 (WDR, 2004; 2012; GBD, 2010).

In addition to the severe health impact of drug use, the illegal setting surrounding the misuse of addictive drugs, places drug use and dependence intrinsically with criminal activity. Maintaining drug use during dependence is often fuelled through criminal activity including robbery, theft and fraud. The magnitude of criminal activity associated with drug use and dependence is illustrated by statistics reporting that within the UK 90% of the social economic costs of drug abuse are accountable to the costs of drug related crimes, estimated at approximately £13.9 billion (NTA, 2012). Illicit drug use and dependence therefore poses a huge monetary, health and social burden within the UK, and throughout the world.

1.1.1 The problem of Amphetamine and Amphetamine-Type-Stimulant (ATS) Misuse and Dependence: Global and UK statistics

Amphetamine type stimulants (ATS) (excluding ecstasy (MDMA)) are the second most frequently used illicit drug group after cannabis world-wide, with up to 1.2% of the adult population (15-64 years) consuming ATS (WDR, 2012). Over the past 20 years amphetamine and ATS use has grown to equalise the use of cocaine and opiates combined world-wide, raising concerns that amphetamine and ATS use may exceed cocaine and opiate use in the future (WRD, 2004; 2009; 2010; 2012). This would prospectively place amphetamine and ATS use above two of the world's largest drug markets combined. Corresponding with this increased use, global seizures of ATS have increased from 2002-2010, with methamphetamine (MA) showing the steepest recent increase from 2007-2010 (WDR, 2012). In addition, a 44% increase in amphetamine laboratories was detected in Europe from 2009 to 2010 (WDR, 2012), indicating that the production of amphetamine in Europe nearly doubled within one year.

Amphetamine and ATS are amongst the most commonly misused illegal drug groups in England. In 2011, 800,000 adults reported use of amphetamines and 300,000 young people aged between 16-24 reported use of amphetamine and ATS (both figures inclusive of MDMA) (Crime Survey for England and Wales (CSEW), 2012). Outside of household surveys, The Drug Treatment Research Outcomes Study (DTORS) surveying 1,796 drug users seeking treatment within community based services found that that 'unprescribed amphetamines' were the 5th most prevalent drug of primary use at baseline, and were the 6th most prevalent problem drug at baseline (Donmall et al., 2012). Injection of amphetamine is also prevalent amongst problem drug users with crack cocaine and heroin dependence. Hope et al., (2008) found that from a sample of crack and heroin users 13% reported injecting amphetamine. Amphetamine use is also highly prevalent within the UK prison population. A large sample of 1009 male adult prisoners found that amongst the 55% of this sample that reported drug use in prison, amphetamine use was the most prevalently misused drug with 75% of this sample reporting use of amphetamine, followed by cocaine at 69% and heroin at 58% (Strang et al., 2006).

Dependence and illegal use of ATS is also highly prevalent within the UK. The CSEW found that out of the 800,000 adults reporting use of amphetamines 500,000 of this figure represent MDMA use, and out of the 300,000 young people aged between 16-24 reporting use of amphetamine and ATS's, 200,000 of this figure represents MDMA (CSEW, 2012). Furthermore, club drug clinics within the UK that treat dependence to ATS's such as, MDMA, MA and

methadrone, have also reported an increase in the number of young adults and adults in treatment for club drug dependence between 2005-2012 (Club Drugs Report, NTA, 2012).

In addition to the misuse of illegal street amphetamine and ATS's, misuse and diversion of prescription amphetamines and ATS's is also rising globally and within the UK. Misuse of prescription amphetamines and ATS's within the USA is highly prevalent, with 13% of young adults (18 – 25 years) in the USA using prescription stimulants for non-medical purposes (National Survey on Drug Use and Health, 2011). This trend is most prevalent amongst college students and the magnitude of this problem is demonstrated by one study reporting that amongst a sample of 9,161 American college students the misuse of prescription amphetamines used to treat ADHD (Adderall, Ritalin) actually exceeded the use of prescription amphetamines amongst students with ADHD (McCabe, Teter, & Boyd, 2006). There is also evidence of rising amphetamine and ATS misuse within the UK. Sumnall et al. (2008) refer to an unpublished survey conducted by the authors finding a lifetime prevalence of illicit methylphenidate (MPH; Ritalin) use amongst 31% young people, second only to cannabis misuse. These findings therefore illustrate trends in diversion and misuse of prescription amphetamine and ATSs globally and within the UK, and highlight potential pathways to amphetamine misuse and dependence.

Collectively, the literature reviewed in this section demonstrates that amphetamine dependence and misuse of ATS's is a current problem globally. Amphetamine dependence is a severely debilitating syndrome that has been likened to heroin and crack cocaine dependence (Churchill et al., 1993; Kramer, Fischman, & Littlefield, 1967). The withdrawal syndrome associated with psychostimulant dependence is typically defined by depression, tiredness, lethargy, insomnia, paranoia, psychosis and increase the risk of suicide (Barr, Markou, & Phillips, 2002). Consequently, amphetamine withdrawal is very difficult to treat and half of amphetamine users go into remission within the first year of abstinence (Calabria et al., 2010). Despite the prevalence and deleterious health consequences of amphetamine use, there remains no effective treatment for amphetamine dependence, defined by successful relapse prevention. Consequently, there is a demand to explore the consequences of amphetamine use and dependence that may render drug users more vulnerable to relapse and poor abstinence rates. Furthermore, exploration of the neural systems underlying amphetamine dependence is a clear research priority in order to identify targets of potential pharmacological treatment.

1.2 Amphetamine: Definitions and Pharmacology

1.2.1.1 Structural properties

Amphetamine is a synthetic psychostimulant containing a phenyl ring bonded to a three-carbon side chain substituted with an amine group at the 2-carbon (IUPAC: 1-phenylpropan-2-amine). The core presence of a phenyl ring, two-carbon side chain and amine group at the 2-carbon represents the molecular structure of β-phentylamine. Amphetamine is therefore classified as a β-phenthylamine and can also be defined as a β-phenthylamine containing an α-methyl group (α-methylphenthylamine). The 2-carbon in amphetamine is chiral and created two enantiomers of amphetamine; *(S) dextro-* and *(R) levo-* amphetamine.

1.2.1.2 Overview of amphetamine pharmacology

Amphetamine induces feelings of euphoria, increased energy, alertness, anorexia, insomnia and, at high doses, can cause auditory and visual hallucinations known as 'amphetamine psychosis' (Curran, Byrappa, & Mcbride, 2004; Leonard, 2004). These symptoms are a consequence of increased synaptic dopamine (DA), noradrenaline (NA) and serotonin (5-HT), that in turn increase signal activity within these neurotransmitter circuits. The primary molecular targets of amphetamine that elevate synaptic catecholamine concentration are plasma membrane transporters responsible for the reuptake of dopamine (DAT), noradrenalin (NAT) and serotonin (SERT), and vesicular monoamine transporters (VMAT). Amphetamine binds competitively to reuptake transporters and consequently inhibits the reuptake of DA, NA and 5-HT and increases synaptic neurotransmitter levels (Kuczenski & Segal, 1989; Segal & Kuczenski, 1994; Sulzer et al., 2005). Acting as a substrate at reuptake transporters also allows amphetamine to receive direct passage through reuptake transporters from the synapse into the nerve terminal in place of DA, NA and 5-HT. Additionally amphetamine is lipophillic and can diffuse across the cell membrane, creating two direct pathways to enter the cytosol (Kahlig et al., 2005; Sulzer et al., 1995). Once inside the nerve terminal, amphetamine competitively binds to VMAT1 and VMAT2 (Peter et al., 1994), inhibiting the packaging of cytosolic DA, NA and 5-HT into vesicles. Alongside additional molecular actions of amphetamine, including vesicle rupture, MAO inhibition, DAT and NA trafficking, and enhanced DA synthesis, collectively, these molecular actions ultimately lead to a high concentration of unpackaged neurotransmitter within the nerve terminal. This concentration coupled with changes in membrane transporter permeability drives the release of DA, NA and 5-HT from the nerve terminal into the synapse via membrane transporters, and is termed amphetamine induced 'reverse transport'. Consequently, amphetamine is termed a catecholamine 'releaser'.

1.2.1.3 Binding affinity

D-amphetamine has a high affinity for the DAT and NAT, and a weak affinity for SERT (for review see Heal et al., 2013). L-amphetamine has a much lower affinity for DAT in comparison to d-amphetamine and has no significant affinity for SERT. L-amphetamine however has a greater affinity for NET than d-amphetamine (Heal et al., 2013). Due to l-amphetamine showing low affinity for DAT, it has a low potential for abuse and induction of addictive behaviours (Jasinski, 1991; Schechter & Rosecrans, 1973; Schechter, 1978). For the remainder of this section, the pharmacology of d-amphetamine will be considered due to d-amphetamine being the focus of this thesis and of greater abuse potential to l-amphetamine. Unless otherwise stated, 'amphetamine' henceforth refers to d-amphetamine.

1.2.2 Amphetamine-type Stimulants: Definitions and pharmacology

'Amphetamine-type stimulants' (ATS) is a collective term for stimulants that are substituted amphetamines. Amphetamine can be substituted at the aromatic ring, the α and β carbons and the amine terminal. This consequently enables a range of structural combinations that ATS can form. Due to amphetamine and ATS containing the same structural core, all ATS act as central and peripheral nervous system stimulants via activating DA, NA and 5HT release. The affinity of ATS at catecholamine receptors, however, varies between ATS depending on their structural substitution. All ATS therefore act as psychostimulants to increase wakefulness and alertness; however the strength of these stimulatory effects varies between ATS. Structural changes between ATS also produce differences in the effects of ATS. For example, ring-substituted amphetamines such as MDMA and methylenedioxyamphetamine (MDA) are associated with low stimulant effects but high empathogenic and hallucinogenic effects, whilst substitutions at the N-terminus, such as methamphetamine (MA) and cathinone, are associated with similar psychomotor and anorectic effects to amphetamine (Carvalho et al., 2012).

1.3**Key stages of drug addiction**

Drug addiction has been conceptualised as a 'chronically relapsing disorder', whereby as drug use increases, a transition from impulsive to compulsive drug use emerges, driving a state of uncontrollable and chronic relapse (Koob & Le Moal, 2008). The transition into this state is thought to occur through distinct stages that include initiation, acquisition, maintenance, escalation, abstinence and relapse.

Initiation refers to the first time engagement with a drug and acquisition marks the transition from this stage in a phase of regular drug use, such as weekly use (Perry, 2008). Following acquisition maintenance marks a phase of steady and regular drug use (Perry, 2008). In contrast, escalation refers to increased frequency and quantity of drug use that is reflective of a loss of control over drug use. This stage marks the transition from controlled to uncontrolled levels of drug use (Perry, 2008). Following the cessation of drug use, a phase of abstinence begins. This stage in addiction marks the period where users refrain from engaging in drug use, which may be of short or long duration. Subsequently, relapse defines the transition from abstinence to the engagement with drug use again (Perry, 2008).

A variety of biological (genetics, neuroadaptive changes), psychological (psychopathology, personality traits) and social (stressful life events, environmental cues, socioeconomic status) risk factors can interact with any or all of these stages of addiction to increase vulnerability to the development of drug addiction. One of the most prevalent risk factors throughout all stages of addiction however is stress (Miczek et al., 2004; Piazza & Le Moal, 1996; Ramsey & Van Ree, 1993; Shaham, Erb, & Stewart, 2000; Stewart, 2000). In addition, common risk factors of relapse during the later stages of addiction include intense drug craving, greater sensitivity and reactivity to drug cues and the aversive state of drug withdrawal (Cook et al., 2010; Koob & Le Moal, 2008; Marra et al., 1998; Niaura et al., 1988; Robinson & Berridge, 1993; Rohsenow et al., 1991). Alongside these common risk factors, greater levels of impulsivity can increase vulnerability to addiction through reducing inhibitory control over the magnitude of these highly emotive states (Jentsch & Taylor, 1999).

1.4 Theories of Addiction

1.4.1 Incentive-Sensitisation Theory of Addiction

The incentive-sensitization theory of addiction (Robinson & Berridge, 1993) addresses central facets of addiction involving craving, relapse and drug induced neural adaptations. Robinson and Berridge (1993) propose that all drugs of abuse share a common neural target of the mesolimbic dopamine system that is responsible for controlling motivation and reward processes. The mesolimbic dopamine system is also proposed to mediate the subjective experience of 'wanting', but not 'liking', of drugs and associated stimuli (cues) via elevating the incentive value attached to these stimuli. Drugs and drug cues are therefore rendered highly attractive due to enhanced incentive value. It is proposed that the continuous activation of this pathway through repeated drug use produces neural adaptations that render this system 'hypersensitive', or 'sensitized', to the incentive value of drug reward and drug cues. Consequently, sensitization of the mesolimbic dopamine system leads to a pathological state of drug 'craving', whereby increased attractiveness (salience) of drugs and conditioned drug stimuli drives pathological 'wanting'. Sensitization to the incentive value of rewards is proposed to persist long-term due to enduring alterations in brain reward and motivation (DA) circuitry. Consequently, enduring neural alterations that cause heightened sensitivity to the incentive value of drugs and drug cues are proposed to underlay relapse even after a prolonged period of drug abstinence (See Fig. 1.1). Incentive-sensitization is also proposed to interact with drug induce changes in fronto-striatal systems that can compromise inhibitory control over drug cravings (Jentsch & Taylor, 1999) and compromise cognitive choice that renders drug users biased to disadvantageous decision-making when coupled with pathological drug cravings (Bechara, Dolan, & Hindes, 2002; Robinson & Berridge, 2008)

The central component to the 'incentive-sensitization' theory of drug addiction is that drugs of abuse sensitize the incentive value of drugs and drug related stimuli. Consequently, evidence for this theory is taken principally from the investigation of behavioural and neural sensitization to drug and drug related cues.

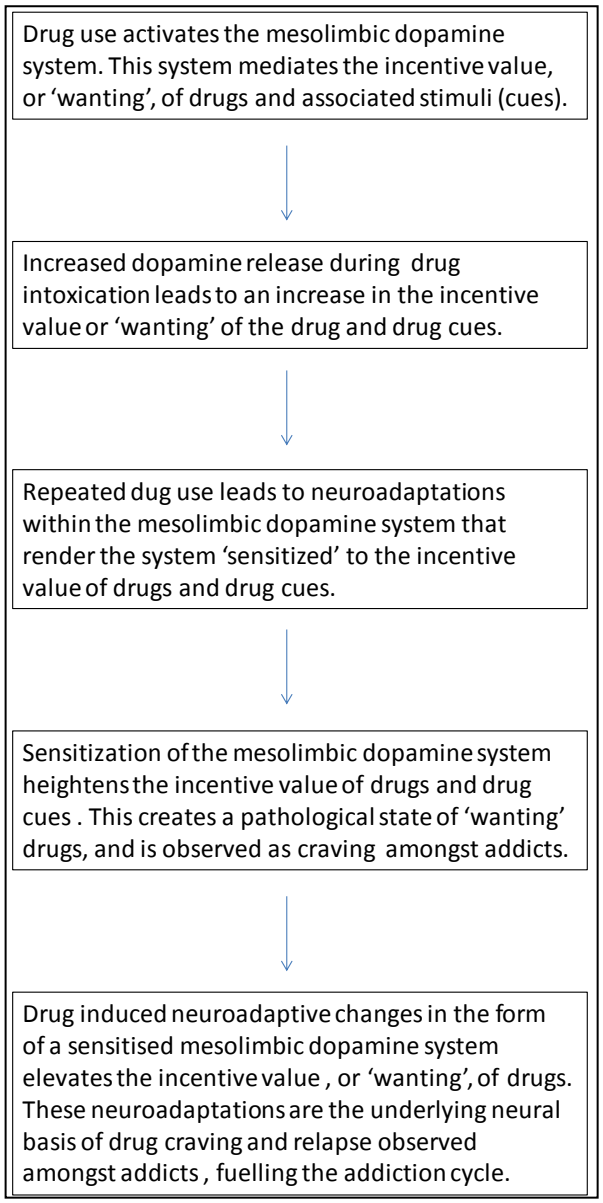

Figure 1.1: Flow diagram summarising key points in the incentive-sensitization theory of addiction.

1.4.1.1 Sensitization in Humans

Evidence of behavioural and neural sensitization as a consequence of drug use in humans has been demonstrated by Boileau et al., (2006, 2007). Boileau et al., (2006) found that repeated amphetamine exposure in healthy volunteers produced increased eye bink reactions and increased dopamine release in the striatum in response to amphetamine up to 1 year following their first amphetamine dose. This study therefore demonstrates that repeated amphetamine treatment in healthy subjects can cause enduring behavioural and neural sensitization. More specifically, neurochemical sensitivity within dopaminergic circuitry supports drug induced sensitization of the mesolimbic dopamine system. In addition, cues

associated with amphetamine can increase dopamine transmission within the ventral striatum and putamen within healthy controls (Boileau et al., 2007), expanding evidence of dopamine sensitivity to drug cues as well as the acute pharmacological effect of drugs.

The mesolimbic dopamine system is also assumed to mediate the psychomotor effects of drugs, such as enhanced locomotor activity, rotational behaviour and stereotyped patterns of behaviour (Wise & Bozarth, 1987). Consequently, a common indirect method of assessing sensitization of addictive drugs in animal models is through measuring psychomotor sensitization following drug exposure. Increased locomotor and stereotypy in response to repeated drug exposure is a well established phenomenon amongst psychomotor stimulants, including amphetamine, cocaine, methylphenidate, methamphetamine, nicotine, MDMA (Benwell & Balfour, 1992; Kalivas, Duffy, & White, 1998; Kuczenski & Segal, 1997; Pierce, Bell, Duffy, & Kalivas, 1996; Pierce & Kalivas, 1997; Segal & Kuczenski, 1997; Segal & Mandell, 1974; Shuster et al., 1982) and amongst non-stimulant drugs including morphine and alcohol (Crow, McWilliams, & Ley, 1979; Kalivas & Duffy, 1987). However, more direct evidence for incentive-sensitization comes from animal models measuring the incentive value of drug paired stimuli (cues) following repeated drug exposure. Assessment of behavioural motivation to seek and respond for drug paired stimuli allows for investigation of the incentive properties caused by primary drug reinforcement.

1.4.1.2 Behavioural sensitization to reward cues: Animal Studies

There is a wealth of evidence demonstrating that repeated administration of psychostimulants including amphetamine, cocaine and nicotine leads to increased pavlovian conditioned approach behaviour and increased sign-tracking of stimuli associated with the delivery of food reward (Doremus-Fitzwater & Spear, 2011; Hall & Gulley, 2011; Palmatier et al., 2013; Shiflett, 2012; Taylor & Jentsch, 2001). Amphetamine sensitization can also increase pavlovian-instrumental transfer (PIT) in comparison to saline treated animals. Wyvell & Berridge (2001) found that amphetamine sensitization can increase instrumental responding for a food-associated cue under extinction conditions in comparison to saline treated animals. This demonstrates that amphetamine can boost the incentive value of a natural reward cue to produce elevated instrumental responding for the cue even under extinction conditions. Amphetamine sensitization can also impair out-come specific pavlovian-instrumental transfer (Hall and Gulley, 2011; Shiflett, 2012). These findings have been interpreted to represent an amphetamine induced increase in transfer of general pavlovian associated incentive motivation of reward, such that specific PIT is superseded (Shiflett, 2012) Additionally, animals treated with a sensitizing regime of cocaine alongside conditioning with a cocaine-paired

stimulus, can acquire a novel instrumental procedure quicker than saline treated animals (Di Ciano, 2007). This indicates that drug cues can act as conditioned reinforcers in their own right to aid learning of novel instrumental behaviour. These studies collectively demonstrate that sensitisation to stimulants, and in particular amphetamine, can increase the incentive value, or 'wanting', for reward cues.

Rats treated with a methamphetamine regime that reliably induces locomotor sensitisation, however, do not increase sign-tracking behaviour for water reward (Michaels, 2012), and repeated administration on MDMA in rats does not increase approach behaviour for food reward (Taylor & Jentsch, 2001). These findings contrarily demonstrate that not all stimulants induce increasing 'wanting' for natural reward cues.

1.4.1.3 Dopaminergic sensitization to reward cues: Animal studies

A central component of the incentive-sensitization theory of addiction is that addictive drugs induce sensitisation towards the incentive value of drug reward through increasing the dopamine releasing properties of addictive drugs and drug related cues. In support of this, increased transmission of dopamine within the ventral striatum, and specifically the nucleus accumbens (NAcb), has been detected alongside locomotor and cue elicited behavioural sensitisation (Bassareo et al., 2013; Benwell & Balfour, 1992; Robinson et al., 1988). Direct elevation of dopaminergic transmission within the NAcb via intra-accumbens amphetamine administration has also been shown to increase the incentive value of a conditioned sucrose cue in comparison to saline treated animals (Wyvell & Berridge, 2000), and 6-hydroxydopamine lesions to the NAcb can severely impair acquisition, and to a lesser extent impair performance, within an appetitive pavlovian approach behaviour procedure (Parkinson et al., 2002). More recently, Bassareo et al., (2013) found that rats treated with a sensitizing regime of morphine developed increased approach behaviour for a drug-CS and non-drug-CS (food-CS), however, only increased dopamine release within the NAcb core and shell was simultaneously recorded during drug-CS approaches, and not non-drug-CS, demonstrating that only drug-CS developed increasing dopamine releasing properties. These findings draw attention to potential differences in dopamine evoked response in animal models measuring the incentive value of non-drug related cues

1.4.1.4 Transcriptional mechanisms of enduring neural sensitization

A further prediction of the incentive-sensitisation theory is that drugs of abuse cause enduring neural changes within reward circuitry that can facilitate heightened drug sensitivity even after

long periods of drug abstinence. Molecular changes in the induction of transcription factors, such as ΔFosB, within targets of the mesolimbic dopamine system have been proposed to underlay enduring changes in neural sensitisation (Renthal & Nestler, 2008). Chronic administration of stimulants and opioids induce accumulation of ΔFosB mRNA within the nucleus accumbens (Murphy et al., 2003; Nye et al., 1995; Nye & Nestler, 1996; Pich et al., 1997), and due to the long-lasting half-life of the ΔFosB isoform, this induction of ΔFosB is suggested to act as a molecular neural mechanism of prolonged sensitization to drugs (Renthal & Nestler, 2008). In addition, transgenic mice with over expression of ΔFosB in the nucleus accumbens show increased locomotor activity in response to cocaine (Kelz et al., 1999) and show greater sensitivity to the reinforcing effects of cocaine (Colby et al., 2003). These studies demonstrate drug induced alterations at the molecular level that can produce both long-term neural and behavioural sensitization.

1.4.2 Opponent Process Theory of Addiction

The opponent process theory of drug addiction (Koob et al., 1997; Koob & Le Moal, 2008) alternatively considers how neural adaptations acting to neutralise (oppose) the effects of drugs might lead to addiction. Conceptually, Koob and Le Moal (2008) propose that from initiating drug use a transition from impulsive to compulsive drug use emerges that drives a chronic state of drug relapse. The motivational framework of this theory builds upon Solomon & Corbit's (1974) proposition that opponent processes, in the form of negative feedback loops, are in place to regulate a normal homeostatic range of motivation. Koob and LeMoal (2008) expand upon this principle by proposing that an 'antireward' system is in place to limit excessive activity of reward circuitry within the central nervous system (CNS). An 'antireward' system is therefore recruited in response to drugs that directly elevate activity of the dopamine reward pathway. Activation of the 'antireward' system consequently counteracts the intensity of excessive dopamine activity, and therefore reward, within the CNS.

Neuroadaptations that limit reward are proposed to manifest via 'within-system' and 'between-system' adaptations. Within-system neuroadaptations desensitise the dopamine reward pathway in attempt to counter act excessive dopamine activity at a cellular level. Between-system adaptations recruit neural stress and emotion systems in attempt to further limit reward through the production of negative reinforcement. Recruitment of the antireward system however comes with a homeostatic pay off that is an elevation in the brains reward 'set point'. Elevation in reward 'set point' consequently means that a larger quantity of a drug is required in order to achieve the threshold for experiencing reward. This is commonly observed as tolerance during the escalation and maintenance of drug dependence amongst addicts. Continuous use of drugs is therefore proposed to drive a feed-forward shift from a homeostatic to an allostatic reward 'set point' (See Fig. 1.2). The persistence of an allostatic reward state, alongside increased sensitivity of stress and affective circuitry consequently creates a highly aversive affective state and persistent change in motivation that drives compulsive drug seeking and taking behaviour in the attempt to alleviate this highly aversive state of drug dependence (Koob and LeMoal, 2008). In following, the opponent process theory addresses how hallmark features of addiction such as tolerance, and the negative affective state of drug withdrawal, contribute towards the compulsive state of drug dependence.

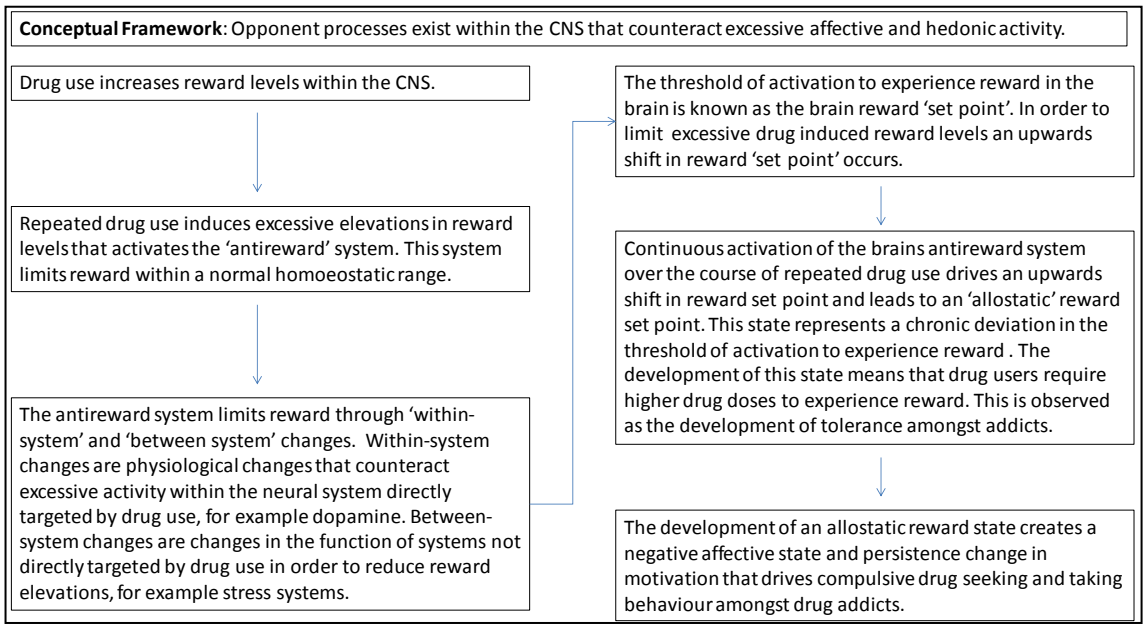

Figure 1.2: Flow diagram summarising key points in the opponent process theory of drug addiction.

1.4.2.1 Within-system neuroadaptations: Human literature

There is considerable evidence from human literature that chronic use of addictive drugs can downregulate activity of the mesolimbic dopamine system, and that such downregulation can persist long-term. For example, cocaine dependent subjects show blunted alpha-methyl-para-tyrosine (AMPT) induced increase in binding of the $D_{2/3}$ antagonist raclopride in comparison to controls (Martinez et al., 2009) indicating that cocaine dependent users have reduced endogenous dopamine transmission at the $D_{2/3}$ receptors within the striatum. Cocaine dependent subjects also show a blunted response to amphetamine induced reductions in raclopride binding within the striatum in comparison to controls (Martinez et al., 2007) indicating reduced drug induced pre-synaptic dopamine release within the striatum amongst cocaine users. Downregulation of the dopamine $D_{2/3}$ receptors within the striatum and ventral striatum is also prevalent amongst stimulant dependent users and detoxified abstinent users. Methamphetamine dependent subjects also show reduced $D_{2/3}$ receptors binding within ventral striatum using the $D_{2/3}$ antagonist fallypride (Lee et al., 2009). In addition, there is a positive correlation between $D_{2/3}$ receptor availability in the ventral striatum and craving in smokers (Fehr et al., 2008) linking reduced activity at the $D_{2/3}$ receptors within the ventral striatum with an important motivational variable in the maintenance of drug use.

Ex-cocaine and methamphetamine users also continue to display reduced $D_{2/3}$ receptor availability into protracted drug withdrawal and into long-term abstinence, supporting that within-system hypo-functioning dopamine is an observable long-term neuroadaptations in drug addicts. Detoxified cocaine subjects display reduced cocaine uptake and a blunted dopamine response within the striatum following MPH (Volkow et al., 1996, 1997). Abstinent methamphetamine subjects (abstinence range 2 weeks up to ~ 3 years) also display reduced $D_{2/3}$ receptor availability within the striatum in comparison to healthy controls (McCann et al., 1998; Volkow et al., 2001), supporting long-term downregulation of the $D_{2/3}$ receptors within the striatum amongst stimulant addicts. However, there is also evidence of some functional dopamine recovery following long-term drug abstinence, for example, abstinent methamphetamine users display increased striatal DAT density at 12-17 months of abstinence in comparison to 1 month of abstinence (Volkow et al., 2001). Striatal DAT recovery was also correlated with duration of methamphetamine abstinence (Volkow et al., 2001) and reduced striatal DAT density has been correlated with years of methamphetamine use (Volkow et al., 2001). Such abstinence associated recovery, and striatal DAT association with years of methamphetamine use, supports the notion that downregulation of dopamine function is a long-term neuroadaptation in association with drug exposure and is not necessarily a consequence of a pre-disposing neural state. Collectively, these studies support, firstly, downregulation of the mesolimbic dopamine pathway amongst chronic drug users, and secondly, the persistence of such dopaminergic downregulation long-term.

1.4.2.2 Within-system neuroadaptations: Animal literature

Evidence for the recruitment of neural opponent processes that can counteract drug induced reward is largely supported by animal models of intracranial self-stimulation (ICSS). Administration of many drugs of abuse can lower reward thresholds during drug treatment and elevate reward thresholds following the termination of drug treatment (Harrison and Markou, 2001; Kornetsky & Esposito, 1979; Markou & Koob, 1991; Paterson, Myers, & Markou, 2000; Schulteis et al., 1995; Wise & Munn, 1995). Drug induced reductions in reward thresholds are thought to reflect the high rewarding properties of the administered drug, resulting in reduced demand for ICSS. Elevated reward thresholds during drug abstinence are believed to be the result of opposing neuroadaptive responses to excessive drug induced reward, thus producing reduced sensitivity to previously reinforcing levels of electrical brain stimulation. Such changes therefore indicate a change in reward 'set point' as predicted by the opponent-process theory. Animals treated with long-access (11 hours) to drug self-administration also show reduced sensitivity to the reinforcing effects of the administered

drug long-term, indicating persistent changes in reward 'set point' following long access to drug self-administration (Ahmed & Koob, 1999). In addition, animals exposed to long-access (6 hours) drug self-administration develop an increase in break point during progressive ratio procedures (Paterson & Markou, 2003) and continue to self-administer more drug than controls even when this is paired with an aversive outcome, such as foot shock, (Ahmed, Walker, & Koob, 2000) indicating that drugs cause significant increases in the motivation to gain drug reward.

In addition to ICSS, measurement of neural adaptations following chronic drug exposure can now be measured using animal PET scans. Such methods are also useful for providing more direct comparisons between animal and human studies investigating drug induced neuroadaptations. Chronic methamphetamine treatment in monkeys has shown reduce $D_{2/3}$ receptor and DAT binding in the striatum for up to seven weeks and reduced DAT bingeing in the ventral striatum for up to two weeks following drug termination (Groman et al., 2012) similar to reduced $D_{2/3}$ receptor and DAT downregulation reported in the striatum of abstinent human methamphetamine users (Volkow et al., 2001; 2001). Such findings therefore support the hypothesis that repeated use of drugs can induce persistent neuroadaptations in dopamine transmission within the striatum. Interestingly, mice bred with gene knockout of arrestin3, a protein responsible for the internalisation of the D_2 receptor, and G protein-coupled receptor associated sorting protein (GASP-1), a protein responsible for moving internalised D_2 receptors to lyosomes, have been shown to display increased $D_{2/3}$ receptor binding throughout drug abstinence in comparison to wild-type mice (Skinbjerg et al., 2010; Thompson, Martini, & Whistler, 2010) suggesting that a physiological mechanism underlying reduced $D_{2/3}$ receptor binding might be increased internalisation of the D_2 receptor. These findings therefore support that downregulation of the D_2 receptors persist within dopamine nerve cells under conditions of increased dopaminergic tone.

1.4.2.3 Transcriptional mechanisms of enduring neural desensitization

Changes in the expression of transcription factors in dopamine cells provide molecular evidence of neuroadaptations in response chronic drug use. The induction of ΔFosB via chronic drug administration has been linked to the induction of epigenetic changes in chromatin packaging in individual cells within the nucleus accumbens (Robison & Nestler, 2011). One example of how an epigenetic mechanism might produce long lasting changes within the mesolimbic dopamine pathway, and that may contribute towards the long-lasting changes in drug desensitisation (tolerance), is through ΔFosB desensitisation of c-fos transcription. Induction of ΔFosB following chronic amphetamine has been found to decrease c-fos

transcription via recruitment of the histone deacetylase 1 (HDAC1) to the promoter region of the c-fos gene (Renthal et al., 2008). The authors interpreted the behavioural significance of this finding in relation to tolerance of drug reward, in consideration of mutant c-fos mice displaying reduced sensitivity to drug induced reward (Zhang et al., 2006). In following, ΔFosB induced suppression of c-fos transcription via an epigenetic mechanism involving histone methylation within chromatin structure, may serve as an example of how nerve cells mediating reward within the nucleus accumbens adapt to produce long-term alterations that can limit reward in response to chronic drug exposure. These molecular changes therefore illustrate how drugs might limit the reinforcing value of drugs following chronic exposure and how these changes persist at a molecular level long-term.

1.4.2.4 Between-system neuroadaptations

Between-system adaptations are proposed to exist in the form of sensitised stress systems within the brain (Koob and LeMoal, 2008). Chronic drug use and withdrawal are associated with increased activation of the HPA axis measured by with increased plasma cortisol and adrenocorticotropic hormone (ACTH) in drug users (Bannan et al., 1984; Contoreggi et al., 2003; Schluger et al., 2001). Similarly, chronic administration of many drugs can lead to an increase in corticotrophin-releasing factor (CRF) within the amygdala during drug administration and drug withdrawal in rats (Ambrosio, Sharpe, & Pilotte, 1997; Heinrichs et al., 1995; Koob et al., 1994; Pich et al., 1995; Richter & Weiss, 1999; Sarnyai et al., 1995). Systemic administration of CRF antagonists can also block increased cocaine and heroin self-administration (Goeders & Guerin, 2000; Greenwell et al., 2009) and stress (foot-shock) induced relapse in animal models (Koob, 2010; Le et al., 2000; Shalev, Erb, & Shaham, 2010). These findings therefore collectively highlight the motivational and affective value of CRF activity and the relationship between HPA axis activation with drug use, withdrawal and relapse.

1.4.3 Impulsivity: Definitions and Measures

Impulsivity can broadly be defined as acting upon internal urges without forethought for the long-term or detrimental consequences. The converse of impulsivity has been suggested to be 'self-control' (Monterosso & Ainslie, 1999), conceptualising impulsive behaviour as a lack of self-control to suppress internal urges in consideration of long-term or detrimental consequences. Whilst occasional behaviour that acts on impulsive urges can serve to produce positive behavioural outcome, continuous selection to act upon impulsive urges in spite of the associated negative consequences can lead to a state of maladaptive and dysfunctional behaviour, during which risky decisions and behaviours are maintained through a lack of impulse control and restraint. This maladaptive state of behavioural control is prominent amongst substance dependent individuals that are continually driven to seek out and take harmful drugs despite the aversive and negative consequences of such decisions and behaviours (Bechara et al., 2002; Goldstein & Volkow, 2002; Jentsch & Taylor, 1999). The clinical relevance of impulsivity to substance dependence is highlighted by the inclusion of several criteria relating to dysfunctional behavioural control within the ICD 10 codes, these include, 'a strong desire or sense of compulsion to take the substance', 'difficulties in controlling substance-taking behaviour in terms of its onset, termination, or levels of use' and 'persisting with substance use despite clear evidence of overtly harmful consequences' (ICD-10 Codes, 4th edition). These codes not only highlight the diagnostic relevance of impulsivity to addiction, but also draw attention to the importance of understanding impulsive behaviour in relation to the aetiology and pathology of substance dependence. Understanding the relationship between impulsivity and drug dependence may therefore create a pathway to novel treatment options for substance dependence.

1.4.3.1 Trait Impulsivity: Self-reported Impulsivity

Impulsivity is a prominent dimension of personality which is reflected through the prevalence of impulsive traits within all major models of personality (Five-factor model; Eysencks E-N-P; Tellegens 3 factor model). The most common measure of trait impulsivity in humans is the Barratt Impulsivity Scale (BIS) (Barratt & Patton, 1983).The BIS-11 is a 30 item questionnaire that probes for an overall score of trait impulsivity through the assessment of impulsive responses within three distinct sub-traits of impulsivity, these are, motor impulsivity (acting without thinking), non-planning impulsivity (lack of 'futuring' or forethought) and attentional impulsivity (attention and cognitive instability) (Patton & Stanford, 1995). BIS has been found to detected differences in trait impulsivity between current, former and recreational drug

users across a range of different drugs (Balodis, Potenza, & Olmstead, 2010; Clark et al., 2006; Lee et al., 2009; Mitchell, 1999; Moreno et al., 2012).

Trait impulsivity is also commonly assessed by the Impulsivity Venturesomess Extraversion Questionnaire (IVEQ; I_7) (Eysenck et al., 1985). This questionnaire measures impulsivity as a two component structure placing impulsivity as a constituent trait of psychoticism and extraversion. The I_7 is therefore related to Eysencks three factor model of personality and locates trait impulsivity within psychoticism and extraversion dimensions of personality. The dichotomy of impulsivity aligning with psychoticism and extraversion within the I_7 broadly related to sub-factors of the BIS measuring for non-planning and motor impulsivity, respectively. However, unlike the BIS, there is no measure within the I_7 that assesses cognitive inflexibility (attention impulsivity). Similar to BIS, the I_7 is sensitive to trait impulsivity within current, former and recreational drug users across a range of ages and different drugs (Clark et al., 2009; Morgan, 1998; Parrott, Sisk, & Turner, 2000; Sher, Bartholow, & Wood, 2000; Thompson et al., 2006).

More recently, Whiteside & Lynam, (2001) proposed a four-factor model of impulsivity incorporating measures of impulsivity through; 'Urgency' (negative urgency), 'Premeditation' (lack of premeditation), 'Perseverance' (lack of perseverance) and Sensation seeking, constituting the UPPS Impulsive behaviour scale. This four-factor model was derived from conducting factor analysis of ten self-report measures of trait impulsivity with 437 subjects, following which the 'UPPS' factors emerged. Negative urgency relates to the tendency to engage in impulsive behaviour under conditions of negative affect in order to alleviate such negative emotion despite potentially harmful consequences. Lack of premeditation refers to difficulty in thinking and reflection of the consequences of an act before engaging in an act, this definition maps onto personality dimensions relating to 'impulsive choice'. Lack of perseverance refers to the ability to remain focused on a task that may be difficult of boring, of which definition can be mapped on to personality dimensions relating to 'behavioural inhibition'. Sensation seeking refers to the pursuit of activities that are exciting and that may be dangerous and can broadly map onto other personality measures of impulsivity such as 'venturesomeness'. Additionally, 'Positive Urgency' was added to the UPPS scale to form the UPPS-P scale to assess impulsive behaviour related to rash and risky behaviours as a consequence of heightened and positive mood (Cyders et al., 2007). The UPPS is a powerful predictive tool of frequency and patterns of drug use across a range of different drugs (Coskunpinar, Dir, & Cyders, 2013; Moreno-López et al., 2012; Verdejo-García et al., 2010; Verdejo-García et al., 2007; Zapolski, Cyders, & Smith, 2009).

1.4.3.2 Behavioural Dimensions and Measures of Impulsivity

Impulsive behaviour spans a range of behaviours that are categorised by *impulsive reflection*, relating to the ability to gather and evaluate information before making a decision (Kagan, 1966), *impulsive choice*, relating to choice preference between immediate over delayed reward (Ainslie, 1975) and *impulsive action*, relating to volitional action restraint usually manifested through the ability to 'wait' to receive reward and the ability to 'stop' an behaviour once initiated.

1.4.3.2.1 Impulsive Reflection

Reflection impulsivity refers to the tendency to gather and evaluate information before making a decision (Kagan, 1966; Clark et al., 2006). Failure to sample all information before making a decision can bias decision making towards immediately rewarding or salient options without evaluation of all information (Clark et al., 2006). This bias can speed up the decision making process and result in rash or risky decision making based upon poor reflection of all available information. This dimension of impulsivity can be measured through the Matching Familiar Figures Test (MFFT) (Kagan, 1966) whereby subjects are required to identify an image from within 6 similar images that identically matches a template image. Impulsivity within this test is indexed through the speed to match images and accuracy, with more impulsive subjects producing quicker reaction times and reduced accuracy to match images due to poor information sampling before decision making. Children with hyperactive disorders make more errors on the MFFT (Sandoval, 1977) and stimulant treatment for ADHD children reduces errors within the MFFT reflecting improved impulsivity within child populations of poor inhibitory control (Brown & Sleator, 1979).

More recently, the Information Sampling Task (IST) was developed by Clark et al., (2006) to measure reflection impulsivity within the adult population. The IST presents subjects with a 5 x 5 square grid within which they must open up boxes to reveal the colour inside the box. Subjects are required to make a judgement on which colour is predominant throughout the whole grid. Within 'fixed win' (FW) trials subjects can open as many boxes as they wish within the grid before making a decision without losing points, however, within 'decreasing win' (DW) trials points correspondingly decrease as subjects select boxes to open, creating a low certainty to reward and high certainty to low reward condition. Consequently, the IST does not depend upon the speed of responding as does the MFFT, which may relate to executive or visual processes, in order to index impulsivity. Reflection impulsivity is assessed through the average number of boxes opened before making a decision of overall grid colour and the number of incorrect judgements, of which demonstrate a subjects requirement for sourcing

information before making a decision and the consequence of poorly evaluated decisions in relation to performance.

1.4.3.2.2 Impulsive Choice

Impulsive choice refers to the preference between receiving a small immediate reward or a large delayed reward (Ainslie, 1975). Decline in the perceived value of delayed reward over time can be termed 'temporal discounting'. Greater preference for immediate reward over delayed reward is termed 'impulsive' as this choice reflects greater discounting of delayed reward value in preference for reward immediacy (Monterosso & Ainslie, 1999). This dimension of impulsivity is interlinked with cognitive decision-making processes and the valuation of reward based on temporal dynamics. Amongst humans and animals, temporal discounting functions in a hyperbolic curve (Madden et al., 1997; Mazur, 1987; Petry, 2001; Richards et al., 1999), whereby steep discounting exists during initial/ early intervals of time, reflecting generalised human and animal motivation bias to gain immediate reward, and shallow discounting exists through longer time delays, reflecting the existence of some long-term reward magnitude and preference but generally to a lesser extent than immediate reward magnitude (Monterosso & Ainslie, 1999). Human and animal measures of impulsive choice can be employed through delayed reward and delay discounting task whereby the individual or animal is given a choice of receiving a small fixed reward immediately or a large delayed reward over varying delays (eg. 10, 20, 40, 60 seconds in an animal model). Impulsive choice can be accessed through measuring the proportion of choice for delayed reward in comparison to choice for immediate reward (Cardinal, Robbins, & Everitt, 2000; Evenden & Ryan, 1996). Another assessment of impulsive choice is through adjusting the delay (Mazur, 1987) or adjusting the amount (magnitude) of fixed delay reinforcers (Richards, Zhang, et al., 1999) until subjects choose both immediate and delayed reinforces equally. This point is referred to as the 'indifference point' and represents the difference, in either delay or reinforcer magnitude, between which choice preference is equal between immediate and delayed reward.

1.4.3.2.3 Impulsive Action

'Impulsive action' refers to poor behavioural restraint. Examples of this dimension of impulsive behaviour include acting prematurely, behaving inappropriately, and difficulty in stopping and changing inappropriate behaviour. This dimension of impulsivity, also termed *behavioural disinhibition*, can be assessed in humans and animals through measuring a subject's ability to withhold from making a prepotent response, assessing action restraint in the form of 'waiting',

and through measuring a subject's ability to inhibit or cancel an on-going behaviour, assessing restraint of initiated action in the form of 'stopping'.

Behavioural inhibition is commonly measured in humans using one of the following tasks: continuous performance task (CPT) (Cornblatt et al., 1988; Rosvold et al., 1956), the immediate memory task/ delayed memory task (IMT/ DMT) (Dougherty, Marsh, & Mathias, 2002), go/no-go task (Go/No-go) (Newman, Widom, & Nathan, 1985), stop-signal task (SST) (Logan & Cowan, 1984).

The CPT (Rosvold et al., 1956) assesses a subject's ability to continuously track and respond to target stimuli, and appropriately inhibit responding to non-target stimuli. This task therefore requires both sustained attention and inhibitory control over prepotent responding for target stimuli. Stimuli usually consist of a series of letters of numbers (eg. AB) that are presented individually across trials. Following the presentation of target stimuli, for example 'X', subjects must make a correct response, recorded as a 'correct detection'. A response made to non-target stimuli is recorded as a 'commission error'. Failure to respond to a trial is recorded as an 'omission error'. Within this task, impulsivity is indexed according to commission errors and attention is indexed according to omission errors.

The IMT/DMT is a more complex variation of the CPT that was developed for use amongst non-clinical populations with higher cognitive capacities than clinical populations with impaired processing (Dougherty et al., 2002). This task maintains the same framework of a CPT in that subjects must respond to target stimuli and inhibit responding to non-target stimuli, however, additional task parameters create greater memory, attention and inhibitory demands on subjects than a CPT. Within the IMT/DMT 2- 7 digits (eg. 20417) are presented individually across trials. In the IMT subjects are required to respond when the numbers currently presented on screen match the numbers presented in the preceding trial, this reflects a 'target' (eg. 20417-20417 = target). In the DMT, three distracter stimuli are presented between target stimuli (eg. 20417 – 12345 – 12345- 12345 – 20417 = target). This therefore extends the length of time subjects must remember initial target stimuli, sustain attention and inhibit inappropriate/ early responding. Within both the IMT and DMT, stimuli that closely resemble the target stimuli are presented, termed 'catches' (eg. 20417-20415). Similarly to the CPT, impulsivity in this task is indexed according to commission errors.

Go/No-go tasks have a similar framework to continuous performance tasks in that subjects must make active and passive responses depending on the presentation of particular targets. However, a Go/No-go task requires passive responding when signalled by specific No-go

stimuli, as opposed to a non-target that may span multiple stimuli. Subjects must respond as fast as possible to 'Go' stimuli and inhibit responding following the presentation of 'No-go' stimuli. In order to bias active responding in subjects, the proportion of presented Go trials is usually greater than that of No-go trials, to enforce prepotent responding for Go trials. Go and No-go stimuli may consist of numbers, letters or colours. Correct and incorrect responses are usually reinforced through written 'correct'/'incorrect' or auditory feedback between trials (Fillmore et al., 2003; Verdejo-García, Perales, & Pérez-García, 2007). The proportion of responses made following No-go stimuli (commission errors) is used as a behavioural index of response disinhibition. High levels of commission errors within Go/No-go tasks therefore represent poor prepotent response inhibition.

In contrast to the CPT, IMT/DMT and Go/No-go tasks, the stop-signal task (Logan & Cowen, 1984) measures a subject's ability to stop or cancel an on-going action. This dimension of behavioural inhibition is hypothesised to function in a 'race model' where inhibitory control processes that control 'stopping' on-going behaviour compete against 'go' processes driving initiated behaviour in order to determine the outcome of behaviour (Logan & Cowan, 1984; Logan, 1994). 'Stop' and 'Go' response mechanisms are assumed to be independent processes meaning that when both processes are activated a race to reach the end point of each process begins before the outcome behaviour can be selected by the organism. The Stop process is assumed to be quicker than the Go process, allowing for inhibitory control mechanisms to successfully inhibit an initiated behaviour under conditions where enough time is available for the inhibitory process to finish before the Go process. The stop-signal theory therefore builds a model of action cancellation whereby if an inhibitory signal is presented at close temporal proximity to the signal to respond stimulus it is likely that the signal to respond action will be successful inhibited and the signalled action 'cancelled', however the further away the stop signal is presented from the signal to respond stimulus the harder the action will be to inhibit because the Go process will finish before the Stop process can be fully executed. Within this paradigm impulsivity can therefore be indexed as an estimation of speed of the internal inhibitory Stop process based on the speed of responding during signal to response trials where no Stop signal is present and the probability of inhibition at any given delay. This calculation is known as the stop-signal reaction time (SSRT) and can be calculated as a function of Go reaction time (RT) and the probability distribution of inhibition at the stop signal delay (SDD). Impulsive behaviour within the stop signal paradigm is reflected in long SSRT which represents a slow internal inhibition processes indicating that it is difficult to inhibit or cancel an initiated behaviour.

Within the stop-signal task for humans, subjects are presented with a series of Go and Stop trials cued by specific stimuli. For example, a dot (•) and letter 'X' may represent Go and stop trials, respectively. Around two thirds of the trials are usually Go trials and one third Stop trials. During a Go trial, the Go stimulus (•) will appear on screen for brief duration of (1- 2 s) during which time subjects must make an active response. This encourages fast prepotent responding to Go trials. During a Stop trial, the Go stimulus will appear on screen (•) followed by the Stop stimulus (X) presented at varying delays from the presentation of the Go stimulus in milliseconds (eg. 100, 200, 300, 400, 500 ms). Using the Go RT and SSD, subjects SSRT can be calculated to index behavioural control of action cancellation.

Behavioural inhibition in animals can be measured using operant based tasks that are conducted in skinner boxes. Many of these tasks employ comparable parameters to that of human behavioural inhibition tasks. The five choice serial reaction time task (5CSRTT) originally designed to measure attention (Carli et al., 1983) has been the most frequently employed in animal studies to measure behavioural inhibition and is comparable to continuous performance tasks. During this task animals are required to constantly scan an array of five apertures during a five second inter-trial interval (ITI) period prior to the random brief illumination (0.5 s) of one of the apertures. The animals then have to respond in the illuminated aperture to receive a food reward. Inappropriate premature responding in the apertures prior to the presentation of the visual stimulus is a measure of the animals behavioural restraint or waiting ability. High levels of premature responding indicates poor levels of behavioural inhibition. In addition, nose pokes made in an aperture immediately after a correct response are recorded as perseverative responses. This type of behaviour is thought to reflect an inability to stop an action despite no programmed consequence, reflecting compulsive repetition.

Another method of assessing behavioural inhibition in the form of 'waiting' is through the differential reinforcement of low rates schedule (DRL) (Evenden, 1999). In this task reinforcement is gained through low levels of behavioural responding. Animals are required to nose poke an active hole following a fixed internal of time, requiring animals to successfully employ timing accuracy and behavioural inhibition during the fixed interval. This interval is called the inter-response time (IRT), and can vary according to the particular demands of an experiment. For example, a DRL30 schedule represents a 30 s schedule of reinforcement whereby nose pokes must be separated by at least 30 s. Responses made during the IRT (30 s) represent non-rewarded responding and are therefore used as an index of behavioural inhibition. Responses made during the IRT can be separated in to bins per second and used to

assess the distribution of premature responding as a function of time. This enables assessment of differential patterns of premature responding over time.

Animal versions of the Go/No-go task are also frequently used to assess behavioural inhibition. Within this task animals are trained to discriminate between Go and No-go stimuli and respond (nose poke/ lever response) or inhibit responding, respectively. The presentation of a Go stimulus constitutes a Go trial and presentation of No-go stimuli constitutes a No-go trial. Trials are usually cued via a light stimulus, for example, light stimulus on and light stimulus off for Go and No-go trials, respectively (Fletcher, 1993). Reinforcement can be delivered either asymmetrically (food reward following correct Go trials only) (Fletcher, 1993), or symmetrically (food reward following correct Go and No-go trials) (Harrison et al., 1999). Go and No-go trials are usually presented equally across the total number of trials. Animals are initially trained to respond under Go trial conditions before receiving No-go trial training, consequently, responding during Go trials is considered the prepotent response behaviour within this task, and thus successful inhibition during No-go trials represents inhibition of a prepotent response. Impulsive behaviour within Go/No-go tasks is therefore indexed by responses made during No-go trials.

Action cancellation can also be measured in rats using a rodent version of the stop-signal task (Eagle & Robbins, 2003). Within this task, animals are required to respond rapidly during Go trials and to cancel an initiated response following the presentation of a stop signal (Stop trial). Stop trials are presented during 20% of the stop-signal task to ensure that animals do not develop an inhibitory response strategy that may invalidate calculation of the SSRT. To initiate a Go trial animals must make a nose poke in a central food well that subsequently presents a lever on the left hand-side of the chamber and illumination of a light above the lever. Animals are required to press the left lever following which the lever is retracted and light extinguished. A lever on the right-hand side of the chamber is then presented and light above the lever is illuminated. Animals must quickly press the right lever to complete the Go trial and receive a food reward, representing a correct go trial. The response speed from left lever press to right lever press is controlled by a limited hold period. The limited hold is calculated per animal in relation to the time required to make a fast Go response and successful action cancellation, for example 1.1- 1.4 s (Eagle et al., 2011) During Stop trials animals initiate the trial in the same manner as a Go trial; however, following left lever press a stop-signal tone is presented. The delay at which the tone is presented varies depending on animals individual GoRT. Subsequently individual SSRT for each rat can be calculated based on individual GoRT and SSD.

1.4.3.3 Correlation between dimensions of impulsivity

These dimensions highlight the complexity of impulsivity, such that impulsivity has been defined as a multidimensional construct (Evenden, 1999). The relationship between self-report, choice and action probing measures of impulsivity has produced contrasting evidence over whether these dimensions are related or not. BIS and I_7 scores correlate with delay and probability discounting, and false alarm rate within the IMT/DMT (Richards et al., 1999; Swann et al., 2002) and delay discounting to loads on to overall BIS scores (Meda et al., 2009). However, BIS and I_7, have also been found to produce no correlation with behavioural measures of impulsive action and choice, including the SST, delay and probability discounting and IMT/DMT (Broos et al., 2012; Dougherty et al., 2009; Lane et al., 2003; Reynolds et al., 2006). A recent meta-analysis of studies exploring the relationship between self-report and behavioural measures of impulsivity in humans identified a small overall correlation (r=0.097) between self-report and behavioural measures impulsivity in humans (Cyders & Coskunpinar, 2011). Authors, however, concluded that generally self-report and behavioural measures do not correlate and that any correlation observed is usually small (Cyders & Coskunpinar, 2011).

The relationship between different behavioural forms of impulsivity has also been found to be unrelated. Delay discounting and SSRT is uncorrelated in humans (Broos et al., 2012; Reynolds, 2006) and delay discounting is uncorrelated with premature responding on the 5CSRTT within the same cohort of rats (Broos et al., 2012) suggesting that impulsive choice and action are independent behaviours. Interestingly, animals displaying high levels of impulsivity in the 5CSRTT do not show longer SSRT in comparison to less impulsive animals (Robinson et al., 2009), indicating that not only are impulsive choice and action dissociable, but also sub-dimensions of impulsive action are behaviourally independent. Additionally, treatment of amphetamine reduces impulsive choice whilst increasing premature responding, and atomoxetine produces an increase in impulsive choice and decreases impulsive action within the same cohort of rats (Broos et al., 2012), indicating that impulsive choice and action might be both behaviourally and pharmacologically dissociable. Global serotonin depletion has also been shown to preferentially disrupt premature responding whilst remaining undisruptive to delay discounting in rats (Harrison, Everitt, & Robbins, 1997; Winstanley et al., 2004), collectively indicating that impulsive choice and action are neurally dissociable within dopamine, noradrenalin and serotonin systems in rats. However, Robinson et al., (2007) reported that atomoxetine reduces delay discounting, premature responding and stop-signal reaction time in separate cohorts of rats, suggesting a possible underlying noradrenalin relationship between these behavioural measures of impulsivity in rats. Additionally, Robinson

et al., (2009) reported that highly impulsive rats on the 5CSRTT also showed steeper discounting of delayed reward than less impulsive animals, suggesting that there might be some overlap in impulsive action and choice in animals that are impulsive at baseline. In conclusion, there appears to be support for orthogonal dimensions of impulsivity, most convincingly between impulsive choice and action; however, there is also evidence for some pharmacological overlap in animal models and behavioural overlap in animals that are highly impulsive.

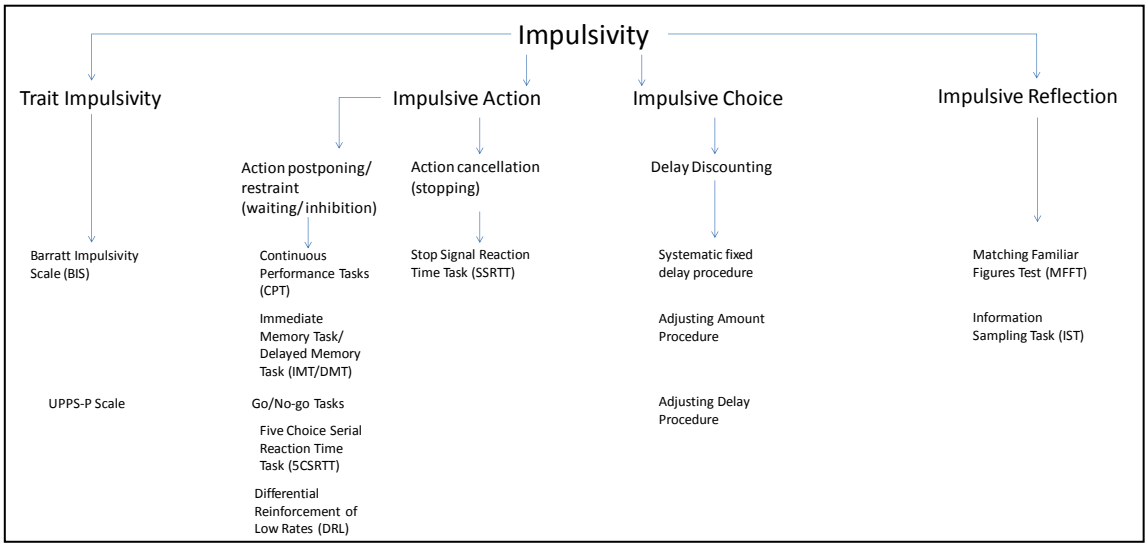

Figure 1.3: Diagram to depict the multi-dimensional concept of impulsivity and the tasks used to assess these dimensions in both humans and animals.

1.4.4 Impulsivity and Drug Addiction: The Relationship

1.4.4.1 Initiation and Acquisition of Drug use

A wealth of evidence has reported that dependent and recreational drug users have elevated trait impulsivity, suggesting that impulsivity might act as a predated risk factor for drug use. However, there is also evidence that drug use can predict changes in trait impulsivity (Quinn, Stappenbeck, & Fromme, 2011) therefore suggesting that drug use per se might contribute towards the differences in trait impulsivity observed in drug users. Longitudinal studies are therefore required to identify whether trait impulsivity is a pre-disposing risk factor for the initiation and acquisition of drug use.

Trait impulsivity at 13 years can longitudinally predict alcohol, cigarette and illicit drug use at 18 years and the age that alcohol, cigarette and illicit drug use was initiated within a sample of 777 adolescent subjects (Hartman et al. 2013). More specifically, high scores of self-reported novelty seeking, defined as self-reported impulsive behaviour and thrill seeking, were associated with a younger age of first time alcohol, cigarette, illicit drug use, and greater experimentation with illicit drugs reported at 18 years (Hartman et al. 2013). This indicates that trait impulsivity in young adolescents can increase the risk of earlier initiation with drugs and the likelihood of initiating experimentation with more than one drug through adolescence. Self-reported novelty seeking was also found to be the best predictor of reported alcohol, cigarette and illicit substance use at 18 years relative to personality traits that can also predict substance use, including, harm avoidance, reward dependence and lack of perseverance (Hartman et al. 2013).

Trait impulsivity predating the initiation and acquisition of drug use can also be exemplified through the relationship between childhood ADHD and the risk of substance use. Adolescents with a diagnosis of ADHD report greater illicit drug use and daily smoking than non-ADHD controls and childhood symptoms of ADHD are associated with the age of first cigarette and illicit drug use (Molina & Pelham, 2003), indicating both greater initiation and acquisition of drug use amongst a population with increased impulsivity. A ten year follow up of children with ADHD also recently reported that ADHD at 10 years predicts cigarette smoking at 20 years (Wilens et al., 2011). Symptoms of hyperactivity and impulsivity, but not inattention, in children with ADHD at 14 years has also been found to increase the odds ratio for nicotine, alcohol and illicit substance use at the age of 18 years (Elkins, McGue, & Iacono, 2007). These studies therefore support that impulsive traits in children can longitudinally increase the chance of initiating drug use and risk of acquisition repeated/ daily drug use.

In addition to evidence that trait impulsivity can predict the initiation and acquisition of drug use, impulsive behaviour has also been linked to these early stages of addiction. Steep discounting of hypothetical monetary reward correlates with the age of first alcohol, cigarette and marijuana use, and the total number of illicit drugs used within a student population (Kollins, 2003). This suggests that the chance of initiating and experimenting with drugs might increase as a result of insensitivity to future rewards that might act as an incentive to prevent drug use initiation. Steep reward discounting in rats also predicts faster acquisition of cocaine self-administration in comparison to less impulsive rats (Perry et al. 2005; Perry et al. 2008) similarly suggesting that reduced value of delayed reward might increase the likelihood of acquiring drug use. However, rats that are screened for high impulsivity (HI) within delay discounting procedures do not differ in comparison to less impulsive rats in acquiring cocaine or nicotine self-administration (Broos et al. 2012b; Diergaarde et al. 2009). This suggests that discounting of future rewards may predispose vulnerability towards faster acquisition of drug use, whilst greater delay discounting per se may not be involved in this early stage of addiction.

Rats displaying trait impulsivity on the 5CSRTT, defined by high levels of premature responding within this task, acquire nicotine and cocaine self-administration faster than rats displaying low levels of premature responding (Dalley et al., 2007; Diergaarde et al., 2009) suggesting that low levels of behavioural inhibition at baseline might increase the risk of acquiring drug use. Rats displaying this high impulsivity phenotype on the 5CSRTT, however, do not show any difference in the acquisition of MDMA self-administration in comparison to less impulsive rats (Bird & Schenk, 2012) and similarly, trait impulsivity measured by BIS in subjects categorised ‘at risk’ of MDMA use does not longitudinally predict MDMA use in these subjects (de Win et al., 2006). This suggests that reduced behavioural inhibition might increase vulnerability to the acquisition of stimulants that have a high, but not low, abuse potential in humans. Subjects with reduced action restraint on the SST also report greater ‘satisfied’ and ‘want more’ feelings following acute nicotine (Perkins et al., 2008), additionally suggesting that individual differences in behavioural inhibition may predict greater sensitivity to nicotine following initiation, increasing the chance of acquisition.

In summary, these studies support that greater trait impulsivity, impulsive choice, and impulsive action that predates drug use might increase the chance of initiating and acquiring drug use during these early stages of the addiction.

1.4.4.2 Maintenance

A pattern of drug use that might be considered exemplary of the 'maintenance' stage is smoking. Smokers typically maintain a steady and regular level of nicotine intoxication throughout waking hours. As mentioned within the initiation/ acquisition section, ADHD status and impulsive symptoms amongst children with ADHD can longitudinally predict daily smoking and nicotine dependence (Elkins et al., 2007; Wilens et al., 2011) suggesting that greater levels of trait impulsivity can predict maintained drug use. Smokers from non-clinical samples also report greater levels of trait impulsivity than never smokers (Mitchell, 1999) and display higher levels hypothetical monetary discounting than ex smokers and non-smokers (Bickel, Odum, & Madden, 1999) suggesting that maintained drug use in non-clinical populations might also be associated with impulsivity.

Animal models examining the relationship between impulsivity and maintenance have, however, generally indicated that higher levels of impulsive action and choice at baseline do not affect maintained drug self-administration. Animals screened for high and low trait impulsivity on the 5CSRTT do not show any difference in the number of cocaine infusions earned during self-administration when access to cocaine is low, for example when cocaine infusions are limited to phases of 40 minute access over 2.75 hours (Belin et al. 2008). Similarly, animals screened for high and low levels of impulsive choice within an adjusting delay procedure do not differ in the number of cocaine infusions earned when self-administration is limited to 20 infusions or a limit of 2 hours (Anker et al. 2009; Perry et al., 2005). This suggests that individual differences in impulsive action and choice might not affect drug use that resembles relatively controlled drug use. Interestingly, however, rats with low trait impulsivity that have limited access (20 infusions per self-administration session) to cocaine self-administration for 14 days become more impulsive (Anker et al., 2009) suggesting that low levels of maintained drug use might actually increase impulsivity in low impulsive rats. Taken together these might suggest that whilst differences in baseline levels of impulsivity do not affect maintained drug use, maintained drug use itself might increase impulsivity, possibly in relation to baseline levels of impulsivity.

1.4.4.3 Escalation

Trait impulsivity measured by the I_7 and BIS can predict binge drinking and the development of alcohol dependence, of which both outcomes represent escalated and uncontrolled drug use (Carlson, Johnson, & Jacobs, 2010; Moreno et al., 2012; Sher, Bartholow, & Wood, 2000; Verdejo-García et al. 2007). The motor sub-scale within BIS has been found to specifically

correlate with the number of drinks consumed per drinking session and was found to be the best predictor of binge drinking within these studies (Carlson et al., 2010; Moreno et al., 2012). This suggests that high self-reported impulsive action, rather than forethought or attention measured by BIS, might act as a risk factor for escalating alcohol consumption. Similarly, a recent meta-analysis by Coskunpinar et al., (2013) found that 'lack of perseverance' within the UPPS scale predicts the quantity of alcohol consumed whilst all other personality dimensions measured by UPPS predict the frequency of drinking. This suggests that whilst overall scores of 'trait impulsivity' measured by both BIS and UPPS can predict frequent drinking, escalation in the quantity of alcohol consumed once drinking is initiated is specifically related to reduced action restraint. Alcohol intoxication also acutely reduces behavioural inhibition measured by Go/No-go and SST tasks (Caswell, Morgan, & Duka, 2013; de Wit, Crean, & Richards, 2000; Fillmore & Vogel-Sprott, 1999; Ramaekers & Kuypers, 2005) but does not affect more cognitive dimension of impulsivity including reflection impulsivity and delay discounting procedures (Caswell et al., 2013; George, Rogers, & Duka, 2005; Ortner, MacDonald, & Olmstead, 2003; Richards, Zhang, et al., 1999). These findings again support impulsive action rather than cognitive dimensions of impulsivity in escalating alcohol consumption, and demonstrate that the acute effects of alcohol might also increase the chance of escalating alcohol consumption once drinking is initiated. Both increased trait impulsivity and drug induced effects on impulsivity might therefore increase vulnerability to escalate alcohol use.

'Negative Urgency' has also been identified as a pathway to problematic drinking and substance dependence. Negative urgency acts as a mediator in the development of high quantity uncontrolled alcohol consumption that is harmful to subjects (Adams et al., 2012) and can predict alcohol, methamphetamine and cocaine dependence (Verdejo-García et al., 2007). Whilst other measures within UPPS were found correlate with and predict problem drinking and substance dependence, negative urgency was identified as the greatest mediator and predictor of both these outcomes. This indicates that escalation of alcohol and stimulant use to harmful and uncontrollable levels can also result through reduced impulse control that is driven by a negative emotional state.

Impulsivity measured by the I_7 are is also associated with 'heavier' MDMA use (30- 1000 occasions) whilst subjects that report less frequent MDMA use (0- 30 occasions) or no MDMA use have lower I_7 scores than subjects reporting heavy MDMA use (Parrott et al., 2000). This indicates that even though both these groups initiate and engage with repeated MDMA use, subjects with greater trait impulsivity actually escalate the frequency of their usage. Frequent monthly (heavy) MDMA use also correlates with reduced behavioural inhibition within the IMT/DMT (F Gerard Moeller et al., 2002) suggesting that subjects with reduced behavioural

inhibition might be more likely to escalate to heavier monthly drug use than subjects with better inhibitory control. Similarly, behavioural disinhibition amongst recreational cocaine users correlates with lifetime cocaine use (Colzato, van den Wildenberg, & Hommel, 2007) and the severity of cocaine use measured as a composite of years, frequency per month and dosing per usage is associated with more errors of commission on a Go/No-go task and can predict more errors on the stroop task (Verdejo-Garćia et al., 2005; Verdejo-García et al., 2007). Subjects that report heavy MDMA use also have reduced reflection capacity within the MFFT in comparison to healthy controls, that also correlates with peak lifetime does of MDMA (Quednow et al., 2007). This suggests that subjects that make faster and less informed decisions are more likely to administer greater quantities of MDMA per dose (pill) taken.

Greater frequency of illicit drug use and quantity of poly drug use amongst substance users also correlates with disadvantageous decision making within the IGT (Hanson, Luciana, & Sullwold, 2008). This suggests that amongst subjects that have all acquired drug use, those that escalate their use to a greater frequency and greater level of polydrug use, make more bad decisions than subjects will less frequent drug use and polydrug use. Similarly, Bechara et al., (2001) found that the severity of dependence calculated using a composite index of: days abstinent, years of dependence, episodes of treatment and employment status, correlates with poor performance on the IGT. This indicates that drug users that make more disadvantageous decisions also show more severe escalation of dependence than drug users with lower dependence severity. Subjects that engage in more frequent drug use, poly-drug use and report more severe dependence, of which all circumstances are symptomatic of escalated drug use, are therefore associated with poor decision making capacity relative to other drug users. Disadvantageous decision making during phases of escalation (increased frequency, quantity and duration of drug use and dependence) might then render drug users more likely to make choices that are associated with negative or detrimental outcomes resulting in fast escalation of drug use.

Animal studies addressing the predictive role of trait impulsivity in the escalation of drug use have identified that rats with trait impulsivity on the 5CSRTT escalate stimulant, but not opiate, use at faster rate than less impulsive animals (Dalley et al., 2007; McNamara et al., 2010). Rats displaying this high impulsivity phenotype also display higher break point to earn stimulant reward during progressive ratio (PR) schedules in comparison to low impulsive animals (Belin et al., 2008; Diergaarde et al., 2009). Taken together, these studies might suggest that highly impulse rats escalate cocaine self-administration faster than low impulsive animals due to increased sensitivity to the reinforcing effects of cocaine. In addition, rats with trait impulsivity on an adjusting delay task also escalate cocaine self-administration under extended access

conditions at a faster rate than rats that are less impulsive at baseline (Anker et al., 2009). This high impulsivity phenotype within impulsive choice models can also predict amphetamine induced conditioned place preference (CPP) and inelastic demand for cocaine and nicotine (Koffarnus & Woods, 2013; Yates et al., 2012). Inelastic demand is an index of 'essential' value of a reward that is measured by consumption and not responding of reward. This further suggests that highly impulsive rats have greater sensitivity to the reinforcing effects of stimulants, and associate greater 'essential' value with drugs that may both leads to faster escalation in drug use.

However, it is also possible that increased impulsivity observed during phases of escalation in dependent and non-dependent drug users is a consequence of direct disinhibitory effects of drugs. Administration of stimulants including amphetamine and cocaine can acutely increase impulsive action in stimulant dependent subjects (Fillmore, Rush, & Hays, 2002; Fillmore, Rush, & Marczinski, 2003) suggesting that the acute effects of these drugs in dependent subjects might directly contribute to reduced inhibitory control during phases of escalation or 'bingeing'. Acute amphetamine also increases premature responding in rats performing the 5CSRTT, increases SSRT and decreases No-go trial accuracy within a symmetrically reinforced Go/No-Go task in rats (Eagle & Robbins, 2003; van Gaalen et al., 2006; Harrison et al., In prep). Similarly, acute administration of cocaine and nicotine increases premature responding within the 5CSRTT and decreases No-go trial accuracy within a symmetrically reinforced Go/No-go task in rats (Kolokotroni, Rodgers, & Harrison, 2011; van Gaalen, Brueggeman, et al., 2006). Acute nicotine also increases impulsive choice within delayed reward and adjusting delay procedures in rats (Dallery & Locey, 2005; Kolokotroni et al., 2011), indicating that nicotine can acutely increase both impulsive action and choice. Similarly, acute morphine administered to rats increases behavioural disinhibition within the 5CSRTT and increases impulsive choice within delayed reward and adjusting amount procedures (Kieres et al., 2004; Pattij et al., 2009).

Such disinhibitory effects of drugs may therefore fuel greater escalation of drug use specifically during phases of bingeing. These drug induced effects might be particularly relevant to patterns of dependence typical of stimulant and opiate dependence, where the binge phase of the addiction cycle is very intense and significant to this pattern of drug use and dependence (Koob & Le Moal, 2008). However, it is noteworthy that not all stimulants increase impulsivity, but can acutely reduce impulsivity. Amphetamine, methamphetamine and methylphenidate acutely improve impulsive choice (Pitts & McKinney, 2005; Richards, Sabol, & de Wit, 1999; van Gaalen et al., 2006b). The direct drug induced effects in relation to impulsivity during this

phase of addiction is therefore particularly complex amongst stimulant drugs that exert both acute inhibitory and disinhibitory actions on subjects.

In addition to the acute effects of drugs, the effects of repeated drug administration can also lead to direct effects on impulsivity in subjects. Long access (6 hrs) to 0.3 mg/kg infusions of amphetamine self-administration over 21 days reduces the mean adjusted delay (MAD) in rats during this phase in comparison to rats receiving short access self-administration, and these rats also escalated amphetamine self-administration during this phase (Gipson & Bardo, 2009a). Non-contingent repeated cocaine administration also lowers indifference points in rats (Paine, Dringenberg, & Olmstead, 2003) and chronic nicotine administration increases impulsive action and choice in rats (Dallery & Locey, 2005; Kolokotroni, Rodgers, & Harrison, 2012). This indicates that repeated exposure to stimulants can increase impulsive action and choice during phases of drug administration during which escalation of drug use takes place. Such disinhibitory actions following acute and chronic exposure to drugs might therefore lead to reduced behavioural control following initial phases of drug acquisition and might subsequently increase the chance of escalating drug use due to reduced control over limiting drug intake.

The acute and chronic effects of drugs on impulsivity have also been shown to interact with baseline levels of inhibitory control, particularly amongst stimulants. This suggests that the combination of both baseline impulsivity and drug induced inhibitory/ disinhibitory actions might contribute towards escalation of drug use. For example, acute amphetamine reduces behavioural inhibition in subjects that exhibit low behavioural inhibition at baseline but does not affect inhibitory control in subjects with displaying 'normal' behavioural control at baseline (de Wit, Enggasser, & Richards, 2002; Fillmore, Kelly, & Martin, 2005). Conversely, amphetamine administered to stimulant users, a subject group that are more impulsive than controls (Clark et al., 2006), increases impulsivity in these subjects (Fillmore et al., 2003). A possible interpretation of amphetamine induced impulsivity amongst stimulant users and amphetamine induced behavioural control amongst healthy controls, is that prior drug exposure may alter the pharmacological-neural relationship underlying amphetamine induced impulsivity. This would mean that the acute effect of amphetamine might not impact the acquisition of amphetamine use in healthy subjects, but may be involved in disinhibited control during phases of bingeing (or escalation) in subjects that have already entered the acquisition or maintenance phases of addiction and thus have a different neural pharmacological-neural relationship following previous amphetamine intoxication. The acute effects of cocaine on impulsivity in cocaine dependent subjects also produce contrasting results. Low dosages of cocaine (50- 150 mg) can reduce inhibitory control in cocaine

dependent subjects (Fillmore et al., 2002) whilst higher dosages of 300 – 400 mg can improve inhibitory control in this group (Fillmore, Rush, & Hays, 2006; Garavan, Kaufman, & Hester, 2008). These studies therefore show that the acute effect of stimulants that might contribute towards escalation and 'bingeing' within this drug group and 'bingeing' may relate both to baseline inhibitory control, prior to drug exposure, and the effects of the drug and dose consumed.

In combination with elevated impulsivity predicting drug escalation, these studies suggests that both high impulsivity phenotypes and drug induced effects on impulsivity during phases of repeated drug use might act as risk factors for escalation. This suggests that both disinhibited behavioural control at baseline and drug induced disinhibition during phases of extended and repeated drug exposure may act as risk factors for escalating drug use.

1.4.4.4 Abstinence/ Relapse

Trait impulsivity has been reported during both short and long term abstinence amongst former substance users in comparison to healthy controls. Higher BIS and I_7 scores have been reported by alcohol, cocaine and heroin dependent subjects tested at or below 1 month of drug abstinence (Coffey et al., 2003; Nielsen et al., 2012; Petry, 2001) and former amphetamine and MDMA use tested at 1 year of drug abstinence similarly report greater levels of impulsivity within these questionnaires in comparison to healthy controls and report levels of impulsivity comparable to that reported by current amphetamine and MDMA users (Clark et al., 2006; 2008). Taken together, these findings suggest that self-reported reduced inhibitory control during early and late stages of drug abstinence may reduce the chance of entering into longer-term or maintaining abstinence.

Former drug users also display impulsive and disadvantageous decision making during short and long-term abstinence. Cocaine and heroin dependent subjects make more disadvantageous choices within the IGT in comparison to healthy controls when tested at 15 days of drug abstinence (Verdejo-García, Perales, & Pérez-García, 2007) indicating that drug users in this early stage of abstinence are more prone to making bad decisions. Similarly methamphetamine, alcohol and cocaine dependent subject tested 14, 39, 46 and days of abstinence show steeper discounting of delayed reward in comparison to healthy controls (Heil et al., 2006; Hoffman et al., 2008; Petry, 2001) indicating that former substance users bias disadvantageous choices due to insensitivity to long-term reward. In addition, cocaine dependent subjects that are less responsive to contingency management techniques, and therefore have steep discounting of the value of future monetary rewards, do not achieve

protracted abstinence during drug treatment, whilst cocaine users that that do respond to contingency management treatment and are more sensitive to future rewards reach protracted abstinence (Martinez et al., 2011; Washio et al., 2011). Former methamphetamine dependent subjects tested at an average of 6 months of abstinence also display steeper discounting of delayed reward in comparison to healthy controls (Hoffman et al., 2006). These findings therefore indicate that former stimulant users continue to bias immediate disadvantageous decisions over advantageous decisions that require tolerance to receiving reward. This might mean that subjects entering drug abstinence that bias gratification of immediate drug reward are more likely to relapse due to insensitivity to the long-term advantages of drug abstinence. In addition, evidence that subjects reaching longer-term abstinence continue to display increased impulsive decision making indicates that bias for immediate gratification may be responsible for triggering relapse even when subjects comply with abstinence.

Former amphetamine and heroin dependent subjects tested at 1 year of drug abstinence also report reduced reflection capacity. Former amphetamine users show comparable deficits on the IST relative to current amphetamine and opiate users and greater deficits in comparison to healthy controls (Clark et al., 2006). Similarly, former heroin users more errors on a porteus maze test in comparison to healthy controls (Lee & Pau, 2002). This suggests that alongside steeper discounting of future rewards during drug abstinence reduced 'in the moment' reflection capacity might also bias fast and disadvantageous decision making amongst former stimulant and opiate users.

Cocaine users in short term abstinence (≥15 days) also display more errors of commission within a Go/No-go task (Verdejo-García et al., 2007) but do not at 8 months of drug abstinence (Bell et al., 2013). Cocaine users tested at a minimum of 14 days abstinence, however, do not show any differences to healthy controls in SSRT (Li et al., 2007). These studies might therefore suggest that behavioural inhibition during abstinence differs between specific dimensions of impulsivity and the time point measures However, heroin users in both short and long-term abstinence do not differ from healthy controls or current opiate users in error of commission of Go/No-go tasks (Constantinou et al., 2010; Verdejo-García et al., 2007). This suggests deficits in impulsive and disadvantageous decision making reported above might be more involved in the initiation of relapse amongst opiate users rather that reduced action control.

Animal that display trait impulsivity on a DRT show greater resistance to stimulant extinction and relapse within self-administration models. Male rats expressing this behavioural phenotype on the DRT show greater resistance to nicotine and cocaine extinction, and greater

cue and context induced reinstatement in comparison to rats that are less impulsive on this task (Broos et al., 2012b; Diergaarde et al., 2009). These findings map onto human literature identifying that greater discounting of future rewards amongst substance users entering abstinence is associated with a greater chance of relapse during treatment (MacKillop & Kahler, 2009; Martinez et al., 2011; Wang et al., 2011). Male rats with low MAD scores, however, do not show greater resistance to cocaine extinction or drug induced reinstatement of self-administration (Perry et al., 2008) suggesting that impulsive decision making relating to the delay intolerance rather reward intolerance might be related to animal models of abstinence and relapse. In contrast, female rats with high MAD scores (low impulsivity) display greater resistance to cocaine extinction and reinstatement (Perry et al., 2008). This indicates potential gender differences in how trait impulsivity might interact with this stage of the addiction. Interestingly, female cocaine dependent subjects show greater sensitivity to cocaine associated cues and greater disruption in cortical-striatal activation than male cocaine users (Volkow et al., 2011) additionally supporting the prevalence if gender differences in triggers of relapse (cue sensitivity) in human drug users.

Rats displaying trait impulsivity on the 5CSRTT have also been shown to display greater resistance to nicotine extinction in comparison to less impulsive rats, although this effect was not as strong as that seen with highly impulsivity rats screened by the DRT (Diergaarde et al., 2009). Trait impulsive rats on the 5CSRTT also show greater cocaine relapse in comparison to less impulsive rats following repeated periods of punishment induced abstinence (Economidou et al., 2009). Interestingly, rats with both high and low trait impulsivity on the 5CSRTT relapse following an initial phase of punishment induced cocaine abstinence, however, only highly impulsive rats continue to relapse following a second abstinence phase (Economidou et al., 2009). This pattern of relapse between rats with high and low trait impulsivity maps on to human studies reporting that subjects with higher levels of behavioural disinhibition do not reach protracted abstinence as quickly as subjects that are less disinhibited (Krishnan-Sarin et al., 2007). In addition, trait impulsive rats performing the 5CSRTT show increased cocaine seeking in comparison to less impulsive rats (Belin et al., 2008) a behaviour that is believed to represent the motivational incentive to relapse (Stewart, 2010).

The effects of drug withdrawal on impulsivity might also affect vulnerability to relapse during drug abstinence. Withdrawal from short access cocaine self-administration (2 hours) has been shown to lead to a transient increase in premature responding in rats (Winstanley et al., 2009), suggesting that greater levels of disinhibited behaviour during short-term drug abstinence might increase the chance of relapse due to reduced behavioural control. In contrast, intermittent withdrawal from 5-day long-access (8 hours) amphetamine self-administration

and withdrawal from 21-day long access amphetamine, methamphetamine and MDMA self-administration does not produce any effects on premature responding during short or long-term withdrawal (Dalley et al., 2005; Dalley et al., 2007b). Taken together, these studies suggest that the relationship between drug withdrawal and impulsivity might relate specifically to the pattern of drug treatment employed.

1.4.5 Summary

In summary, there is evidence that impulsivity can interact with all stages of the addiction to increase the chance of drug use though a loss of inhibitory control over behaviour. From the literature reviewed, however, amphetamine appears to have a have a particularly paradoxical relationship with impulsivity in that amphetamine can both increase and decrease impulsivity. These effects also appear to differ across different dimensions of impulsivity (choice vs. action) and in relation to baseline levels of impulsivity.

1.5 Neuroanatomical framework of Impulsivity

Impulsivity has been proposed to manifest as a consequence of poor 'top-down' frontal control over subcortical striatal and midbrain regions (Aron et al., 2007; Dalley et al., 2008; Jentsch & Taylor, 1999). Indeed, circuits connect the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) to subcortical regions of the basal ganglia including the dorsal and ventral striatum and to midbrain targets including the globus pallidum, substantia nigra and thalamus (Alexander & Crutcher, 1990; Alexander, DeLong, & Strick, 1986; Alexander, 1994). These circuits can therefore regulate 'top-down' signalling from frontal cortical regions to subcortical targets via direct passageway between higher cortical to subcortical regions. In contrast, subcortical circuits originating in the spinal cord, hindbrain and midbrain regions, such as the mesolimbic dopamine (DA), noradrenalin (NA) and serotonin (5-HT) systems, project ascending fibres to striatal and prefrontal regions and drive 'bottom-up' processing of primary motivation, reward and affect (Ikemoto, 2010; Leonard, 2004; Moore & Bloom, 1979; Moore, Halaris, & Jones, 1978). Activity through these ascending pathways can bias processing of primary motivational states through weakening the strength of descending 'top-down' frontal control systems. This bias can subsequently compromise 'top-down' frontal control leading to disinhibited behaviour. The major neural site of convergence between these circuits occurs within the striatal complex. Within the striatum, cortical, limbic and midbrain projections can converge on the same inhibitory medium spiny neuron (MSN) (Sesack & Pickel, 1990) allowing multiple cortical and subcortical activity to give rise to a single output. Hypothetically, disruption at any stage of these circuits that can alter the balance of 'top-down' processing of inhibitory control in favour of 'bottom-up' processing that might cause a behavioural output of increased impulsivity. 'Impulsivity' can therefore be conceptualised as a neural balancing act between 'top-down' and 'bottom-up' activity within these circuits.

1.5.1 Neural correlates of inhibitory control

fMRI studies have demonstrated that successful inhibition of a prepotent responding frequently activates the right DLPFC and right inferior frontal gyrus (IFG) in healthy subjects performing Go/No-go tasks (Garavan et al., 2002; 1999; Kelly et al., 2004; Liddle, Kiehl, & Smith, 2001; McNab et al., 2008; Menon et al., 2001). Activation of right prefrontal regions during No-go trials has also been detected when increasing and reducing inhibitory demand within No-go trials (Garavan et al., 2002; Kelly et al., 2004), proportionally distributing No-go trials with Go trials (Chikazoe, 2010; Steele et al., 2013) and when implementing low working

memory demand during a Go/No-go task (Steele et al., 2013). These studies therefore demonstrate that the right frontal cortex is involved in successful No-go trial inhibition regardless of alterations in stimulus frequency, salience or attentional demand. However, lesions to the left inferior frontal gyrus have also recently been found to impair performance with a Go/No-go task (Swick, Ashley, & Turken, 2011). In addition, activation the OFC has also been found during No-go trials as well as DLPFC activation (Horn et al., 2003).

Alongside the involvement of right frontal regions, successful response inhibition during Go/No-go procedures also activates the ACC, inferior parietal cortex (IPC), pre supplementary motor area (preSMA), and sub-cortical regions including the caudate nucleus and putamen. Activation of the ACC has been linked to error and conflict monitoring during challenging No-go trials (Garavan et al., 2002; Kelly et al., 2004) but has also been shown to activate during both Go and No-go trials, suggesting a role for the ACC in general response monitoring (Liddle et al., 2001). Sub-divisions of the ACC have however been found to differentially activate during successful inhibition (dorsal anterior ACC) and unsuccessful inhibition (rostral right ACC) (Menon et al 2001), suggesting that distinct regions of the ACC may be differentially involved in response inhibition and error monitoring. Activation of the IPC and preSMA are commonly reported during successful No-go trial inhibition, and as such fronto-parietal networks have been associated with successful response inhibition (Garavan et al., 1999; Steele et al., 2013). Activation of sub-cortical regions, mainly the caudate nucleus and putamen, during successful inhibition of No-go trials (Kelly et al., 2004; Steele et al., 2013), however, is also indicative of frontal-striatal networks in the regulation of inhibitory control. The fronto-parietal network is known to modulate attention in humans whilst fronto-striatal networks have been more closely associated with inhibitory control (Dalley et al., 2008; Ptak, 2012). Consequently the recruitment of fronto-parietal simultaneous to fronto-striatal activation might represent interaction of both these networks at a cortical level.

Successful action cancellation in healthy controls during the SST is also associated with activation of the right IFG and patients with damage to the right IFG show specific impairments in performing the SST (Aron et al., 2003). Similarly, transcranial magnetic stimulation (TMS) applied over the pars opercularis region of the right IFG produces a specific impairment in the ability of healthy subjects to perform stop trials within the SST but does affect Go reaction time (Chambers et al., 2006). Furthermore, a 'hyperdirect' frontal-subcortical pathway originating in the right IFG and projecting to the preSMA and subthalamic nucleus (STN) has been identified in regulating action cancellation. Diffusion-weighted imaging (DWI) conducted in addition to fMRI imaging identified a direct frontal trajectory of white matter axons originating in the right IFG and projecting directly to the preSMA and STN in subjects

performing the SST (Aron et al., 2007). Within this study, regional brain activity collected from fMRI scans during stop trials was found to overlap with frontal-subcortical trajectories identified during DWI. These findings therefore provide insight into the circuitry underlying activation of the right IFG during fMRI scans. In addition these findings provide evidence for direct fronto-subcortical circuits in the regulation of response cancellation.

The neural correlates of delay discounting in healthy subjects have also been associated with activation of frontal, striatal and parietal structures. Activation of the medial and lateral prefrontal cortex has been differentially associated with choice of immediate and delayed reward, respectively. The medial orbitofrontal cortex (mOFC), medial prefrontal cortex (mPFC) and medial posterior cingulate cortex (mPCC) are activated during choice for immediate reward, whilst the lateral OFC (lOFC), DLPFC, ventrolateral prefrontal cortex (VLPFC) and posterior parietal cortex (PPC) are activated during choice for large delayed reward (Ballard & Knutson, 2009; McClure et al., 2004). Volumes of the dorsolateral and inferiolateral prefrontal cortex have also been found to negatively correlate with choice for small immediate reward (Bjork, Momenan, & Hommer, 2009), indicating that increased lateral prefrontal activity is associated with preference for delayed reward.

In addition to lateral prefrontal regions (McClure et al., 2004) reported activation of separate cortical and sub-cortical neural systems for choice of immediate vs delayed reward. Choice of small immediate rewards was found to correlate with limbic-striatal activity whilst choice off large delayed reward correlated with lateral prefrontal and parietal activation (McClure et al., 2004). Similarly, striatal-basal ganglia regions are activated during learning of trials that deliver immediate reward whilst activation of the DLPFC and IFC are activated during learning of delayed reward trials (Tanaka et al., 2004). These studies therefore support that greater prefrontal activity is associated with impulse control whilst greater sub-cortical striatal activity is associated with impulsive choice. A recent study using tract-based diffusion tensor imaging also found that high diffusion and low fractional anisotropy (FA) frontal-striatal white matter, representing reduced tract integrity, predicted high rates of discounting in healthy subjects (Peper et al., 2013). These results expand upon the fMRI studies conducted by McClure et al., (2004) and Tanaka et al., (2004) to further implicate reduced connectivity and processing between frontal to sub-cortical striatal structures in impulsive choice. Collectively these studies support fronto-subcortical systems, and most frequently fronto-striatal systems, in the regulation of behavioural control.

1.5.2 Neural correlates of inhibitory control in substance users

Neuroimaging studies of substance users have typically demonstrated a reduction in the volume of frontal cortical regions (Goldstein & Volkow, 2002). Reduced frontal grey matter volume has been found in stimulant, opiate and alcohol dependent users (Daumann et al., 2011; Jernigan et al., 1991; Koester et al., 2012; Liu et al., 1998; Pfefferbaum et al., 1997). More specifically, reduced grey matter volume has been found in frontal regions including the OFC, ACC and insula in cocaine, amphetamine and methamphetamine users (Ersche et al., 2011; Matochik et al., 2003; Thompson et al., 2004). In addition, volume reductions have been reported in temporal and parietal regions is cocaine and amphetamine users (Ersche et al., 2011; Daumann et al., 2011). Female cocaine users also show hypoactivity within frontal, cingulate, parietal cortical regions and subcortical regions of the thalamus during exposure to a cocaine-cue video indicative of hypoactive 'control circuits' (Volkow et al., 2011).

Conversely, cocaine and methamphetamine dependent subjects show increased grey matter volume in the striatum and other sub-cortical regions of the basal ganglia including the globus pallidus (Chang et al., 2005; Ersche et al., 2012; Jernigan et al., 2005; Thompson et al., 2004). However, greater duration of cocaine use and greater cumulative methamphetamine use has been correlated with reduced volume of the putamen and globus pallidus (Chang et al., 2005; Ersche et al., 2011) and stimulant dependent subjects show reduced substantia nigra volume (Todd et al., 2013). This suggests that greater stimulant exposure may actually reduce, not enlarge, the volume of grey matter within components of the basal ganglia. The enlargement of striatal volume commonly reported in stimulant dependent users has been proposed to act a consequence of reactive glial inflammation following heavy stimulant intoxication (Chang et al., 2005) and does not necessarily reflect long-term enhancement of striatal regions. In following, shrinking of both frontal and subcortical areas may represent volumetric changes within neural circuits that contribute towards drug maintenance. This neural hypoactivity in drug users maps on to fronto-striato-thalamic circuits and suggests that multiple nodes of inhibitory processing may be disrupted in addicts at both the cortical and subcortical level.

Whether or not volume reduction in cortical and subcortical grey matter is specifically disruptive to inhibitory circuitry can be better elucidated via DWI and diffusion-tract imaging (DTI) imaging studies that explore the connectivity of white matter tracts between particular brain regions. Reduced FA in frontal white matter tracts connecting cortical to sub-cortical regions has also been reported in stimulant dependent subjects (Ersche et al., 2012) and reduced FA in white matter tracts originating in the right frontal sub-gyrus negatively correlates with the duration of heroin use in subjects (Liu et al., 2008). Reduced FA in the

anterior corpus callosum (genu) connecting frontal and striatal structures has been reported in alcohol and methamphetamine dependent subjects (Alhassoon et al., 2012; Salo et al., 2009). Interestingly, these studies specifically identify compromised white matter tracts connecting higher order prefrontal and cortical regions to subcortical regions via the anterior corpus callosum in substance dependence. However, to establish whether hypoactivity within frontal regions and thinning of white matter tracts from frontal to subcortical regions is directly linked to a loss of inhibitory control in substance users, assessment of these areas whilst performing inhibitory control tasks in required.

Stimulant users performing tasks of action inhibition typically display reduced activity in comparison to controls within the ACC and insula (Hester & Garavan, 2004; Kaufman et al., 2003; Li et al., 2008). Hypoactivity of the ACC has been linked to poor behavioural and error monitoring whilst the insula has been linked to reduced self-reflection (Modinos, Ormel, & Aleman, 2009). Consequently, hypoactivity of both these areas could lead to reduced awareness of poor behavioural monitoring in stimulant users. Both the ACC and insula constitute nodes of fronto-striatal connectivity, therefore hypoactivity at both of these locations in fronto-striatal connections may impair behavioural control via deleterious effects on top-down behavioural monitoring of sub-cortical structures. However, given that the ACC and insula are nodes in numerous neural circuits relating to executive control and attention processes that are additionally engage during the go/no-go and SSRT (Dosenbach et al., 2008) the specific contribution of these regions to poor inhibitory control is difficult to certify. Li et al., (2008) addressed this issue by controlling for 'attention', 'post-error processing' and 'task frustration' during the correlation analysis of neural activation and SST performance and a measure of emotional regulation (Difficult in Emotional Regulation Scale). Li et al., (2008) reports that hypoactivity of the ACC and preSMA after controlling for attention, post error processing and task frustration were correlated with emotional inhibitory control and short SSRT, respectively in cocaine users. These findings provide a useful insight into the specific contribution of hypoactive cortical brain regions in substance users in relation to reduced behavioural inhibition, and suggest that functional activity of the ACC may specifically regulate 'inhibitory control' whilst activity of the preSMA may specifically regulate 'motor inhibitory control' (Li et al., 2008). In addition, commission errors within the IMT/DMT task negatively correlate with FA in the anterior corpus callosum (Moeller et al., 2004) and long SSRT correlate with reduced FA in white matter IFG and preSMA tracts (Ersche et al., 2012). These findings therefore confirm that poor frontal-striatal tract integrity is directly related to poor prepotent inhibitory control.

Within delay discounting procedures, MA users display reduced activity within regions of the right prefrontal cortex, ACC and parietal regions including the precuneus and inferior parietal sulcus whilst making more impulsive decisions within 'hard' temporal discounting choices (Hoffman et al., 2008; Monterosso et al., 2007). Cocaine dependent subjects with HIV also have reduced right prefrontal activity within the DLPFC, VLPFC and OFC whilst making more impulsive decisions during 'hard' temporal discounting choices in comparison to men diagnosed with HIV with no reported drug use (Meade et al., 2011). These studies suggest that impulsive decision making in stimulant users is correlated with reduced activity of prefrontal and parietal regions.

Reduced activity of subcortical regions including the right caudate nucleus have also been reported during impulsive choices made by MA dependent subjects (Hoffman et al., 2008), indicating that hypoactivity of both frontal cortical and subcortical regions are involved in impulsive choice decision in MA users. Amphetamine dependent subjects also show decreased activity within the ventral striatum in anticipation of reward (Schouw et al., 2012), and area linked to the processing of reward value in temporal discounting procedures (Ballard & Knutson, 2009). In following, the combination of reduced cortical frontal activity (Monterosso et al., 2007; Hoffman et al., 2008; Meade et al., 2011) coupled with reduced ventral striatal sensitivity to reward (Schouw et al., 2012) reported in stimulant dependent subjects may drive and bias behaviour in favour to sub cortically processing to receive immediate reward. This interpretation is consistent with correlations between limbic-striatal activity and preference for immediate reward, and lateral prefrontal activity and preference for delayed reward, reported by McClure et al., (2004).

From human literature reviewed it is clear that fronto-striatal and fronto-thalamic circuits are involved in the regulation of impulsivity and drug induced impulsivity. The following sections will further explore the more specific functional involvement of fronto-striato-thalamic circuits in the mediation of impulsivity and drug induced impulsivity through reviewing animal models of drug addiction and impulsivity.

1.5.3 Neuroanatomical framework of Impulsivity in Animal Models

1.5.3.1 Ventral Striatum

The ventral striatum contains the nucleus accumbens (NAcb), consisting of the core and shell sub-divisions, and the olfactory tubercle. The NAcb contains two subregions, the medial and dorsal region named the core, and the lateral and ventral region named the shell. The NAcb

core receives afferent connections from prefrontal regions including the dorsal prelimbic cortex and lateral OFC, limbic regions including the rostral amygdala, midbrain regions including the ventral tegmental area (VTA), ventral pallidum and medial thalamus, and sends efferent connections to the dorsal subcommissural ventral pallidum, the entopeduncular nucelus and substantia nigra pars reticular (Basar et al., 2010; Brog et al., 1993; Ikemoto, 2010). The dorsal ventral pallidum is in reciprocal connection with the dorsomedial subthalamic nucleus that in turn projects to the entopeduncular nucelus and substantia nigra pars reticular. Consequently the entopeduncular nucelus and substantia nigra pars reticular are under both direct and indirect control from the core. The entopeduncular nucelus and substantia nigra pars reticular send efferent projections to the ventromedial subthalamic nucelus and mediodorsal thalamic nucelus, respectively (Basar et al., 2010; Brog et al., 1993; Ikemoto, 2010). At the most caudal region of this circuit, the ventromedial subthalamic nucelus and mediodorsal thalamic nucleus are in reciprocal connection with the medial prefrontal cortex that in turn sends efferent projections to the core, thus creating a ventral striato-pallido-thalamo-cortical circuit. In contrast, the NAcb shell receives afferent connections from prefrontal regions including the subgenual ACC and the medial OFC, limbic regions including the caudal amygdala and hippocampus, midbrain regions including the VTA and paraventricular thalamic nucleus, and projects efferent connections to the ventromedial ventral pallidum, lateral hypothalamus, VTA and dorsal substantia nigra (Basar et al., 2010; Brog et al., 1993; Ikemoto, 2010). The ventromedial ventral pallidum projects to the mediodorsal thalamic nuclei that in turn projects to the medial prefrontal cortex and insula, thus creating a ventral striato-pallido-thalamo-cortical circuit incorporating the shell (Basar et al., 2010; Ikemoto, 2010).

The NAcb core therefore receives prefrontal input predominantly from dorsal prefrontal regions and can target a multiple number of midbrain regions via output to the ventral pallidum. The NAcb shell in contrast predominately receives input from ventral prefrontal regions and receives dense innervation from the hippocampus. The shell is also in direct reciprocal connection with the VTA and substantia nigra meaning that the output from the shell can directly regulate ascending mesolimbic and mesocortical dopamine release. In contrast, the core does not directly project to the VTA but has greater control over regions of the midbrain, such as the subthalamic nucelus. All output signals sent from the core will ultimately reach the thalamus and affect thalamo-cortical signalling. A lesser proportion of shell output reaches the thalamus to influence the processing of thalamo-cortical signals but the shell will have a greater impact on the regulation of cortical and limbic DA release than the core. In following, the NAcb core is anatomically located to enable indirect processing of

behavioural output through interaction with multiple midbrain regions, whilst the shell is anatomically located to enable fast and direct processing of behavioural output through direct connection with the VTA and substantia nigra and more direct pathway to the thalamus than the core (See Fig. 1.4).

1.5.4 Behavioural Literature examining the involvement of cortical-subcortical structures in impulsivity

1.5.4.1 The Basal Ganglia and Midbrain

Lesions to the NAcb can produce changes in both impulsive action and choice. Although lesions to the NAcb core or shell do not affect premature responses made under normal test or behavioural challenge conditions within the 5CSRTT, core lesions potentiate amphetamine induced premature responding whereas shell lesions attenuate this elevation in premature responding (Murphy et al., 2008). This supports a role for the core in processing inhibited and controlled behaviour and the shell in processing disinhibited and immediate response behaviour following stimulant treatment and is consistent with the involvement of the ventral striatum in impulsivity amongst stimulant users (Lee et al., 2009). In addition lesions to the NAcb core have been shown to increase impulsive responding measured by DRL task (Pothuizen et al., 2005).

However, lesions to the NAcb core do not affect the SSRT or stop signal accuracy under normal test conditions in rats, and neither do they affect SSRT or stop trials in rats treated with amphetamine (Eagle and Robbins, 2003). These findings therefore draw an anatomical distinction between stimulant induced prepotent inhibition and action cancellation in animal models and further support human literature demonstrating that action cancellation does not involve processing via ventral striatal regions but direct pathways from frontal regions to the thalamus (Aron et al., 2007b).

Lesions to the NAcb core have also been found to increase preference for immediate over delayed reward in rats (Cardinal et al., 2001), implicating a role for the core in processing delay discounting. These findings have been interpreted to represent a role for the core in mediating tolerance to delayed reinforcement (Cardinal et al., 2001). These findings are therefore consistent with human literature identifying the involvement of striatal activation during delay discounting procedures in healthy subjects (McClure et al., 2004).

In contrast to the lack of effect that core lesions have on SSRT, lesions to the medial striatum in rats performing the SST produces an increase in SSRT whilst causing no change in the accuracy

of Go or Stop trial performance (Eagle and Robbins, 2003). Lesions to the medial striatum also reduces amphetamine induced elevations in SSRT at a low amphetamine dose (0.3 mg/kg) and an increase in amphetamine induced elevated SSRT at a high amphetamine dose (1.0 mg/kg) (Eagle and Robbins, 2003) indicating that the medial striatum is not only involved in mediating action cancellation under normal conditions but is also a potential target of stimulants that can alter action cancellation.

Lesions to the subthalamic nucleus (STN) can increase premature responding in the 5CSRTT and reduced Stop trial accuracy in the SST (Baunez & Robbins, 1999; Chudasama & Muir, 2001; Eagle et al., 2008) linking this region with the regulation of behavioural disinhibition and action cancellation similar to that observed during imaging studies in humans performing Go/No-go and SST (Steel et al., 2013; Aron et al., 2007b). However, STN lesions do not affect SSRT (Eagle et al., 2008) suggesting that lesions to this region of the midbrain produces a generalised disruption in the ability to perform stop trials and inability to wait but not the processing of the stopping response that would be reflected in the SSRT. This therefore suggests that the STN might be involved in the final output of stopping an on-going action. In following, reduced stop trial accuracy in STN lesioned animals supports the existence of an action cancellation pathway to the STN, within which the STN is the circuitry node mediating behavioural execution (Aron et al., 2007b). Collectively, the involvement of the STN in both waiting and stopping behaviour supports involvement of thalamo-cortical loops as well as fronto-striatal loops in the regulation of behavioural inhibition.

1.5.4.2 Prefrontal Cortex

Lesions to the medial prefrontal cortex do not affect SSRT or performance in rats performing the SST (Eagle and Robbins, 2003). However, temporary inactivation of the ACC and dorsal prelimbic cortex increase SSRT (Bari et al., 2011) linking dorsal prefrontal regions to action cancellation in rats. Lesions to the OFC have also been found to increase SSRT in rats but inactivation of the OFC via gaba agonists does not affect SSRT (Eagle et al., 2008; Bari et al., 2011) suggesting that specific involvement of the OFC in action cancelation remains slightly unclear. In following, the involvement of specific prefrontal regions in action cancelation is not wholly clear from animal models, however, inactivation of the ACC and dorsal prelimic cortex increasing SSRT is consistent with the involvement of the right IFG and ACC in action cancelation and error detection reported in human imaging studies (Aron et al., 2003; Garavan et al., 2002).

In contrast to specific prefrontal lesions in the involvement of action cancellation, lesions to the ACC, infralimbic and prelimbic have all been found to increase premature responding in the 5CSRTT (Chudasama & Muir, 2001; Muir, Everitt, & Robbins, 1996). However more dorsal prefrontal regions do not appear related to premature responding in the 5CSRTT (Chudasama & Muir, 2001), suggesting that more ventral regions are involved in processing of behavioural waiting signals. These findings are consistent with human literature implicating the inferior frontal gyrus and ACC in CPT and Go/No-go tasks (Garavan, 2000; 2002; Kelly, 2004; Steele et al., 2013).

Lesions to the OFC have also been associated with temporal discounting. Lesions to the OFC however have been found to both increase and reduce impulsive choice, depending on the presence of delay to reward cues (Mobini et al., 2002; Rudebeck et al., 2006; Winstanley et al., 2004). These discrepancies between results have been suggested to represent differences in the activation of the OFC that is related to environmental cues (Winstanley, 2007; Zeeb, Floresco, & Winstanley, 2010). Although the exact role of the OFC remains elusive, the OFC is activated during presentation of drug cues and seeking behaviour, and environmental cues associated with reward (Hutcheson & Everitt, 2003; Zeeb et al., 2010). This suggests that the OFC might contribute towards impulsive decision making and behavioural disinhibition in the 5CSRTT in relation to processing of environmental cues that signal reward. These studies also expand on human literature reporting hypofunctioning within OFC of stimulant users that also discount delayed reward and display disinhibited behaviour (Coffey et al., 2003; Colzato et al., 2007; Kirby & Petry, 2004; Volkow et al., 2001).

In summary, lesions to specific nodes of inhibitory circuitry in rats confirm a relationship between fronto-striato-thalamic activity in the regulation of impulsivity. Inhibitory control over a prepotent response appears to engage more ventral prefrontal regions, whilst action cancelation engages more dorsal prefrontal regions. This might be because action cancellation utilises a direct hyper direct pathway to the thalamus, thus recruiting only a specific prefrontal region to initiate this hyperdirect pathway. In contrast, waiting behaviour recruits more ventral regions, comparable to the DLPFC and ACC circuits that have been linked to behavioural disinhibition in humans. In regards to delay discounting the OFC and NAcb core appear to be involved in this choice paradigm but the direction of this involvement in relation to the OFC might relate to differences in chemical circuitry or the extent of cues that can guide delay. This again maps onto human OFC circuits in decision making and impulsivity.

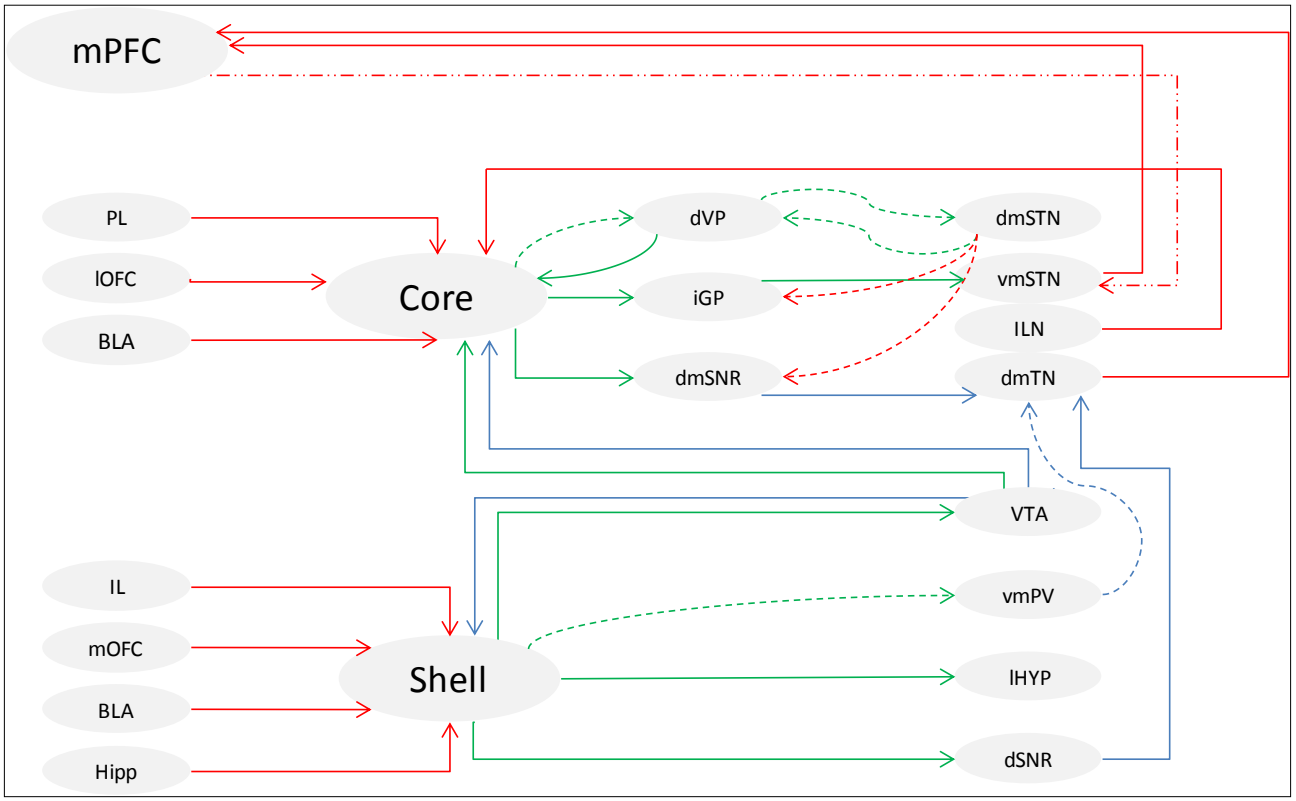

Figure 1.4 Cortical and sub-cortical afferent and efferent connections of the nucleus accumbens core and shell. Abbreviations: BLA, basolateral amygdala; dSNR dorsal substantia nigra pars reticular; dmSNR, dorsomedial substantia nigra pars reticular; dVP, dorsal ventral pallidum; dmSTN dorsomedial subthalamic nuclei; Hipp, hippocampus; IL, Infralimbic cortex; lLN, Intralaminar thalamic nuclei; iGP, internal segment globus pallidum; lHYP, lateral hypothalamus; lOFC, lateral orbitofrontal cortex; mOFC, medial orbitofrontal cortex; mPFC, medial prefrontal cortex; mdTN, mediodorsal thalamic nuclei; PL, prelimbic cortex; VTA; ventral tegmental area. →, glutamate; →, dopamine; →, GABA. →, direct; - -> indirect; −···−> , hyperdirect**.**

1.6 Neurochemical framework of impulsivity

1.6.1 Neurochemical Connections of the Nucleus Accumbens Core and Shell

The NAcb core and shell receive dense dopaminergic innervation from the A10 cell group in the VTA, 5-HT innervation from the median raphé nucleus and NA innervation from the locus coeruleus (Ikemoto, 2010; Leonard, 2004; Moore & Bloom, 1979; Moore, Halaris, & Jones, 1978). The NAcb core and shell also receive GABA afferents from the VTA (Van Bockstaele & Pickel, 1995) meaning that both subregions receive excitatory and inhibitory afferents from the midbrain. In addition, both regions receive excitatory glutamate afferents from limbic and prefrontal regions. Within the NAcb, GABAergic medium spiny projection neurons and aspiny interneurons are the predominant cell type and make up 95% of accumbal cells (Gloria E Meredith, Pennartz, & Groenewegen, 1993). Excitatory D_1 and inhibitory D_2 receptors within the NAcb are located post-synaptically on medium spiny projection neurons (Albin, Young, & Penney, 1989; Surmeier et al., 2007) and there is evidence to suggest that the D_2 receptors are located on presynaptic glutamate terminals innervating the accumbens (Goto & Grace, 2005). D_1-like receptors (D_1 and D_5) are located on GABAergic interneurons (Centonze et al., 2002, 2003). Consequently dopamine release into the NAcb can 'gate' converging afferent connections and efferent projections within the NAcb through actions at the D_1 and D_2 receptors (Goto & Grace, 2005; Groenewegen & Trimble, 2007).

1.6.2 Serotonin and Impulsivity

Reduced 5-HT activity has historically been linked to impulsivity and clinical impulse control disorders (Hollander & Rosen, 2000; Soubrie, 1986). Low levels of circulating 5-HT has an established relationship with impulsive aggression (Seo, Patrick, & Kennealy, 2008) and selective serotonin reuptake inhibitors (SSRI) can reduce impulsive choice in violent male offenders (Cherek et al., 2002; Cherek & Lane, 2000), a population known to have reduced CSF levels of 5-HT metabolite 5-hydroxyindoleactic acid (5-HIAA) (Virkkunen et al., 1994). Polymorphisms in the 5-HT transporter gene (5HTT) and genes that encode for 5-HT receptors are also associated with impulsivity and aggression. The 5-HT transporter promoter gene, 5HTTLPR, is associated with ADHD (Davidge et al., 2004) and the S allele of the 5HTTLPR also interacts with childhood adversity to predict emotional impulsivity in children (Carver et al., 2011). Adolescent psychiatric patients expressing the t/t allele combination of the HTR2a gene, an allele combination associated with increased binding of the 5-HT2a receptors, are less impulsive and aggressive than patients with the t/c allele (Zalsman et al., 2011). These findings

therefore collectively support the involvement of 5-HT systems in impulsivity in clinical populations with co-morbid with mood related symptoms such as aggression.

The role of 5HT activity in impulsivity within healthy subjects has produced mixed results. 5-HT transmission does not appear to be involved in the mediation of action cancellation. Increasing 5-HT transmission via administration of the SSRI citalopram, and decreasing 5-HT transmission via acute tryptophan depletion, the amino acid precursor to 5-HT, both have no effect of SSRT in healthy subjects (Chamberlain et al., 2006; Clark et al., 2005). However, acute tryptophan depletion increases errors in a CPT and reduces right OFC activation during No-go trials in healthy subjects (Rubia et al., 2005; Walderhaug et al., 2002), suggesting that reduced 5-HT transmission might be involved in inhibition of prepotent responding. However, the CPT is primarily a task of attention, therefore, whether 5-HT is specifically involved in response inhibition in non-clinical populations remains inconclusive. The most convincing evidence for 5-HT involvement in impulsivity therefore comes from clinical populations that have dysfunctional impulse control comorbid with emotional symptoms such as aggression.

Central 5-HT depletion can lead to disinhibited behaviour on the 5CSRTT and symmetrically reinforced Go/No-go task in rats (Harrison et al., 1997, 1999). 5-HT has also been shown to interact with amphetamine induced elevations in premature responding and reductions in impulsive choice, indicating serotonin-dopamine interaction in impulsivity (Harrison et al., 1997; Winstanley et al., 2003). Administration of 5-HT_{2a} and 5-HT_{2c} receptors antagonists both systemically and centrally into the NAcb have also been found to mediate changes in ipulsivity on the 5CSRTT (Robinson et al., 2007; Winstanley et al., 2004). Blockade of the 5-HT_{2a} and 5-HT_{2c} receptors however produces opposing effects on impulsivity (Robinson et al., 2007; Winstanley et al., 2004), indicating that activation of the 5-HT_{2a} and 5-HT_{2c} receptors might lead to opposing effects on impulsivity. Animal studies therefore implicate a role for reduced 5-HT tranismission in mediating impulsivity, which might function through indirect effects on dopamine activity.

1.6.3 Noradrenalin and Impulsivity

There is a clear relationship between NA transmission and impulsivity that is highlighted by the therapeutic success of NA reuptake inhibitors (NARI) for the treatment of ADHD. Atomoxetine, a NARI, and guanfacine, a noradrenalin α2a agonist, both increase release of NA and successfully reduce ADHD symptoms in adolescents and adults (Michelson et al., 2003; Sallee et al., 2009). Atomoxetine also improves action cancellation and increases right inferior frontal gyrus activity during stop trials in adults with ADHD performing the SST (Chamberlain et al.,

2007, 2009), suggesting that increasing NA release into prefrontal regions can improve regulation of behavioural inhibition. In addition, the dopamine-noradrenalin reuptake inhibitor MPH reduces delay discounting in children with ADHD (Shiels et al., 2009) and has been found to occupy a significant number of NA reuptake transporters at clinically therapeutic dosages (Hannestad et al., 2010). These findings collectively suggest that reduced NA transmission might increase impulsivity, and more specifically, that hypoactive NA in the right inferior frontal gyrus might contribute towards behavioural disinhibition in subjects with ADHD.

Unlike clinical groups with ADHD, healthy subjects that are acutely administered with atomoxetine become more impulsive. The α2a adrenoreceptor antagonist yohimbine that presynaptically blocks the α2a receptors and increases NE transmission similarly increases commission errors within IMT/DMT and Go/No-go tasks (Graf et al., 2011; Swann et al., 2005). In addition, yohimbine induced behavioural disinhibition in the IMT correlates with increased NA metabolites in healthy subjects, demonstrating that the increased impulsivity observed is related to an increase in NA transmission (Swann et al., 2013). Furthermore, the P300 event-related-potential amplitude that has been proposed to reflect LC-NA activity (Nieuwenhuis, Aston-Jones, & Cohen, 2005) increases during No-go trials in a Go/No-go task and stop trials in the SST (Enriquez-Geppert et al., 2010; Falkenstein, Hoormann, & Hohnsbein, 1999), linking NA activity to behavioural inhibition. In addition, the NA β-blocker propanolol, reduces discrimination between the magnitude of wins and losses within a laboratory gambling task in healthy subjects (Rogers et al., 1999). This would suggest that NA signalling in involved in information processing of reward value under conditions of arousal.

In summary, subjects taken from clinical populations with impaired inhibitory control appear to have hypo-functioning NA and treatment of this via NARI can produce positive clinical effects on symptoms. However, increasing NA in healthy controls appears to increase behavioural disinhibition and increase risky decision making amongst these subjects. These findings therefore support the involvement of NA in inhibitory control in both clinical and healthy populations.

Interestingly, systemic administration of atomoxetine has been found to reduce impulsivity in rats when measured by the 5CSRTT, SSRT and DRT (Robinson, Eagle, et al., 2007) indicating the possibility of common neural substrate of impulsive action and choice being NA. Central administration of atomoxetine has also been shown to reduce impulsivity on the 5CSRTT when infused into the NAcb shell, but not the NAcb core (Economidou et al., 2012). These studies therefore support a relationship between reduced NA transmission and impulsivity in animal models.

1.6.4 Dopamine and Impulsivity

Impulsivity in healthy subjects has been associated with fronto-striatal dopamine activity. SSRT in healthy subjects negatively correlates with $D_{2/3}$ receptor availability within the striatum and subjects with short SSRT show greater activation of frontal structures in comparison to subjects with a long SSTT (Ghahremani et al., 2012). This demonstrates that subjects with greater difficulties in action cancellation have lower $D_{2/3}$ availability within the striatum and reduced frontal activation. Trait impulsivity also negatively correlates with $D_{2/3}$ availability in the VTA, and positively correlates with DA release in the ventral striatum following acute amphetamine (Buckholtz et al., 2010). This additionally indicates that differences in dopamine transmission in midbrain regions are related to impulsivity. Although still limited, these studies support a relationship between DA and impulsivity in healthy subjects.

The relationship between DA and impulsivity has also been demonstrated, similarly to NA, through the therapeutic success of dopaminergic treatment of impulse control disorders, such as ADHD. Drugs that successfully treat impulsive symptoms amongst ADHD patients, such as Adderall (Mixed D- and L-amphetamine salts), Dexedrine (D-amphetamine) and Ritalin (MPH), all act either as a substrate at, or bind to and block, the DAT, indicating that changes in synaptic DA can alleviate impulsive symptoms. Furthermore, MPH administered to subjects with ADHD increases activity within neural regions associated with impulsivity, including frontal (right PFC, dmPFC, right and left IFG, vmPFC) and striatal (caudate nucleus, putamen) regions whilst patients perform inhibitory tasks (Cubillo et al., 2012; Lee et al., 2010; Rubia et al., 2009; Rubia et al., 2011) and activation of frontal and striatal areas following MPH has also been observed alongside a reduction in impulsive errors within Go/No-go tasks (Epstein et al., 2007; Vaidya et al., 1998). These findings therefore indicate that changes in dopamine within fronto-striatal regions can improve impulse control in a clinical population with increased levels of impulsivity.

Downregulation of dopamine synthesis, the DAT and the $D_{2/3}$ receptors are common features of addiction pathology observed amongst stimulant, opiate and alcohol addicts (Lee et al., 2009; Martinez et al., 2004, 2009; McCann et al., 1998; Volkow et al., 1997; Volkow et al., 1993, 2002; Volkow et al., 2001; Volkow et al., 2001; Wang et al., 1997). These changes in dopamine function have been found to exist primarily in the striatum (Lee et al., 2009; Martinez et al., 2004, 2009; McCann et al., 1998; Volkow et al., 1997; Volkow et al., 1993, 2002; Volkow et al., 2001; Volkow et al., 2001; Wang et al., 1997) and in cocaine and methamphetamine users downregulation of striatal D_2 receptors has been observed alongside reduced frontal metabolism (Volkow et al., 1993; Volkow et al., 2001) demonstrating that

downregulation of dopamine function occurs within fronto-striatal regions relating to impulsivity amongst drug addicts.

Increased impulsivity observed amongst addicts (Coffey et al., 2003; Colzato, van den Wildenberg, & Hommel, 2007; Fillmore & Rush, 2002; Hoffman et al., 2006; Kirby & Petry, 2004; Madden, Petry et al., 1999; Monterosso et al., 2005; Petry & Bickel, 1999; Petry, 2001; Verdejo-García et al., 2007) might then relate to the wealth of evidence demonstrating changes in dopaminergic function within regions of inhibitory control. Studies directly assessing the relationship between dopamine and impulsivity amongst drug addicts have identified that reduced $D_{2/3}$ receptor availability within the striatum is associated with increased levels of trait impulsivity and delay discounting in stimulant dependent subjects (Lee et al., 2009; Martinez et al., 2011; Wang et al., 2011) and furthermore that this reduction in $D_{2/3}$ receptor availability can predict earlier relapse in cocaine and methamphetamine subjects (Martinez et al., 2011; Wang et al., 2011). In addition, increasing dopamine levels amongst cocaine addicts via administration of methylphenidate can reduce SSRT in these subjects alongside increasing frontal activity in the vmPFC and inhibition-related activation of the middle frontal cortex (Li et al., 2010). This demonstrates that changes in prefrontal dopamine are also associated with impulsivity in cocaine addicts. These studies therefore collectively confirm that changes in dopamine activity in frontal and striatal regions are associated with increased levels of trait impulsivity, impulsive choice and impulsive action observed in drug addicts, and furthermore that the extremity of these changes can predict relapse in drug addicts.

Animal studies that have explored the neurochemical substrates of impulsivity and drug induced impulsivity have identified that dopamine depletion within the NAcb can reduce behavioural inhibition (Cole & Robbins, 1989) and drug induced changes in behavioural inhibition and delay discounting in rats (Cole & Robbins, 1989; Winstanley, Theobald, Dalley, & Robbins, 2005). Evidence that direct manipulation of dopamine transmission within the NAcb can change impulsivity therefore confirms a relationship between ventral striatal dopamine and impulsivity and supports human literature implicating a relationship between striatal dopamine and impulsivity in addicts (Martinez et al., 2009; Volkow et al., 1997). Confirmation that NAcb dopamine can mediate impulsivity in rats (Cole & Robbins, 1989; Winstanley et al., 2005) alongside evidence that reduced $D_{2/3}$ receptor availability within the ventral striatum is a common pathology of drug addiction (Martinez et al., 2004; Volkow et al., 1993, 2002; Volkow et al., 2001) and impulsivity amongst addicts (Lee et al., 2009; Martinez et al., 2011; Wang et al., 2011) has led to investigation into the roles of the dopamine sub-receptors in mediating impulsivity and drug use in animal models.

Similar to that observed in human PET studies, reduced $D_{2/3}$ receptor availability within the ventral striatum, specifically within the NAcb, has been identified in highly impulsive rats that escalate cocaine use faster than less impulsive rats (Dalley et al., 2007). This overlap between human and animals literature therefore further confirms that neurochemical changes in the dopamine system at the level of the NAcb are involved in impulsivity and drug use. Direct manipulation of the D_1, D_2 and D_3 receptors within the NAcb core and shell has further elucidated the specific functions of these dopamine sub-receptors within the ventral striatum in mediating impulsivity. The D_1 receptors within the NAcb core and shell have been found to mediate impulsivity in rats that display normal levels of inhibitory control at baseline, but do not mediate drug induced impulsivity (Pattj et al., 2007). In contrast, the D_2 receptors within the NAcb core, but not shell, have been found to mediate impulsivity in highly impulsive rats and drug induced impulsivity, but not 'normal' levels of impulsivity in rats (Moreno et al., 2013; Pattij et al., 2007). The D_3 sub-receptor within the NAcb core, similarly to the D_1 sub-receptor, does not mediate high levels of impulsivity in rats, but the D_3 receptors in the NAcb shell do mediate hyperactivity in highly impulsive rats (Moreno et al., 2013). These findings therefore implicate a specific role for the D_2 sub-receptor within the NAcb core in mediating high levels of impulsivity, and suggest that the functional sub-receptor mediating impulsivity in PET studies reporting reduced ventral striatal $D_{2/3}$ receptor availability in highly impulsive addicts and rats might be the D_2 sub-receptor. Consequently, evidence from animal studies to date has most convincingly implicated a role for the D_2 sub-receptor within the NAcb core in the mediating elevated impulsivity in rats.

1.6.5 Summary

Literature reviewed in the 'neurobiology of impulsivity' section of this thesis has demonstrated the involvement of fronto-striatal-thalamic circuits in mediating both the normal expression of impulsivity and drug induced impulsivity. What is clear from human literature examining the neuroanatomical and neurochemical systems involved in impulsivity is that changes in the $D_{2/3}$ receptors within striatal regions, and particularly the ventral striatum, are associated with drug use and impulsivity. In addition, animal literature has similarly identified that changes in the $D_{2/3}$ receptors within the ventral striatum are associated with impulsivity in rats and in the escalation of drug use, further supporting a relationship between $D_{2/3}$ receptor activity in the ventral striatum with impulsivity and addiction.

1.7 General Summary

Drug addiction is a global monetary, health and social burden. Amphetamine is amongst the most harmful and prevalent of recreationally abused drugs both within the UK and throughout the world (Nutt, King, & Phillips, 2010; WDR, 2010; 2011; 2012). Consequently, research efforts aimed at elucidating this disease process are required. To date, two large theories have been the focus of addiction research; these are the 'incentive-sensitisation' and 'opponent process' theories of drug addiction, both of which address how central facets of addiction, including craving, drug seeking, tolerance, withdrawal and relapse, develop pathologically (Robinson & Berridge, 1993; Koob & Le Moal, 2008). However, more recent conceptualisations of addiction as a disorder of impaired impulse control has lead to theories proposing that changes in impulse control systems that heighten levels of 'impulsivity' are involved to the development and maintenance of addiction (Bechara, Dolan, & Hindes, 2002; Jentch & Taylor, 1999).

Previous research attempting to test the hypothesis that reduced impulse control is involved in addiction has found substantial evidence that drug users and addicts have greater levels of both trait and behavioural forms of impulsivity in comparison to healthy subjects (Clark et al., 2006; 2008; Coffey et al., 2003; Ersche., 2008; Fillmore & Rush, 2002; Hoffman et al., 2006; 2008; Kirby & Petry, 2004; Kjome et al., 2010; Madden et al., 1997; Mitchell, 1999; Moeller et al., 2002; Monterosso et al., 2005; Morgan, 1998; 2002; Parrott et al., 2002; Petry & Bickel, 1999; Quednow et al., 2007; Verdejo-Garcia et al., 2007). Animal research, that can more directly assess the cause and effect relationship of impulsivity and addition, has found that animal's displaying greater levels if impulsivity at baseline acquire drug use, escalate drug use and relapse earlier than animals with lower levels of impulsivity at baseline (Anker et al., 2009; Broos et al., 2012b; Dalley et al., 2007; Diergaarde et al., 2009; Economidou et al., 2009; Perry et al., 2005; 2008). These findings suggests that greater levels of trait impulsivity predating drug use can increase the chance of developing addiction, supporting a causal relationship between pre-existing levels of impulsivity and addiction. However, acute and chronic administration of drugs can also increase impulsive behaviour in animals and humans (Blondel et al., 2000; Dallery & Locey, 2005; Fillmore, Rush & Hays, 2002; Fillmore, Rush & Marczinski, 2003; Harrison, Everitt & Robbins, 1997; Kolokotroni, Rodgers & Harrison, 2011; 2012; Paine et al., 2003; Richards, Sabol & de Wit, 1999; Setlow et al., 2009; Simon, Mendez & Setlow, 2007; Winstanley et al., 2009). These studies alternatively suggest that elevations in impulsivity observed in addicts might be a direct consequence of drug use.

Interestingly, amphetamine has been found to both increase and decrease impulsive behaviour in both animals and human studies. These effects appear to specifically depend on

baseline levels of impulse control within the subject group, with amphetamine reducing impulsive behaviour in subjects displaying poor impulse control at baseline (de Wit, Crean & Richards, 2000). Additionally, the effects of amphetamine on impulsivity appears to depend on the dimension of impulsivity measured, for example, acute amphetamine administration in rats can reduce impulsive choice but increase behavioural disinhibition in rats (Cardinal, Robbins & Everitt, 2000; Harrison, Everitt & Robbins, 1997; Harrison et al., In prep; Eagle et al., 2009; van Gaalen et al., 2006; 2006b). These studies therefore suggest that the relationship between amphetamine use and impulsivity might be paradoxical based on the dimension of impulsivity measured.

Investigations into the neurobiology of impulsivity has found that fronto-striato-thalamic regions are recruited by healthy subjects when performing tasks requiring inhibitory control (Aron et al., 2007; Chikazoe, 2010; Garavan et al., 2002; Kelly et al., 2004; Liddle, Kiehl & Smith, 2001; McClure et al., 2004; McNab et al., 2008; Menon et al., 2001; Steele et al., 2013; Tanaka et al., 2004). Interestingly, changes in the volume of frontal (prefrontal cortex, orbitofrontal cortex, cingulate cortex, insula cortex) and striatal (caudate and putamen) regions have been observed in addicts in comparison to healthy controls (Chang et al., 2005; Daumann et al., 2011; Ersche et al., 2011; 2012; Jernigan et al., 1991; Koester et al., 2012; Liu et al., 1998; Matochik et al., 2003; Pfefferbaum et al., 1997; Thompson et al., 2004). Addicts also tend to display lower prefrontal activation in comparison to controls when performing tasks requiring behavioural inhibition and delay discounting (Hester & Garavan, 2004; Hoffman et al., 2008; Kaufman et al., 2003; Li et al., 2008) indicating that changes in the activation of frontal control systems connected with striatal regions are linked to impulsive behaviour observed in addicts.

Investigations into the neurochemical mediators of impulsivity has implicated changes in dopamine, serotonin and noradrenalin transmission to changes in impulsive behaviour amongst healthy subjects (Rubia et al., 2005; Chamberlain et al., 2007; Graff et al., 2011; Swann et al., 2005; Ghahremani et al., 2012; Buckholtz et al., 2010). PET studies conducted with drug addicts have more specifically identified hypoactive dopamine transmission and reduced D_2 receptor availability within the striatum of addicts in comparison to healthy controls (Lee et al., 2009; Martinez et al., 2004; 2009; McCann et al., 1998; Volkow et al., 1993; 1997; 1999; 2002; Wang et al., 1997). Reduced D_2 receptor availability within the ventral striatum has also been found to correlate with trait impulsivity reported by addicts, and can predict treatment outcome amongst addicts engaged in contingency management therapy, where successful treatment outcome is reflective of reductions in impulsive choice behaviour (Lee et al., 2009; Martinez et al., 2011; Wang et al., 2012). These findings have subsequently

led to the recent proposition that the D_2 receptors within the striatum are biomarker of impulsivity in drug addiction (Trifilieff & Martinez, 2013).

1.7.1 Future Research Directions and Thesis Aims

Whilst a clear relationship exists between impulsivity and drug addiction, breaking this relationship down to establish its direction (eg. cause vs. consequence) remains the focus of much research. To date, previous research that has investigated the direct effects of repeated amphetamine administration on impulsivity has found that amphetamine produces paradoxical effects on impulsivity depending on the dimension of impulsivity being measured (action vs. choice paradigm). More specifically, repeated amphetamine has been found to increase impulsive choice behaviour but does not produce any effects on behavioural inhibition in rats when tested with the 5CSRTT (Dalley et al., 2005; Dalley et al., 2007b; Gipson et al., 2009).

The 5CSRTT is predominantly an animal model of attention and as such is known to rely on attentional mechanisms (Carli et al., 1983). This primary measure of the 5CSRTT can handicap the potential to measure behavioural inhibition in rats since changes in attention can produce behaviour similar to that observed as behavioural disinhibition. For example, reduced attention on the 5CSRTT might increase premature responses (the index of response disinhibition) due to the inability to employ the attention required to monitor light cues signalling correct response behaviour, as opposed to a change in the ability to inhibit a premature response. An additional caveat of the 5CSRTT for measuring behavioural inhibition is that premature responding on the 5CSRTT does not have a specific consequence in relation to the reinforcement parameters of the task. For example, if an animal increases premature responding in the 5CSRTT there is not necessarily a change in the amount of overall food reward gained. This means that an animal can make more premature responses without receiving any significant change in reinforcement. Consequently, the actual significance of changes in premature responding is difficult to ascertain from the 5CSRTT. In consideration of these factors, it is unclear whether repeated amphetamine exposure does not directly affect response inhibition in rats, or whether any amphetamine induced changes in response inhibition are not detected from this paradigm. Future research investigating the effects of repeated amphetamine exposure on response inhibition paradigms that are more direct measures of behavioural inhibition are therefore required. Research targeted at this area will, firstly, help to further disentangle the complex relationship between amphetamine and impulsivity, and, secondly, give a clearer picture of how repeated amphetamine specifically affects response inhibition.

The current thesis aims to address this research objective through assessing the effects of repeated amphetamine in rats on response inhibition measured by the Go/No-go symmetrically reinforced task. In the Go/No-go symmetrically reinforced task, response inhibition results in the direct gain of food reward whilst behavioural disinhibition results in the direct loss of food reward. This task also employs continual light cues guiding behaviour enabling low attentional demand. These parameters consequently allow for a more direct assessment of response inhibition in rats, where increases in response disinhibition can be interpreted as a motivationally significant change in prepotent inhibitory control.

Previous research conducted into the neurobiology of impulsivity and addiction has implicated changes in fronto-striatal systems in drug addiction and impulsive behaviour (Chang et al., 2005; Daumann et al., 2011; Ersche et al., 2011; Jernigan et al., 1991; Koester et al., 2012; Lee et al., 2009; Liu et al., 1998; Matochik et al., 2003; Martinez et al., 2004; 2009; McCann et al., 1998; Pfefferbaum et al., 1997; Thompson et al., 2004; Volkow et al., 1993; 1997; 1999; 2002; Wang et al., 1997). The ventral region of the striatum has been identified as an important neuroanatomical site in relation to impulsivity and drug induced impulsivity in both human and animal research (Lee et al., 2009; Cardinal et al., 2001; Murphy et al., 2008). The ventral striatum is a site of neural convergence where prefrontal, limbic and midbrain neurons can synapse onto a single interneuron, allowing integration of prefrontal, limbic and midbrain processing to take place. Changes in the balance of this 'prefrontal-midbrain' integration can consequently weaken prefrontal top-down control over 'impulsive urges' originating from midbrain activity. Neurochemically, the ventral striatum is heavily innervated with dopamine nerve terminals and predominantly consists of GABAergic medium spiny interneurons; consequently, an important aim of future research will be to elucidate the specific role of both of dopamine and GABA systems within the ventral striatum in impulsivity and addiction.

In order to address this research objective, investigating the role of system (dopamine and GABA) sub-receptors within the ventral striatum in impulsivity and drug induced impulsivity will be essential for establishing how neurochemical changes manifest at the level of the ventral striatum to affect impulse control. To date, human research has identified that reduced D_2 receptor availability within the ventral striatum is associated with increased impulsivity and a greater tendency to discount future rewards associated termination of drug use in addicts (Lee et al., 2009; Martinez et al., 2009; Wang et al., 2011). Whilst such findings clearly indicate the involvement of the D_2 receptors in impulsivity and addiction, it is difficult to establish from human research what the specific role of the D_2 receptors within the ventral striatum is in relation to impulsive behaviour observed in addicts. Consequently, future research assessing the effects of direct D_2 receptor manipulation within the ventral striatum on specific

behavioural dimensions of impulsivity and drug induced impulsivity is required. This thesis aims to address these research objectives by investigating the effects of D_2 receptor antagonism within the ventral striatum, specifically within the nucleus accumbens core, on impulsivity and amphetamine induced impulsivity within the Go/No-go symmetrically reinforced task. Furthermore, given that GABA constitutes a major neurochemical network within, and projecting from, the ventral striatum, future research assessing the involvement of GABA within the ventral striatum in impulsivity and drug induced impulsivity is required. This thesis aims to address these research objectives by investigating the effects of $GABA_A$ receptor agonism within the ventral striatum, specifically within the nucleus accumbens core, on impulsivity and amphetamine induced impulsivity within the Go/No-go symmetrically reinforced task. Conducting these research aims will help to build up a more specific (sub-receptor) and broader (systems) understanding of the neurochemical changes within the ventral striatum involved in impulsivity and addiction.

1.7.2 Aims and Hypotheses

In consideration of the future research directions discussed, this thesis has three main research aims:

1) To further disentangle the relationship between repeated amphetamine use and response inhibition through testing the effects of subchronic (4-day) and chronic (11-day) amphetamine treatment on a task that more directly measures response inhibition in rats. This aim sought to test the hypothesis that repeated amphetamine treatment affects response inhibition in rats performing the Go/No-go symmetrically reinforced paradigm.

2) To assess the role of the D_2 receptors within the ventral striatum in response inhibition and amphetamine induced response disinhibition in rats. This aim sought to test the hypothesis that the D_2 receptors within the nucleus accumbens core mediate amphetamine induced response disinhibition in rats measured by the Go/No-go symmetrically reinforced paradigm.

3) To assess the involvement of GABA within the ventral striatum in response inhibition and amphetamine induced response disinhibition in rats. This aim sought to test the hypothesis that the $GABA_A$ receptors within the nucleus accumbens core mediate amphetamine induced response disinhibition in rats measured by the Go/No-go symmetrically reinforced paradigm.

Chapter 2 General Methodology

The following methodology was employed across all behavioural experiments in this thesis. Any adaptations of the below methods adopted for specific experiments are highlighted in the method section at the beginning of each experimental chapter.

2.1.1 Ethics

All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986. Home Office training modules 1-4 were undertaken at The University of Leeds prior to the initiation of experimentation. All experiments were conducted under the author's personal licence (PIL 40/9836) and under the project licence of Dr. Amanda Harrison (PPL 40/2711).

2.1.2 Subjects

All experiments employed male lister hooded rats (Charles River, UK) weighing between 300-320 grams (g) upon arrival into the laboratory. An initial habituation week was implemented to familiarise animals to holding rooms and daily handling. All animals were housed in pairs received free feeding in their home cages until animals reached full adult body weight (320-340g). Prior to behavioural training in the operant chambers animals were habituated to sucrose food pellets (Sandown Scientific, Middlesex, UK), used as food reward during operant training and testing. During behavioural training and testing animals were maintained on a mild food restriction schedule (15-20 g per day), to induce motivation for the food reward. The animals were weighed daily throughout behavioural studies to monitor healthy growth. Water was available ad libitum in their home cages throughout all behavioural studies.

Animals were housed under a 12 hour light/ dark (LD) cycle (lights on at 0700hrs; lights off at 1900hrs), in a temperature (21°C ± 2°C) and humidity (50% ± 5%) controlled environment. During the light phase artificial lights (193 lux) illuminated holding rooms.

2.1.3 Apparatus (Operant Chambers)

Two sets of 8 operant chambers (Med Associates Inc., St Albans, VT, USA) and one set of 4 operant chambers (Campden Instruments Ltd, Lafayette, IN, USA) were employed to conduct the symmetrically reinforced go/no-go paradigm (dimensions: 30.5 X 24.1 X 29.2cm, 30.5 X 24.1 X 21 cm). All chambers were enclosed in wooden sound attenuating cubicles (63.5 x 49.1 x 39.4 cm, Med Associates Inc., USA) and were air ventilated via an 115V AC wall fan.

Chambers consisted of aluminium panels at the front and rear of the chamber with clear polycarbonate joining walls, one of which was hinged making a door to place animals in the chamber. The front panel of the chamber contained two stainless steel levers located symmetrically on the left and right hand side of the panel. Each lever protruded 1.9 cm from the front panel when activated. Flat lens circular stimulus lights (2.5 cm diameter) were located symmetrically on the left and right of the front panel 6 cm below the top of the chamber. Stimuli lights were illuminated by 100 mA bulbs. Food pellets were dispensed into a central hole in the front panel referred to as the magazine which was equipped with an infrared bean across the front of the magazine to record head entries made into the magazine. The magazine (5.1 cm x 5.1 cm) was located 2.5 cm from the bottom of the front panel and could be illuminated by a light located at the top of the magazine. Each chamber was illuminated by a house light (100 mA) located centrally at the top of the rear chamber panel. All apparatus was controlled, and data recorded, from chambers using MED-PC IV software (Med Associates Inc., USA).

2.1.4 Symmetrically reinforced Go/No-go conditional visual discrimination task

Behavioural training commenced with magazine training which occurred in two stages. Initially animals were placed into test chambers whilst the magazine was illuminated and contained food pellets. The second stage of magazine training involved a continuous reinforcement schedule (CRF) whereby the magazine light was illuminated every 10 or 15 seconds (s) for a duration of 5 s across a 20 minute test session. Animals were required to nose poke the magazine upon illumination in order to receive the delivery of a single food pellet. CRF magazine training continued until animals collected a pellet on more than 80 trials during two consecutive test sessions.

Upon successful completion of magazine training, animals began CRF lever training. Animals were required to make a lever response in order to illuminate the magazine for a duration of 5s, during which time animals could nose poke the magazine to receive a food reward. All animals were assigned to either a left or right lever in a counterbalanced design and were required to make at least 100 lever responses over the course of 30 minutes. Upon completion of lever training, usually two consecutive sessions, animals progressed onto the full symmetrically reinforced Go/No-go conditional visual discrimination task.

The symmetrically reinforced Go/No-go conditional visual discrimination task consisted of 80 trials, within which there are 40 go and 40 no-go trials. The visual discriminanda were fast (0.1 s pulses presented at 5 Hz) and slow (0.4 s pulses at 0.83 Hz) flashes of light made in temporal

synchrony via both the left and right light stimulus lights. Animals therefore, were exposed to one of two contingencies; 1) Go(fast)/ No-go(slow), or, 2) Go(slow)/ No-go(fast). Go and No-go trials were subsequently presented in a random sequence during each test session.

Upon initiation of the task, the house light was illuminated and an inter-trial interval (ITI) of 5 s began. During each trial, either fast or slow flashing lights were presented for a period of 11.2 s. During the first 1.2 s of this stimulus presentation (prediscrimination period) it is difficult to discriminate between the two visual stimuli, therefore any lever response made within this period is recorded as an *early anticipatory response*, but had no further consequence. All correct or incorrect behavioural responses were consequently registered during the following 10s (discrimination period) of stimulus presentation. Magazine entries made prior to making a correct lever response, or prior to the termination of No-go discriminanda, were registered as *inappropriate panel responses*, and resulted in a 5 s time out period during which the houselight was extinguished, this was followed by the start of the same trial.

During Go trials, a lever response made during the discrimination period of Go visual discriminanda, resulted in the stimulus lights being switched off, and the illumination of the magazine light for 5 s, during which time a magazine entry resulted in the delivery of a food reward and a *correct go trial* was recorded. *Correct Go response latencies* were measured from the end of the prediscrimination period of the stimulus presentation, to the time of lever response. After the delivery of a food pellet an ITI began, the duration of which was calculated from adding the standard ITI length of 5 s to the residual number of seconds of stimuli presentation after a lever response was made (*5 s + (10 s – correct response latency) = ITI*). *Go trial magazine latencies* were recorded from the time of the lever response to the time of the magazine entry. If an animal failed to enter the illuminated magazine to collect a food reward, a *Go trial magazine omission* was recorded. Following no lever response during a go trial, the 5 s ITI began prior to the next trial and an *incorrect go trial* was recorded. Lever responses made within the prediscrimination period were recorded as *Go trial anticipatory responses*.

During No-go trials, animals were required to withhold from making a lever response during the 10s stimulus presentation of No-go visual discriminanda. Following successful behavioural inhibition, the magazine light was illuminated for 5 s during which time a magazine entry resulted in the delivery of a food reward and the trial was recorded as a *correct No-go trial*. After the delivery of a food pellet an ITI of 5 s began before the start of the next trial. A lever response made within the 10 s discrimination period of a No-go trial resulted in immediate termination of the trial and the initiation of a time out period of 5 s during which the house light was extinguished and the trial was recorded as an *incorrect No-go trial*. *Incorrect No-go*

latencies were measured from the end of no-go prediscrimination period to the time of lever response. *No-go magazine latencies* were recorded from the cessation of the stimulus presentation to the time of the magazine entry. All response latencies were recorded in centiseconds (cs). Lever responses made within the prediscrimination period of No-go trials were recorded as *No-go trial anticipatory responses*. For a full outline of dependent variables measured during performance of the task see Table 2.1.

2.1.5 Statistical Analysis

All data was formatted in Microsoft Office Excel 2007 and was input into Statistical Package for the Social Sciences (SPSS) version 18 for analysis.

All data was initially checked for homogeneity of variance and normality before conducting the main experimental analysis. Homogeneity of variance was assessed via Mauchly's test of sphericity and the normality of data was assessed though Shapiro-Wilk and Kolmogorov-Smirnov tests. Any data deviating from normality were appropriately transformed with square root, log10 or inverse transformations. All data expressed as a percentage values (proportional data) were subject to arcsine transformations. Due to rarity, omissions were not analysed and are therefore not included in this thesis.

Table 2.1: Behavioural measures of the symmetrically reinforced Go/No-go conditional visual discrimination task

Behavioural Measure	***Dependent Variable***
Accuracy of Responding(%)	Total percentage correct Total percentage correct Go trials Total percentage correct No-go trials
Speed of Responding (cs)	Go-trial correct response latency No-go incorrect response latency Go magazine latency No-go magazine latency
Anticipatory Responding	No. of Go trials with an anticipatory response No. of No-go with an anticipatory response No. of Go trials with an inappropriate panel responses No. of No-go trials with an inappropriate panel response
Omissions	No. of magazine omissions following correct Go-trials No. of magazine omissions following correct No-Go trial

Chapter 3 The effects of amphetamine on food intake, water intake and body weight

3.1 Introduction

Amphetamine is an anorexigenic agent that has historically been administered for the treatment of obesity. Amphetamine induced alterations in appetite are biphasic in nature, manifesting initially through reducing food intake (hypophagia) and more longer term through increases in food intake (hyperphagia), also known as the 'rebound' effect (Jones & Caul, 1989). The switch between hypo- and hyperphagia has been suggested to mimic the initial stimulant and later withdrawal phases of amphetamine where alterations in motivation are markedly pronounced (Barr & Markou, 2005a; Der-Avakian & Markou, 2010). Specifically, hypophagia appears to predominantly occupy the early stimulant phase and hyperphagia more closely occupies the later 'withdrawal' phase. However, a secondary phase of hypophagia has also been reported to occur between 13- 27 hours following acute amphetamine administration (White, Sherrill, & White, 2007) suggesting that amphetamine induced hypophagia manifests during both early and more long term phases following amphetamine administration. The relationship between amphetamine and appetitive behaviours therefore appears to reflect at least several different motivational states following amphetamine administration.

The co-occurrence of amphetamine-induced hypophagia and stereotypy has led to theories of early hypophagia (1-3 hrs following drug) which incorporate mechanisms of both anorexia (reducing motivation for food) and stereotypy (increasing behavioural competition with feeding). Animals receiving milk through intraoral cannulas, thus removing the demand to recruit consummatory eating behaviours, show a 50% increase in milk intake in comparison to bottle fed animals following amphetamine (2 mg/kg), suggesting that the amphetamine induced disruptions in the recruitment of consummatory behaviour affects food intake (Wolgin, 2000). However, cannulated animals also showed an increase in hypophagia as the amphetamine dose increased (up to 16 mg/kg) in spite of behaviourally un-restricted access to food, indicating that high dosages of amphetamine reduce food intake by reducing appetite (Wolgin, 2000). Similarly, amphetamine has been shown to disrupt feeding within the behavioural satiety sequence (BSS) in rats (Blundell & McArthur, 1981). More specifically, amphetamine disrupts the BSS via increasing locomotor activity that subsequently delays the rest phase in the BSS. It has therefore been suggested that satiety is not a primary mechanism

of amphetamine-induced hypophagia, but is impacted in-directly following the inability to engage in the eating phase of the BSS (Blundell & McArthur, 1981; Halford, Wanninayake, & Blundell, 1998). Alternatively, amphetamine has also been suggested to induce hypophagia via lowering the body weight 'set point' in rats (Levitsky, Strupp, & Lupoli, 1981; Stunkard, 1982; Wolgin, 1983). This view proposes that the reduction in food intake observed following amphetamine administration is the result of a homeostatic mechanism that is recruited to achieve a lower body weight set point.

Longer-term hypophagia that may continue following the termination of amphetamine treatment has been less extensively studied. Interestingly, a secondary phase of hypophagia has been observed at 13-27 hrs following acute amphetamine administration (1 and 2 mg/kg) (White et al., 2007) indicating that hypophagia can continue to occur well past the clearance of amphetamine in rats (Fuller, Baker, & Molloy, 1977; Hutchaleelaha, Sukbuntherng, Chow, & Mayersohn, 1994). Time to consume food did not differ between saline and amphetamine animals when animals were required to lever press once to receive six pellets representing low response cost to receive reward. Interestingly, therefore, the influence of consummatory behaviours and appetitive motivation appear to be less implicated in the mechanisms underlying the secondary phase of hypophagia (White, Hundley, & White, 2010; White et al., 2007). However, animals treated with a 4-day escalating dose regime of amphetamine exhibit lower break points in progressive ratio self-administration paradigms in comparison to saline controls up to withdrawal day three following drug treatment (Barr & Phillips, 1999; Orsini, Koob, & Pulvirenti, 2001) and a lower break point on food reward progressive ratio tasks for up to four weeks following drug termination (Der-Avakian & Markou, 2010) suggesting a reduction in appetitive motivation to gain food reward through these withdrawal phases. However, it was also found that animals did not differ in their consumption of freely available sucrose solution (Barr & Phillips, 1999) highlighting that this reduction in the animals motivation for food reward is only apparent when the response cost is high, such as during later stages of progressive ration schedules of reinforcement.

Repeated administration of amphetamine leads to a slow reversal of amphetamine-induced hypophagia whereby animals gradually increase their food intake demonstrating tolerance to this drug effect. Tolerance to amphetamine-induced hypophagia however is contingent on preprandial administration of amphetamine (Carlton & Wolgin, 1971). The absence of this effect following postprandial administration of amphetamine has led to the suggestion that such tolerance is in part modulated by contingent drug-induced conditioning (Poulos, Wilkinson, & Cappell, 1981) accounting for the build up of tolerance only when feeding follows amphetamine. Tolerance to amphetamine's hypophagic effects has also been suggested to

reflect instrumentally learnt suppression of stereotypic behaviour that is controlled via the prevailing contingencies of reinforcement (Wolgin, Thompson, & Oslan, 1987). In other words, animals learn to suppress stereotypic behaviours that are interfering with their ability to eat and gain food. Alternatively, Stunkard (1982) has argued that tolerance to amphetamine induced hypophagia reflects a method of homeostatic regulation to meet the new body weight settling point. This argument is supported by data demonstrating that whilst animals become tolerant to the hypophagic effects of amphetamine and subsequently increase food intake, body weight remains significantly lower than saline controls throughout the 'tolerance' phase (Wolgin, 1983). Furthermore, animals receiving chronic amphetamine over the course of 7 days demonstrate increased motivation to obtain food reward through increased break points in a progressive ratio task to receive sucrose pellets whilst simultaneously displaying reduced body weight in comparison to saline controls (Der-Avakian & Markou, 2010). These findings support suggestions that alterations in the appetitive motivational state of animals receiving chronic amphetamine co-exist with a significant reduction in body weight. However, White et al., (2010) recently found that tolerance to the longer-term hypophagic effects (13- 27 hrs) is only produced by repeated administration of 1 mg/ kg of amphetamine and not an alternating 1 and 2 mg/ kg regime, suggesting that tolerance to is less likely to develop under unstable drug dose administration regimes. The lack of tolerance displayed under alternating amphetamine regimes may reflect a deficit in learning due to the unpredictable drug regime. It could be, therefore, that it is harder for animals to learn how to suppress amphetamine induced hypophagia when the hypophagic effect of amphetamine is unpredictable.

Alongside amphetamine-induced alterations in food intake, amphetamine is also known to alter water intake. Typically animals exhibit a reduction in water intake (hypodipsia) for around 1- 2 hrs following amphetamine administration which is later compensated for via increases in water intake (hyperdispsia) at 2- 5 hrs, and subsequently becomes normalised after approximately 5 hrs (Camanni & Nencini, 1994). Repeated intermittent administration of amphetamine however leads to an increase in water intake in rats (Badiani & Stewart, 1993; Camanni & Nencini, 1994). Amphetamine induced hyperdispsia appears to be independent of the hypophagic effects of amphetamine. Amphetamine treated animals have been shown to drink the same amount of water when receiving free access to food as they do in the absence of food, suggesting that the excessive drinking following amphetamine administration is not related to the concomitant reduction in food intake (Rowland, Antelman, & Kocan, 1981).

It is clear that amphetamine exerts substantial effects upon eating and drinking behaviour in animals, both in the short and longer term, subsequently altering the motivational state of animals. Due to the nature of operant tasks employing food reward as a behavioural

reinforcer, the possibility arises that amphetamine induced changes in primary motivation may directly impact behaviour observed in these operant tasks. Studies investigating the effects of pre-feeding on performance of the 5CSRTT have indicated that reducing the motivational state of animals prior to behavioural testing increases the latencies to respond and omitted trials, and significantly reduces premature responses (Grottick & Higgins, 2002; Harrison et al., 1997). Food intake has also been found to correlate with premature responding in the 5CSRTT, whereby the more food consumed by animals prior to operant testing the less number of premature responses were observed (Harrison et al., 1997). Animals maintained at 90 and 95 % of their free feeding body weight (reduced motivation) display significantly lower levels of premature responding in comparison to animals maintained at 80 % (increased motivation) and to amphetamine treated animals (Bizarro & Stolerman, 2003). Motivational changes have also been shown to affect performance on the symmetrically reinforced Go/No-go tasks. Animals that received pre-feeding prior to operant testing (reduced motivation) failed to collect the food rewards during no-go trails more frequently than during baseline testing, whereas animals that received a 50% reduced feed 24 hours prior to operant testing (increased motivation) displayed a reduction in no-go trial incorrect response latencies in comparison to baseline, whilst causing no effect on go and no-go trial performance accuracy (Kolokotroni et al., 2011). Such studies highlight the importance of understanding the motivational state of animals prior to proceeding with operant food reward tasks.

The current thesis aims to investigate the effects of acute and chronic amphetamine on behavioural disinhibition via a symmetrically reinforced Go/No-go task, within which impulsive behaviour is measured as increased inappropriate responding within no-go trials which results in the loss of food reward. The effects of the employed acute and chronic drug regimes on food intake, water intake and body weight have not yet been extensively studied. Thus, in order to effectively interpret the behavioural effects of amphetamine on impulsivity it is important to directly measure and identify the motivational state of animals following acute and 4-day amphetamine.

3.1.1 Objectives

In order to investigate potential alternations in the motivational state of animals following acute and repeated (4-day) amphetamine administration, the objectives of Experiments 1a and 1b were:

i) To determine the effect of acute amphetamine on food intake, water intake and body weight in rats (Experiment 1a)

ii) To determine the effect of 4-day amphetamine and withdrawal on food intake, water intake and body weight in rats (Experiment 1b)

3.2 Method

3.2.1 Subjects

Subjects were 21 male Lister Hooded rats (Charles River, UK). Upon arrival animals weighed between 300-320g and were housed in pairs for experiment 1 and singly housed in experiment 2 under a light/dark cycle of 12 hrs (lights on 07:00 hrs, lights off 19:00 hrs) in a temperature (21 +/- 2°C) and humidity (50 +/- 10%) controlled environment. Following habituation to the laboratory animals were separated and housed independently. Animals were maintained on a free-feeding schedule receiving unlimited access to rodent chow (Sandown Scientific, UK) within home cages for the duration of experimental procedures. Water was available ad libitum.

3.2.2 Drugs

Amphetamine (Sigma Aldrich, UK) was dissolved in 0.9% NaCl and was injected i.p. in a volume of 1 ml/kg body weight.

3.2.3 Experiment 1a: The effect of acute amphetamine on primary motivation

3.2.3.1 Procedure

Upon reaching adult body weight (320- 340g) animals (n=8) entered the experiment. Animals received 0, 0.2, 0.4, 0.8, and1.2 mg/kg of amphetamine in a latin-square design, constituting five test sessions with seven days between each session.

Prior to each test session animals' were food restricted for 12 hours. On the morning of each test day animals were weighed before receiving amphetamine injections. 15 minutes prior to the test, and were subsequently placed back into their home cages and transported to the test room. Water bottles were removed from home cages for 15 minutes pre-test to ensure that test sessions captured water intake following amphetamine absorption. Pre-weighed rodent chow was placed into cage hoppers, and pre-weighed water bottles were placed in animals cages at the start of each test session. Each 1 hour test session took place between 12:00-13:00 hrs. At the end of each test session animals were weighed again and all food and water was re-weighed. Animals were then transported back to holding rooms where they received free access to food and water.

Food intake, water intake and body weight were all measured via change in grams from the beginning to the end of a test session.

3.2.4 Experiment 1b: The effects of 4-day amphetamine on primary motivation

3.2.4.1 Procedure

Upon reaching adult body weight (320- 340g) animals entered the experiment and were assigned to either the amphetamine (n=7) or saline (n=6) treatment group. Animals were assigned to the group based on their body weight, food intake and water intake during the week prior to the study, to ensure that prior to drug treatment the groups were evenly matched across baseline in each dependent variable. Intake of rodent chow, water intake and body weight were subsequently recorded across 31 days containing the following four test phases: baseline (7 days), drug administration (4 days), withdrawal week one (7 days), withdrawal week two (7 days) and withdrawal week three (7 days). Food and water intake were measured from animals' home cages. Following baseline, amphetamine was administered three times a day (0800 hrs/ 1400 hrs/ 2000 hrs) over the course of four days in a rising dose regime. A total of 50mg/kg of amphetamine was administered per animal at the following doses; Day 1 (1, 2, 3 mg/kg), Day 2 (4, 5, 5 mg/kg), Day 3 (5, 5, 5 mg/kg), Day 4 (5, 5, 5 mg/kg). Control animals followed the same administration regime receiving three saline injections a day (0800 hrs/ 1400 hrs/ 2000 hrs) over 4 days. All measures were recorded at the same time daily, prior to the first amphetamine injection, during the administration phase. The data for baseline day 7 was recorded prior to the first drug treatment, on drug day 1, and therefore represents the final baseline measure of the dependent variables. On Figures 3.4 – 3.6 drug day 1 therefore represents changes in food intake, water intake and body weight following amphetamine administration of 1, 2, 3 mg/kg or saline treatment on the previous day (baseline day 7).

3.2.5 Statistical Analysis

Before conducting the main analysis, all data were checked for normality and homogeneity of variance via Shaprio-Wilk tests and Mauchley's test of Sphericty. Any data found to be abnormally distributed was subsequently transformed via log10, square root or inverse transformations (Field, 2005). Data violating sphericity was adjusted via Greenhouse-Geisser correction.

Acute amphetamine data (Experiment 1a) was analysed in a 1 x 5 repeated measures ANOVA, with amphetamine dose entered as the within-subjects factor and test dosages (0, 0.2, 0.4, 0.8, 1.2 mg/kg) constituting 5 dose levels. Further analyses of main effects of dose were conducted using Bonferroni post-hoc analyses. Data was analysed as the change in grams from the beginning to the end of test sessions.

Chronic amphetamine data was split into baseline, drug treatment, withdrawal week 1, withdrawal week 2 and withdrawal week 3 phases, and was subsequently analysed independently via 5 independent analyses. A 2 x 7 repeated measures ANOVA was conducted to analyse baseline data with treatment group entered as the between-subjects factor and baseline day entered as the within subjects factor. All remaining drug treatment and withdrawal data were analysed via ANCOVA with the subjects average baseline data acting as the covariate. Homogeneity of regression between the covariate and treatment groups, and the significance of the covariate were checked before proceeding with ANCOVA. In summary, one 2 x 3 repeated measures ANCOVA was conducted to analyse drug treatment (although there were four days of drug treatment the drug day 1 data was recorded prior to any drug treatment and was therefore represented during baseline (drug free), three 2 x 7 repeated measures ANCOVAs were conducted to analyse withdrawal week 1, withdrawal week 2, and withdrawal week 3 for each dependent variable. Interaction effects were further analysed via simple effects analysis of between and within subjects-effects utilising the pooled error term (Howell, 2010). Between-subjects effects on individual test days were analysed using a Univariate ANCOVA with the subject's average baseline entered as the covariate. Within-subjects effects were analysed using two 1-way repeated measures ANCOVA (one ANCOVA performed for each group). Significant F-Values resulting from within-subjects simple effects analyses were further investigated using post hoc comparisons between within-subject levels (drug days/ withdrawal days).

3.3 Results

3.3.1 Experiment 1a: The effect of acute amphetamine on primary motivation

3.3.1.1 Food Intake

Acute amphetamine was found to significantly affect food intake in rats ($F(4, 28) = 15.946$, $p<0.001$). Post hoc bonferroni analysis of this main effect revealed a significant reduction in food intake at 0.8 ($p<0.05$) and 1.2 mg/kg ($p<0.01$) in comparison to the saline control treatment. Furthermore, animals ate significantly less food after 1.2mg/kg in comparison to both 0.2 ($p<0.05$) and 0.4 mg/kg ($p<0.05$) and ate significantly less food after 0.8 mg/kg in comparison to 0.4 mg/kg ($p<0.05$). There was no difference in food intake between 0.8 mg/kg and 1.2 mg/kg ($p>0.05$) (Fig. 3.1).

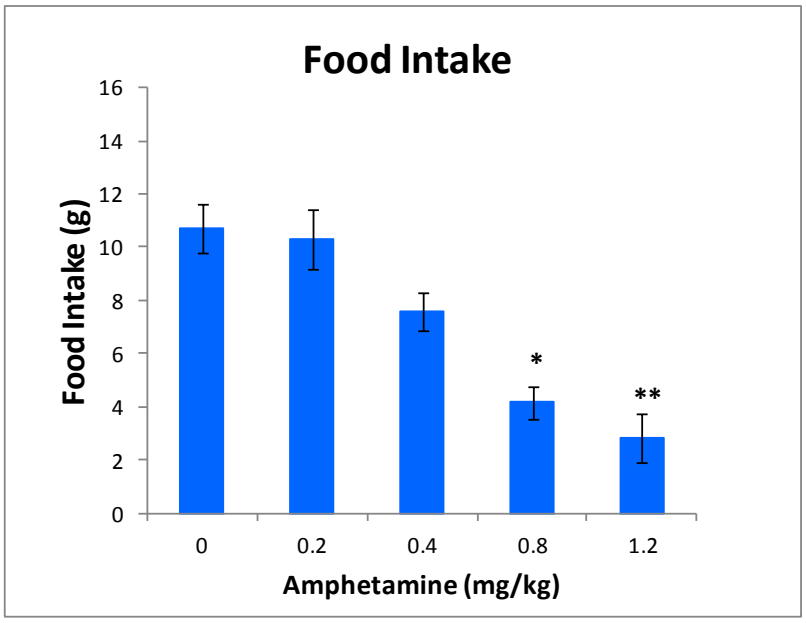

Figure 3.1: Effects of acute amphetamine on food intake. $^{**}p<0.01$, $^{*}p<0.05$ vs saline dose. Values represent means and error bars represent the SEM.

3.3.1.2 Water Intake

Acute amphetamine significantly reduced water intake ($F(4, 28)= 9.774$, $p<0.001$). Post hoc Bonferroni analysis of this main effect revealed a significant reduction in water intake following 0.8 mg/kg ($p<0.05$) and 1.2 mg/kg amphetamine ($p<0.001$) in comparison to the saline control. 1.2 mg/kg of amphetamine was also found to produce significantly lower water intake in comparison to 0.2 mg/kg amphetamine ($p<0.05$) (Fig 3.2).

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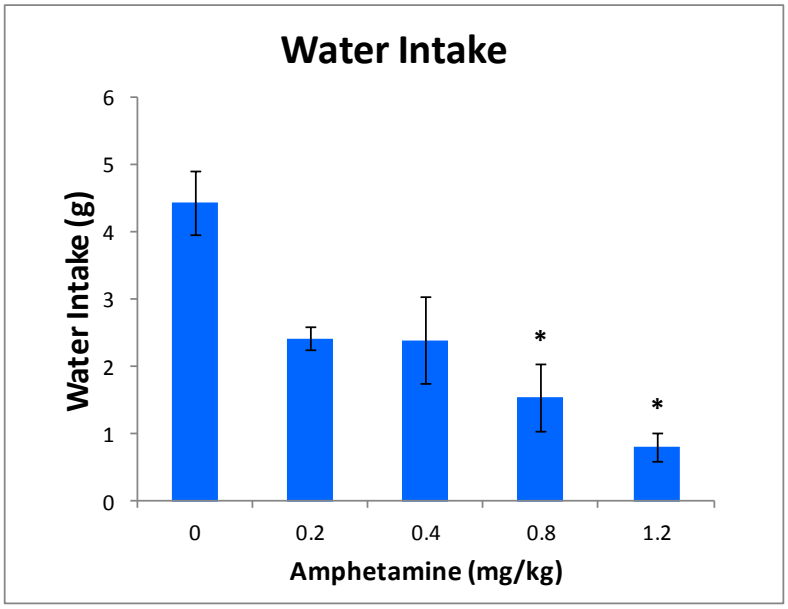


Figure 3.2: Effects of acute amphetamine on water intake. $^{**}p<0.01$, $^{*}p<0.05$ vs saline dose. Values represent means and error bars represent the SEM.

3.3.1.3 Body Weight

Analysis of body weight data revealed that acute amphetamine significantly affected body weight in rats ($F(4, 28) = 9.383$, $p<0.001$), however post hoc bonferroni analysis revealed only an approaching significant difference between 0 mg/kg and 1.2 mg/kg ($p=0.051$, NS) (Fig. 3.3).

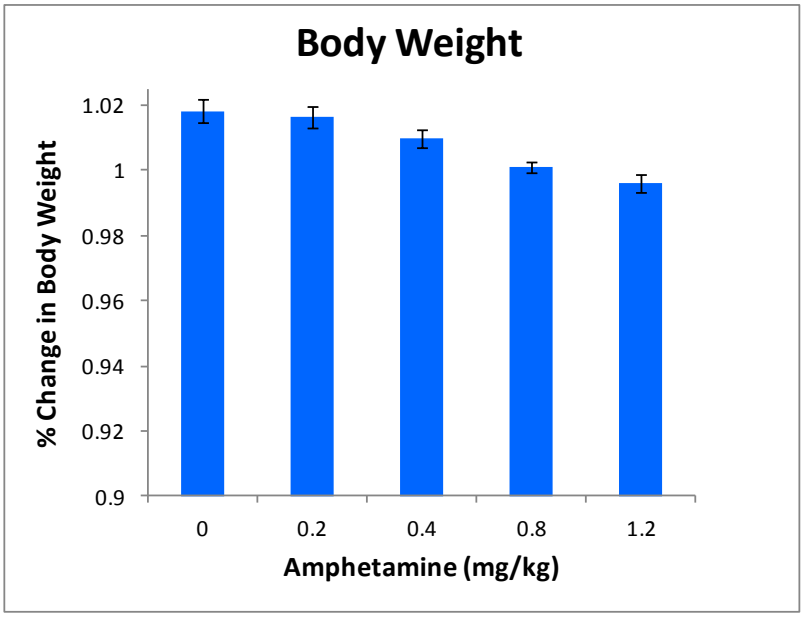


Figure 3.3: Effects of acute amphetamine on body weight. Values represent means and error bars represent the SEM.

3.3.2 Experiment 1b: The effects of 4-day amphetamine on primary motivation

Homogeneity of regression was violated within body weight data during withdrawal weeks two and three. In following this data was analysed using ANOVA. Homogeneity of regression was confirmed for all remaining variables employing ANCOVAs (covariate x treatment group, $p > 0.05$, NS) and the baseline covariate was found to significantly affect treatment groups ($p > 0.05$), confirming the need to control for baseline differences between groups in food intake.

3.3.2.1 Food Intake

3.3.2.1.1 Baseline

Analysis of food intake during baseline revealed a main effect of baseline day on food intake ($F(6, 66) = 2.611$, $p<0.05$), however post hoc bonferroni analysis revealed no significant differences in food intake between baseline days 1-7 ($p>0.05$) (Fig. 3.4). No main effect of group ($F(1, 11) = 0.404$, NS), or baseline day x treatment group interaction was found in the analysis of food intake during baseline ($F(6, 66) = 0.942$, NS) (Fig. 3.4).

3.3.2.1.2 Amphetamine treatment

Analysis of food intake during drug treatment revealed no main effect of drug day ($F(2, 20) = 2.324$, NS), however, a main effect of drug group indicated that amphetamine treated animals ate less food during drug treatment in comparison to saline treated animals ($F(1, 10) =$

336.916, p < 0.001) (Fig. 3.4). In addition, analysis of food intake produced a significant drug day x drug treatment group interaction (F(2, 20) = 336.916, p < 0.001), with post hoc simple effects analysis revealing that amphetamine treated animals ate less food relative to the controls on day 1 (F(1, 30) = 78.960, p < 0.001), day 2 (F(1, 30) = 196.423, p < 0.001), and day 3 (F(1, 30) = 254.316, p < 0.001) (Fig. 3.4). Analysis of within-subjects simple effects revealed no main effect of drug treatment day in either saline (F(8, 20) = 0.284, NS) or amphetamine animals when analysed independently (F(10, 20) = 0.662, NS) (Fig. 3.4).

3.3.2.1.3 Withdrawal week 1

Analysis of food intake during withdrawal week one revealed no main effect of withdrawal day (F(6, 60) = 0.638, NS), however, a main effect of drug treatment indicated that amphetamine treated animals continued to demonstrate less food intake in comparison to controls during withdrawal week one (F(1, 10) = 66.652, p < 0.001). In addition, a significant withdrawal day x drug treatment group interaction was detected (F(6, 60) = 40.627, p < 0.001) with simple effects analysis revealing that amphetamine treated animals ate less food than controls following 12 (F(1, 70) = 229.625, p < 0.001), 36 (F(1, 70) = 6.735, p < 0.001) and 60 hours of amphetamine withdrawal (F(1, 70) = 16.440, p =< 0.001) (Fig. 3.4). Simple effects analysis of withdrawal day effects however revealed no main effect of withdrawal day on food intake in either saline treated animals when analysed independently (F(24, 60) = 0.872, NS) or amphetamine treated animals (F(30, 60) = 0.907, NS) (Fig. 3.4).

3.3.2.1.4 Amphetamine Withdrawal Week Two

Analysis of food intake during withdrawal week two revealed no main effect of withdrawal day (F(1, 60) = 1.007, NS) and no difference in food intake was found between amphetamine and saline treated animals throughout this later phase of withdrawal (F(1, 10) = 1.693, NS). A significant withdrawal day x treatment group interaction was however detected during withdrawal week two (F(1, 60) = 2.561, p < 0.05). Within-subjects simple effects however failed to detected a main effect of withdrawal day within either amphetamine (F(30, 60) = 1.451, NS) or saline treated animals (F(24, 60) = 0.203, NS). In addition between-subjects simple effects revealed no differences in food intake between amphetamine treated animals at specific withdrawal days (df = 1, 70, all F ≤ 4.804, NS) (Fig. 3.4).

3.3.2.1.5 Amphetamine Withdrawal Week Three

Analysis of food intake during withdrawal week three revealed a main effect of withdrawal day (F(1, 60) = 3.283, p = 0.01) with post hoc bonferroni analysis indicating that animals ate more food on withdrawal day 21 in comparison to both withdrawal day 16 (p<0.01) and withdrawal day 18 (p<0.05) (Fig. 3.4). No difference in food intake however was found between

amphetamine and saline treated animals (F(1, 10) = 1.152, p = 0.308, NS) and no interaction between withdrawal day x treatment group was by the analysis of food intake during this late withdrawal period (F(1, 60) = 0.656, NS) (Fig. 3.4).

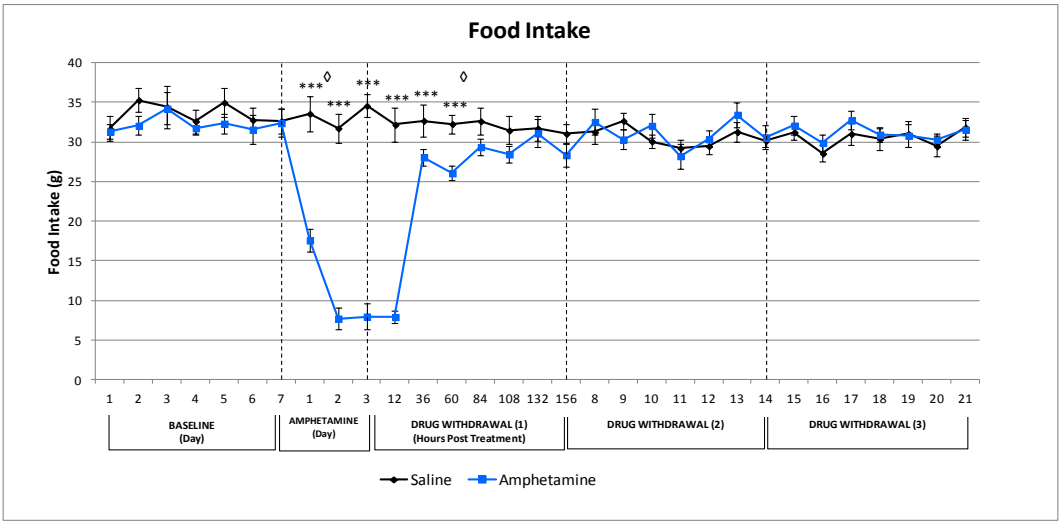


Figure 3.4: Effects of 4-day amphetamine and amphetamine withdrawal on food intake. ◊ = main effect of group. **p*<0.05 and ***p*<0.01, ****p*<0.001 = significant difference between amphetamine and saline groups. Values represent means and error bars represent the SEM.

3.3.2.2 Water Intake

3.3.2.2.1 Baseline

Analysis of water intake during baseline revealed no main effect of baseline day (F(1.614, 17.753) = 1.760, NS), drug treatment group (F(1, 11) = 2.762, NS) and no baseline day x treatment group interaction (F(1.641, 17.753) = 0.549, p = 0.550) (Fig. 3.5).

3.3.2.2.2 Amphetamine treatment

Analysis of water intake during drug treatment revealed no main effect of drug treatment day (F(2, 20) = 1.214, NS), drug treatment group (F(1, 10) = 0.120, NS) or drug day x drug treatment group interaction (F(2, 20) = 2.012, NS) (Fig. 3.5).

3.3.2.2.3 Amphetamine Withdrawal Week One

Analysis of water intake during withdrawal week one revealed no main effect of withdrawal day (F(6, 60) = 0.767, NS), drug treatment group (F(1, 10) = 1.018, NS) and no withdrawal day x treatment group interaction (F(6, 60) = 0.260, NS) (Fig 3.5).

3.3.2.2.4 Amphetamine Withdrawal Week Two

Analysis of water intake during withdrawal week two revealed a main effect of withdrawal day ($F(6, 60) = 2.427$, $p < 0.05$), however, post hoc bonferroni analysis revealed no significant differences in water intake between individual withdrawal days 7-14 (all $p > 0.05$) (Fig. 3.5). In addition, analysis revealed no main effect of drug treatment group on water intake ($F(1, 10) = 0.008$, NS) and no withdrawal day x drug treatment group interaction was detected ($F(6, 60) = 1.018$, NS) (Fig. 3.5).

3.3.2.2.5 Amphetamine Withdrawal Week Three

Analysis of water intake during withdrawal week three revealed no main effect of withdrawal day ($F(6, 60) = 0.887$, NS), drug treatment group ($F(1, 10) = 0.103$, NS) or withdrawal day x drug treatment group interaction ($F(6, 60) = 0.603$, NS) (Fig. 3.5).

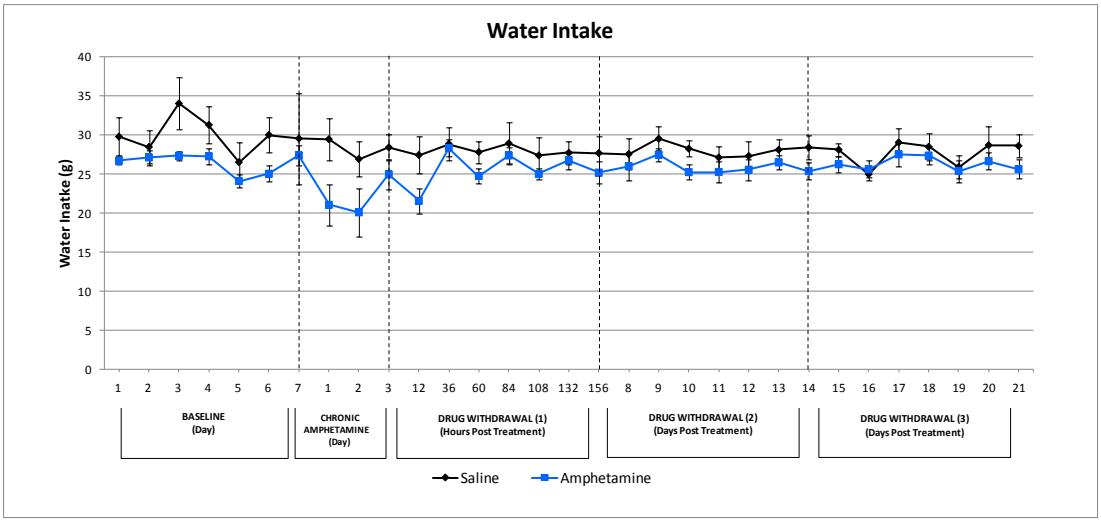


Figure 3.5: Effects of 4-day amphetamine and amphetamine withdrawal on water intake. ◊ = main effect of group. **p*<0.05 and ***p*<0.01, ****p*<0.001 = significant difference between amphetamine and saline groups. Values represent means and error bars represent the SEM.

3.3.2.3 Body Weight

3.3.2.3.1 Baseline

Analysis of body weight during baseline revealed a main effect of baseline day was ($F(1.243, 13.674) = 53.097$, $p < 0.001$) (Fig. 3.6). Post hoc bonferroni analysis revealed a significant increase in body weight across all baseline days 1-7 (all $p<0.05$). There was, however, no difference in body weight between treatment groups ($F(1, 11) = 0.008$, NS) and no baseline day x drug treatment group interaction was detected ($F(1.243, 13.674) = 0.162$, NS) (Fig. 3.6).

3.3.2.3.2 Amphetamine treatment

Analysis of body weight during drug treatment revealed no main effect of drug day ($F(2, 20) = 0.016$, NS), however, amphetamine treated animals were found to have lower body weight throughout drug administration ($F(1, 10) = 22.026$, $p < 0.001$). In addition, analysis revealed a significant drug day x treatment group interaction ($F(2, 20) = 69.314$, $p < 0.001$). Within-subjects simple effects revealed no main effect of drug day in either amphetamine ($F(10, 20) = 0.03$, NS) or saline ($F(8, 20) = 0.017$, NS) during drug administration, however, between-subjects simple effects analysis revealed that amphetamine treated animals had lower body weight than saline treated animals on drug days 1 ($F(1, 30) = 9.140$, $p < 0.01$), 2 ($F(1, 30) = 33{,}948$, $p < 0.001$), and 3 ($F(1, 30) = 58.889$, $p < 0.001$) (Fig. 3.6).

3.3.2.3.3 Withdrawal Week One

Analysis of body weight during withdrawal week one revealed no main effect of withdrawal day ($F(2, 60) = 0.509$, NS), however, amphetamine treated animals maintained a lower body weight than saline treated animals during withdrawal week one ($F(1, 10) = 20.119$, $p < 0.001$). In addition, a withdrawal day x treatment group interaction was detected ($F(2, 60) = 3.457$, $p < 0.05$). Within-subjects simple effects analysis detected no main effect of withdrawal day within amphetamine ($F(6, 23) = 0.007$, NS) or saline ($F(6, 23) = 0.009$, NS) treated animals, however, between-subjects simple effects analysis revealed that amphetamine treated animals had lower body weight relative to controls at the following hours post drug termination: 12hrs ($F(1, 33) = 11.978$, $p < 0.01$), 36 hrs ($F(1, 33) = 10.381$, $p < 0.01$), 60 hrs ($F(1, 33) = 10.752$, $p < 0.01$), 84 hrs ($F(1, 33) = 8.417$, $p < 0.01$), 108 hrs ($F(1, 33) = 8.018$, $p < 0.01$), 132 hrs ($F(1, 33) = 7.002$, $p < 0.05$), and 156 hrs ($F(1, 33) = 6.380$, $p < 0.05$) (Fig. 3.6).

3.3.2.3.4 Withdrawal Week Two

Analysis of body weight during withdrawal week two revealed a main effect of withdrawal day ($F(1.339, 14.733) = 11.618$, $p < 0.01$) with post hoc bonferroni analysis revealing that animals increased body weight across all days ($p < 0.05$) apart from withdrawal day 11 ($p > 0.05$) (Fig. 3.6). Analysis revealed no main effect of drug treatment group ($F(1, 11) = 4.762$, NS) or withdrawal day x drug treatment group interaction on body weight during withdrawal week two ($F(1.339, 14.733) = 2.339$, NS) (Fig. 3.6).

3.3.2.3.5 Withdrawal Week Three

Analysis of body weight during withdrawal week three revealed no main effect of withdrawal day ($F(6, 60) = 3.622$, NS) or drug treatment group ($F(1, 10) = 2.531$, NS) and no withdrawal day x drug treatment group interaction ($F(6, 60) = 0.806$, NS) (Fig. 3.6).

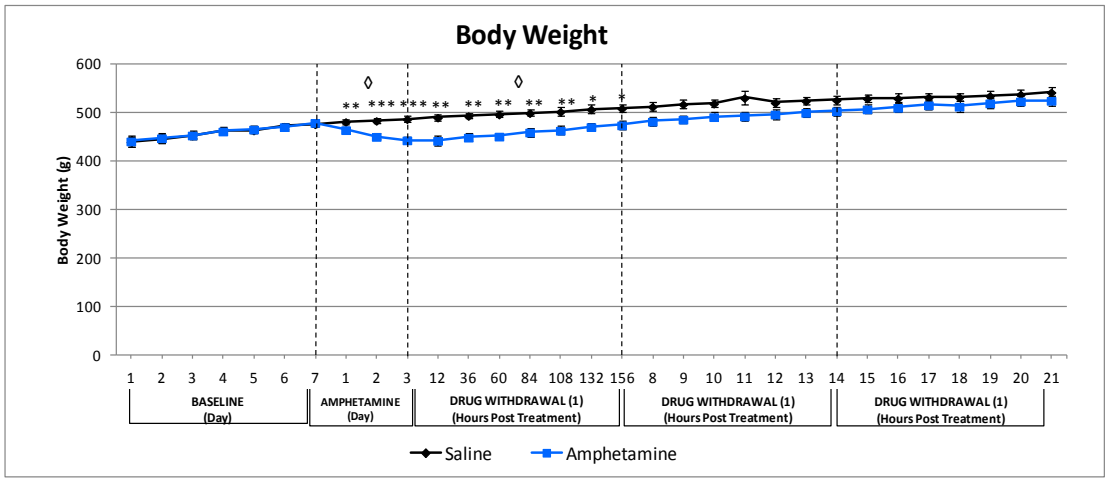


Figure 3.6 Effects of 4-day amphetamine and amphetamine withdrawal on body weight. ◊ = main effect of group. **p*<0.05 and ***p*<0.01, ****p*<0.001 = significant difference between amphetamine and saline groups. Values represent means and error bars represent the SEM.

3.4 Discussion

Animals treated with acute amphetamine demonstrated amphetamine-induced hypophagia and hypodipsia following 0.8 and 1.2 mg/kg amphetamine alongside a general reduction in body weight. This suggests that dosages at and above 0.8 mg/kg are required to induce short term hypophagia of up to 1 hour following acute amphetamine. These findings mimic previous studies reporting a reduction in food intake acutely following amphetamine dosages at or above 0.5 mg/ kg (Wellman, Davis, Clifford, Rothman, & Blough, 2009; White et al., 2010, 2007; Wolgin, Oslan, & Thompson, 1988). The reported short term hypophagia could be attributed to the influence of amphetamine induced stereotypy at higher dosages. Amphetamine-induced stereotypy predominantly occurs around dosages of 1.0 mg/kg (Wolgin, 2000), it is therefore possible that increases in stereotypic behaviour following 0.8 and 1.2 mg/kg of amphetamine began to compete with consummatory behaviours, thus making it more behaviourally challenging for animals to eat food.

Animals drank significantly less water following 0.8 and 1.2 mg/kg of amphetamine in comparison to the vehicle control, thus entering a hypodipsic state following 0.8 and 1.2 mg/kg amphetamine in line with previous reports that acute dosages of amphetamine initially inhibits water intake (Stolerman & D'mello, 1978). Animals did not demonstrate an increase in water intake (hyperdipsia) within the one hour test session following acute amphetamine, suggesting that such compensatory hyperdipsia following the early hypodipsic affect of acute amphetamine do not occur within the first hour following amphetamine administration. Previous studies investigating hyperdipsia have measured water intake up to 7 hours following amphetamine administration (Rowland et al., 1981), it is therefore likely that the one hour test session employed in the current study only captured the initial hypodipsic effects of amphetamine. Furthermore, amphetamine-induced hyperdipsia has previously been reported following higher doses of amphetamine than those employed in the current study, ranging from 1- 10 mg/kg (Cioli, Caricati, & Nencini, 2000; Rowland et al., 1981; Stolerman & D'mello, 1978). Furthermore, in light of the fact that higher doses of amphetamine are usually recruited in order to produce a disruptive effect upon drinking behaviour, it is probably not unsurprising that the employed dosages below 0.8 mg/ kg did not produce a significant effect on water intake.

Acute amphetamine was found to lower body weight across all doses. Although post-hoc analyses failed to reveal significant differences between the body weights of the animals following the saline control treatment and any of the doses of amphetamine, there was a non-significant trend for body weight to be reduced following administration of 1.2 mg/kg ($p =$

0.051). This suggests that following 0.8 mg/kg, amphetamine is beginning to rapidly alter mechanisms regulating body weight. Wolgin (1983) has previously reported weight loss in rats during 30 minute trials following 2 mg/kg of amphetamine, similarly supporting a fast acting effect of amphetamine upon body weight at high doses. Not surprisingly, food intake and body weight were found to highly correlate indicating that the observed reduction in body weight is related to the hypophagic effects of amphetamine. Furthermore, in consideration of the stimulatory effects of amphetamine on metabolism and stereotypy, it is plausible that acute amphetamine induced increases in gastric metabolism and motor activity alongside a reduction in food intake may have tipped animals into a negative energy balance causing a rapid reduction in weight loss. Doong et al. (1998) reported an increase in gastric emptying between 30 - 45 minutes following amphetamine dosages at and above 0.75 mg/kg. Similarly, amphetamine-induced increases in behavioural activity is initially exhibited within the first hour after 0.5 mg/kg amphetamine (Leith & Barrett, 1976). Thus, illustrating fast acting mechanisms that may cumulatively produce body weight loss during the first hour following acute amphetamine administration.

Animals treated with 4-day amphetamine demonstrated hypophagia throughout drug treatment (Fig. 3.4). The magnitude of amphetamine induced hypophagia has previously been shown to be dose dependent (White et al., 2010; Wolgin & Hertz, 1995). For example, 1 and 2 mg/kg of amphetamine have been shown to causing a greater reduction in food intake in comparison to 0.5 mg/kg (White et al., 2010) whilst 4 and 8 mg/kg cause a greater reduction in food intake than 1 and 2 mg/kg (Wolgin & Hertz, 1995). This suggests that as amphetamine doses increased during drug days within experiment 1a, the severity of hypophagia might have accordingly increased. Rats treated with 2.0 mg/kg amphetamine have previously been reported to display hypophagia for between 1 - 6 hours and resume eating between 7 - 12 hours (White et al., 2007). During the administration schedule in experiment 1a there were 6 hours between each daily injection of amphetamine with food intake measured 12 hours after the last daily amphetamine injection. In light of the possibility that from amphetamine administration at and above 2 mg/kg animals may have induced hypophagia for up to 6 hours, this would suggest that animals receiving amphetamine in experiment 1a might have only had approximately 6 hours (between 19- 24 hours following the last daily injection) within the 24 hours food intake was measured to engage in normal feeding behaviour. The magnitude and time course of chronic amphetamine (2 mg/kg per day/ 24 days) induced hypophagia has also been shown to map onto chronic amphetamine induced stereotypy (Wolgin et al., 1987), suggesting that greater amphetamine induced stereotypy with rising amphetamine dosages might have also contributed towards inhibition of feeding. Furthermore, high doses of

amphetamine such as 4 and 8 mg/kg have been shown to reduce intraoral milk consumption as well as completely inhibiting intake of bottle milk (Wolgin & Hertz, 1995) suggesting that both consummatory behaviours and appetitive motivation might have been inhibiting feeding behaviour following administration of 5 mg/kg in experiment 1a during prolonged phases of hypophagia.

Tolerance to amphetamine-induced hypophagia did not develop within the employed 4-day amphetamine regime. Tolerance has previously been reported to devlop at around seven days following repeated administration of a stabilised amphetamine dose (e.g. 2 mg/kg) (Carlton & Wolgin, 1971; Wolgin & Hertz, 1995; Wolgin, 2000). Due to the fact that amphetamine was administered across four days in experiment 1a, it is possible animals were not exposed to the hypophagic effects of amphetamine long enough to develop tolerance. Furthermore, it has previously been shown that tolerance does not develop to amphetamine-induced long-term hypophagia following an alternating dose regime of amphetamine (White et al., 2010). This would suggest that it is harder for animals to learn how to override amphetamine-induced hypophagia when the severity of drug induced hypophagia is unstable. Across drug days one and two in the current experiment animals were subjected to escalating doses of amphetamine three times a day. Thus, the employed unstable regime across drug days one and two may have inhibited tolerance from developing. This would leave only two days of a stable regime for animals to learn how to suppress amphetamine-induced hypophagia, which is likely to be too short a duration for animals to develop tolerance to amphetamine induced hypophagia.

Following the termination of 4-day amphetamine treatment, animals continued to display hypophagia for up to 60 hours during amphetamine withdrawal, followed by normalisation of food intake by 84 hours after cessation of drug administration. These findings suggest that the employed amphetamine escalating-dose regime compromises motivation for food reward for up to 60 hours following the termination of amphetamine. Animals previously treated with the same 4-day amphetamine regime have been similarly reported to exhibit deficits in motivation for food reward for up to 60 hours. Specifically, animals treated with a similar 4-day escalating amphetamine regime to that employed in experiment 1a have been shown to display reduced break points to receive sucrose solution (Barr & Phillips, 1999) and greater negative contrast for sucrose reward for up to 60 hours following amphetamine termination (Barr & Phillips, 2002). Brain reward thresholds are also elevated following the administration of the employed 4-day amphetamine regime for up to 84 hours of amphetamine withdrawal indicating deficits in brain reward function during this period (Harrison, Liem, & Markou, 2001). It would appear,

therefore, that amphetamine withdrawal induced alterations of food intake normalises around 24 hours earlier than amphetamine withdrawal induced dysphoria.

Drug treatment effects persisted into withdrawal week two, as demonstrated by a significant withdrawal day x treatment group interaction during this later phase of withdrawal. However, post-hoc analysis of this interaction only revealed a trend towards an increase in food intake in amphetamine treated animals on day 13 relative to controls ($p = 0.031$, bonferroni correction $p = 0.007$). This suggests that amphetamine treated might have been showing signs of feeding compensation during withdrawal week two following the earlier anorexic effects of amphetamine observed during amphetamine treatment and withdrawal week on. However, by withdrawal week three animals had normalised food intake to a comparable levels as saline controls, indicating that amphetamine treatment did not cause long-term changes in feeding behaviour.

4-Day amphetamine administration did not affect water intake either during amphetamine administration or throughout amphetamine withdrawal. Whilst amphetamine is known to induce hyperdipsia following repeated administration, this has been observed following more maintained and longer durations of chronic amphetamine, such as 4mg/kg once a day for 30 days (Rowland et al. 1981). In light of the fact amphetamine treated animals demonstrated no hyperdipsia following 4 days of a high dose amphetamine regime, this would suggest that a longer chronic regime is required to stimulate significant alterations to water intake. Camanni & Nencini (1994) previously reported amphetamine induced hypodipsia between 3 - 4 hours following 2 mg/kg of amphetamine but no difference in water intake at 24 hours following amphetamine administration, indicating that acute amphetamine induced alterations in water intake may shift the distribution of water intake without impacting the overall level of water intake measured across 24 hours. It is possible therefore that the employed 4-day amphetamine regime did affect water intake, however, due to the fact water intake was measured over a 24 hour period, acute alternations in water intake may have been compensated over the course of 24 hours and not captured in the current data set. The lack of effect of amphetamine upon water intake is particularly interesting in light of the severe hypophagia demonstrated across amphetamine administration and withdrawal. If animals are still able to engage in drinking behaviours, this would suggest that amphetamine treated animals were not completely inhibited by amphetamine-induced stereotypy. However, as Wolgin et al., (1998) demonstrated, the more complex the consummatory behaviour that is required to drink milk, e.g. drinking from milk bottle vs. from an intraoral cannula, the more likely behaviour is to be disrupted by the effects of amphetamine. will manifest upon food intake. Furthermore, the lack of effect of amphetamine on water intake alongside observed

hypophagia supports previous research demonstrating that amphetamine-induced hypophagia and hypodipsia are not related for the manifestation of each effect (Rowland et al., 1981).

Amphetamine treated animals exhibited a significant reduction in body weight in comparison to saline treated animals during 4-day amphetamine treatment, in line with hypophagia observed in animals. Interestingly, the reduction in body weight throughout withdrawal week one was the longest residual effect of all variables measured, suggesting that amphetamine induced alterations in body weight were affected most significantly long-term. Amphetamine treated animals did not differ in body weight in comparison to saline throughout withdrawal week two and three, confirming that amphetamine treated animals had fully compensated the earlier anorexic effects of amphetamine on body weight. However, although non-significant, it is noteworthy that animals only regained body weight comparable to saline controls across withdrawal week two when there was a trend indicating that animals began to simultaneously consume more food (Fig. 3.4), suggesting that animals may have regained body weight by compensating with food intake.

3.4.1 Key Findings

The main findings from this study are that acute amphetamine at and above doses of 0.8 mg/kg significantly reduce food intake and water intake, whilst more chronic 4-day amphetamine treatment significantly reduces food intake and body weight during drug treatment and withdrawal week one in comparison to controls in rats. These findings suggest that both acute and chronic regimes of amphetamine might impair primary motivation in rats. This study was conducted in order to assess how amphetamine regimes later employed in this thesis directly affect primary motivation in rats, with a view to considering how these changes might affect measures of response inhibition on the Go/No-go symmetrically reinforced task. Changes in food intake, water intake and body weight indicate that any amphetamine induced changes in impulsivity observed on this task should be interpreted in consideration of possible changes in primary motivation impinging on task performance.

3.4.2 Limitations

The main limitation in this study is that gross measures of food intake, water intake and body weight were employed. Firstly, measurement of all variables was conducted manually which increases the chance of error in data collection. For example, it would have been more reliable to measure food and water intake through using food and water dispensers. This would have enabled a more exact and accurate measurement of food consumed by rats during drug

treatment. Secondly, measurement of food intake, water intake and body weight were only measured over 24 hours periods. This means that any subtle changes in any of these measures may not have been captured when being measured over a period of 24 hour. In order to more accurately measure changes in food and water intake it would have been useful to employ food and water dispensers that log the displacement of food and water at specific time points. This would enable more precise measurement of any drug induced changes in these measures.

3.4.3 Conclusions

In conclusion, acute amphetamine leads to hyperphagia that can be triggered by amphetamine dosages at or above 0.8 mg/kg, whilst hypodipsia requires a slightly higher dosage of 1.2 mg/kg. Body weight, similarly, appears to be more impacted by higher dosages of amphetamine at 1.2 mg/kg to produce an effect on weight change. Treatment with 4-day amphetamine similarly induced hypophagia and causing weight loss in rats, however no hypo-/ hyperdipsic effects were observed following this longer-term regime. 4-day amphetamine also induced long-term alterations in hypophagia for up to 60 hours of drug withdrawal and reduced body weight for up to 154 hours of withdrawal. However, no significant differences between amphetamine and controls were observed during withdrawal weeks two and three confirming that residual effects on hypophagia and body weight were restricted to withdrawal week one. Collectively, these findings indicate that treatment with acute and 4-day amphetamine can cause alterations in the motivation for food.

Chapter 4 The effects of a 4-day amphetamine binge on behavioural inhibition in rats

4.1 Introduction

The acute effects of drugs in healthy subjects have been shown to alter impulsivity during measures of impulsive action, choice and reflection (Caswell et al., 2013; de Wit et al., 2000; Fillmore, Carscadden, & Vogel-Sprott, 1998; Fillmore et al., 2002, 2003; George et al., 2005; Ortner et al., 2003; Ramaekers & Kuypers, 2005; Reynolds, Richards, & de Wit, 2006). These effects typically increase impulsivity, however, stimulant drugs show a slightly more paradoxical profile on impulsivity and can reduce impulsive choice and action in healthy subjects when measured by delay discounting, SSRT and Go/No-Go performance (de Wit et al., 2002). However, these effects were found in subjects with reduced inhibitory control at baseline, suggesting that the effects of amphetamine interact with baseline level of inhibitory control (de Wit et al., 2002).

In addition to the acute effects of drugs on impulsivity, substance dependent subjects display increased levels of impulsivity. Opiate, cocaine, methamphetamine, nicotine and alcohol dependent users display poor inhibitory control (Verdejo-García et al. 2007; Kjome et al. 2010; Colzato et al. 2007; Moeller et al. 2002; Moeller et al. 2004; Lawrence et al. 2009; Monterosso et al. 2005) and discount delayed reward to a greater extent than healthy controls (Coffey et al., 2003; Hoffman et al., 2006; Kirby & Petry, 2004; Madden et al., 1997; Mitchell, 1999; Petry, 2001; Vuchinich & Simpson, 1998). Reduced action restraint on the SST, reduced reflection on the IST and increased delay discounting have also all been observed in drug users during phases of short and long-term drug abstinence (Clark et al., 2006; Heil et al., 2006; Hoffman et al., 2006; Lee & Pau, 2002; Li et al., 2006; Petry, 2001) indicating that elevated impulsivity is associated with drug use and drug withdrawal.

Consistent with human literature, the acute administration of drugs in rats alters impulsivity. Alcohol, amphetamine, cocaine, nicotine and morphine have been shown to acutely reduce behavioural inhibition in rats (Van Gaalen, Brueggeman, et al. 2006; Harrison et al. 1997; Pattij et al. 2007; Bizarro et al. 2004; Pattij et al. 2009; Paine & Olmstead 2004). Acute nicotine and morphine can also acutely increase delay discounting (Kolokotroni et al. 2011; Kieres et al. 2004; Pitts & McKinney 2005) whilst stimulants including amphetamine and MPH produce contrasting effects that actually reduce delay discounting and improve impulse control in rats (Wade et al. 2000; van Gaalen et al. 2006b; Cardinal et al. 2000; Pitts & McKinney 2005).

Amphetamine induced reductions in delay discounting have however been shown to depend on the presence of light cues during the delay to reward (Cardinal et al., 2000). In addition, the effects of amphetamine in rats have also been shown to depend upon baseline levels of impulsive behaviour, for example, acute amphetamine does not affect impulsive choice on a cued delayed reward task in rats that are impulsive at baseline, but does increase impulsive choice in rats that are not impulsive at baseline (Hand, Fox, & Reilly, 2009). These data indicate that similar to human literature, the effects of amphetamine on impulsivity depends on both baseline levels of inhibitory control and the specific dimension of impulsivity measured.

Chronic drug administration can also alter impulsivity during drug administration and withdrawal in rats. Studies utilising chronic drug administered non-contingently and through self-administration procedures have shown that cocaine, methamphetamine and nicotine can increase impulsive action, and or, impulsive choice during drug administration (Winstanley et al. 2009; Richards et al. 1999; Dallery & Locey 2005; Kolokotroni et al. 2012) and chronic cocaine, methamphetamine, nicotine and MDMA can increase impulsive action, and or, impulsive choice during drug withdrawal in rats (Dallery & Locey, 2005; Dalley et al., 2005; Dalley et al., 2007b; Winstanley et al., 2009).

Interestingly, despite the acute effects of amphetamine reliably altering impulsive behaviour in rats (Pattij et al. 2007; Harrison et al. 1997; van Gaalen et al. 2006; van Gaalen et al. 2006b; Wade et al. 2000; Cardinal et al. 2000) long term amphetamine administration has only been found to directly increase impulsivity throughout drug administration and acute drug withdrawal within one study measuring the effects of amphetamine self-administration on an adjusting delay procedure (Gipson & Bardo, 2009). Additional studies investigating the relationship between long-term amphetamine treatment and impulsivity have found no effects of chronic amphetamine on long-term changes in impulsive choice measured by a delayed reward task (Slezak, Krebs, & Anderson, 2012; Stanis et al., 2008) and withdrawal from amphetamine self-administration does not affect behavioural inhibition on the 5CSRTT in rats (Dalley et al., 2005; Dalley et al., 2007b). However, the 5CSRTT primarily measures accuracy of attention in rats (Carli et al., 1983) and subsequently the index of impulsivity, assessed through the number of premature responses made before the presentation of a brief light stimulus, is intertwined with attentional demand. Consequently, if amphetamine does increase impulsivity during withdrawal this would make it difficult to assess within the 5CSRTT if attention is also disrupted, which it appears to be (Dalley et al., 2005; Dalley et al., 2007b). Within the Go/No-go symmetrically reinforced task attentional demand is relatively low due to the continuous presentation of visual light cues guiding behaviour during Go and No-go trials. In addition,

failure to complete a correct No-go trial results in the direct loss of reward meaning the animal has an incentive to successfully inhibit behaviour in contrast to the 5CSRTT where reward for behavioural inhibition is intertwined with attentional accuracy and does not necessarily result in a loss of reward. In following, an investigation into the effects of chronic amphetamine on behavioural inhibition during drug treatment and subsequent drug withdrawal was conducted in rats using the symmetrically reinforced Go/No-go task. In consideration of the fact stimulant drugs, including amphetamine, are commonly administered in binge patterns, amphetamine was administered in a 4-day binge pattern. In addition, the long-term effects of amphetamine treatment on impulsivity were assessed through acute amphetamine challenges administered following prolonged drug withdrawal.

4.1.1 Objectives

In order to investigate the effects of a 4-day amphetamine binge on behavioural inhibition within the symmetrically reinforced Go/No-go task the objectives of Experiments 2a, b, c and d were:

i) To determine the effects of a 4-day amphetamine binge on behavioural inhibition during drug treatment within the symmetrically reinforced Go/No-go task (Experiment 2a)

ii) To determine the effects of spontaneous short-term drug withdrawal from a 4-day amphetamine binge on behavioural inhibition within the symmetrically reinforced Go/No-go task (Experiment 2b)

iii) To determine the effects of long-term drug withdrawal from a 4-day amphetamine binge on behavioural inhibition within the symmetrically reinforced Go/No-go task (Experiment 2c)

iv) To determine the effect of acute amphetamine challenges on behavioural inhibition within the symmetrically reinforced Go/No-go task following prolonged withdrawal from a 4-day amphetamine binge (Experiment 2d)

4.2 Methods

4.2.1 Subjects

20 male Lister Hooded rats (Charles River, UK) were housed in pairs and maintained under a 12 hour light/ dark cycle, in a temperature (21°C ± 2°C) and humidity (50% ± 5%) controlled environment. Throughout experimentation animals were maintained on a food restriction schedule of 18.6g per day (inclusive of food received during testing), maintaining animals at 85% of their adult free feeding body weight. Water was available ad libitum and feeding took place in the morning after testing. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996. All procedures were covered by Home Office Project Licence No. PIL 40/2711 and Home Office Personal Licence No. 40/9836.

4.2.2 Drugs

d-Amphetamine sulphate (Sigma Aldrich, UK) was dissolved in 0.9% saline solution and was administered intraperioneally (i.p.) in a volume of 1 ml/kg body weight.

4.2.3 Apparatus

Behavioural testing took place in eight aluminium operant chambers (30.5 x 24.1 x 21 cm, Med Associates Inc., USA) placed inside sound attenuating and ventilated cubicles (63.5 x 49.1 x 39.4 cm, Med Associates Inc., USA). Apparatus was controlled, and all data was recorded from chambers, using MED-PC IV software (Med Associates Inc., USA). For a more detailed description of all apparatus refer to Chapter 2, section 2.1.3, page 60.

4.2.4 Behavioural Testing

Behavioural disinhibition was assessed via the symmetrically reinforced go/no-go visual discrimination paradigm. Behavioural training took place for approximately 8 weeks until animals reached a total of 85% correct or more over seven consecutive training sessions. Following stable baseline performance, animals subsequently entered the experiment. All behavioural training and testing took place between 0700-0930 hours during the light phase of animals Light/ Dark cycle. For a detailed outline of the task refer to Chapter 2, section 2.1.4, page 61.

4.2.5 Design and Procedure

A mixed design was employed to assess the effects of amphetamine treatment on behavioural disinhibition, with 'drug treatment group' (saline and amphetamine) acting as the between-subjects factor and 'test day' acting as the within-subjects factor. Following stable performance, animals were assigned to groups receiving either systemic saline or amphetamine treatment. Groups were counterbalanced according to their baseline performance accuracy, programmed levers, and fast/ slow- Go/ No-go contingencies.

4.2.6 Experiment 2a: The effects of a 4-day amphetamine binge on behavioural inhibition

The chronic drug regime employed was a modified version to that of (Harrison et al. 2001). Animals were entered into two groups receiving amphetamine (n=9) or saline (n=10) treatment. Amphetamine was administered i.p. three times a day (0800 hrs/ 1400 hrs/ 2000 hrs) over the course of four days in a rising dose regime. Dosages started at 1 mg/kg increasing up to and stabilising at 5 mg/ kg. A total of 50 mg/ kg of amphetamine was administered per animal in the following daily regimes; Day 1 (1, 2, 3 mg/kg), Day 2 (4, 5, 5 mg/kg), Day 3 (5, 5, 5 mg/kg), Day 4 (5, 5, 5 mg/kg). Drug treatment started immediately after behavioural testing on baseline day 7. Control animals were administered with saline i.p. three times a day (0800 hrs/ 1400 hrs/ 2000 hrs) over the course of four days, mimicking the administration regime of amphetamine treated animals. Behavioural testing took place prior to the first morning injection of amphetamine or saline, thus behavioural inhibition was measured during the treatment phase 12 hrs after the final amphetamine injection on the previous day.

4.2.7 Experiment 2b and c: The effects of short- and long-term spontaneous amphetamine withdrawal on behavioural inhibition

Spontaneous amphetamine withdrawal was initiated after the fourth day of amphetamine treatment. Animals were subsequently tested for the next 14 days to assess the effects of short- and long-term amphetamine withdrawal on behavioural inhibition. In order to more thoroughly examine behavioural disinhibition throughout acute amphetamine withdrawal, animals were tested at 12 and 18 hrs post drug termination; constituting two test sessions on day 1 of drug withdrawal. Animals were tested once per day across all remaining withdrawal days. Specifically, animals were tested at the following hours post drug treatment; 12, 18, 36, 60, 84, 108, 132, 156 hrs (withdrawal week 1) (Experiment 2b), and across the following days post drug treatment; 8, 9, 10, 11, 12, 13, 14 (withdrawal week 2) (Experiment 2c).

4.2.8 Experiment 2d: The effects of acute amphetamine challenges on behavioural inhibition

Following two weeks of drug withdrawal and when animals performance had returned to baseline levels (85% correct, +/- 5%), both amphetamine and saline treated animals received five acute amphetamine challenges (0, 0.2, 0.4, 0.8 and 1.2 mg/kg, i.p.) in a latin square design 20 minutes before behavioural testing. The acute amphetamine dose range was chosen based on findings by Harrison et al., (pers. comm.). Animals were required to achieve a minimum of 85% correct (+/- 5%) across two consecutive days between acute injections in order ensure that animals had returned to baseline levels in their performance before receiving their next amphetamine injection. A wash out period of a minimum of 72 hrs was employed between each drug injection. Performance had returned to baseline levels prior to all drug injections.

4.2.9 Statistical Analysis

All data was initially checked for normality through Shaprio-Wilk tests and was appropriately transformed via arcsine, log10, square root and reciprocal transformations following any violations to normality. Homogeneity of variance was checked via Mauchley's test of Sphericity and following any significant violation of equal variances, the GLM degrees of freedom were adjusted using the Greenhouse- Geisser correction. For all employed ANCOVAs, homogeneity of the regression slope was assessed via testing the interaction between baseline performance and independent variables. Following a significant baseline x treatment group or baseline x test day interaction, the use of ANCOVA was considered no longer appropriate, and subsequently data was re-expressed a percentage of baseline performance and entered into 2 x x mixed ANOVA (Field, 2005). The validity of using average baseline performance as a covariate was also assessed through checking that baseline performance yielded a main effect upon treatment groups within the ANCOVA model (Field et al., 2005). On the rare occasion that baseline performance was found to not significantly affect treatment groups, data was re-expressed a percentage of baseline performance and entered into a 2 x x mixed ANOVA (Field, 2005).

In order to maintain power when conducting multiple post hoc comparisons the sidak correction was applied to all within-subjects post hoc tests (Field, 2005) and the Benjamini-Hochberg correction was applied to between-subjects post hoc comparisons (Benjamini-Hochberg, 1995). The sidak correction controls for false positivity in combination with increased power in comparison to traditional post hoc tests such as the bonferonni correction (Sidak, 1967; Liu et al, 2010), and the Benjamini-Hochberg test has been shown to enforce

stronger control over both type 1 and type 2 errors in comparison to traditional approaches controlling for familywise error rate when conducting multiple comparisons (Thissen et al, 2002; Noble 2009). The Benjamini-Hochberg test achieves this control though conducting a test of 'false discovery rate' across all comparisons controlling for the chance of incorrectly accepting the null hypothesis as well as incorrectly rejecting the null hypothesis, as oppose to post hoc tests such as the bonferonni correction, controlling solely for the probability of making at least one incorrect rejection of the null hypothesis. Furthermore, through employing ANCOVA an additional proportion of the variance is accounted for within the analysis, enforcing further control over the variance analysed and subsequent p value generated.

Unless otherwise stated $\alpha < 0.05$ was employed. Subsequently, the following four sets of data analyses were performed:

4.2.10 Assessment of Baseline Performance

After checks of normality, the following transformations were performed before conducting baseline analysis: $\log_{10}$ transformation (Go magazine latency).

Baseline performance was analysed via 2 x 7 mixed ANOVAs with 'drug treatment group' entered as the between- subjects factor, and 'baseline day' entered as the within-subjects factor. Main effects of 'baseline day' were followed up via sidak corrected post hoc tests, and significant treatment group x baseline day interactions were deconstructed via simple effects analysis. Simple effects analysis was conducted in two steps. Firstly, two one-way repeated measures ANOVAs were performed for each treatment group to assess individual group within-subject effects, with subsequent significant main effects of 'baseline day' followed up via sidak corrected post hoc tests. Secondly, seven independent t-tests were performed to examine between-treatment group effects within individual baseline days (Benjamini-Hochberg correction of $\alpha = (i/k)*0.05$)[1]).

4.2.11 The Effects of a 4-Day amphetamine binge, short- and long-term spontaneous amphetamine withdrawal on behavioural inhibition

After checks of normality, the following transformations were applied across chronic drug and withdrawal data: $\log_{10}$ transformation (Go trial response latency, Go trial magazine latency).

Performance across amphetamine treatment, short- and long-term spontaneous amphetamine withdrawal was assessed via mixed ANCOVAs, with 'drug treatment group'

[1] "*i*" = Order of p values ranked from highest to lowest, "*k*" = number of tests

entered as the between-subjects factor and 'test day', 'withdrawal hour', or withdrawal day' entered as the within-subjects factor. In summary, the following ANCOVAs were performed across all behavioural parameters; 2 x 4 mixed ANCOVA (drug treatment), 2 x 8 mixed ANCOVA (withdrawal week one), and 2 x 7 mixed ANCOVA (withdrawal week two). In order to control for within-subjects variation in behavioural performance, average baseline performance across baseline was entered as a covariate into the ANCOVA model. Further analysis of within-subjects main effects was conducted via post hoc sidak corrected tests and significant interaction effects were deconstructed via post hoc simple effects analysis. Simple effects were performed using the pooled error term from the main ANCOVA model (Howell, 2010). However, under circumstances of violated sphericity, a separate error term for each simple effect was generated to avoid the use of an unreliable post-hoc F value generated from adjusted degrees of freedom (Boik, 1981; Howell, 2010). Simple effects were subsequently conducted in two steps as reported in section 4.2.10 with the addition of entering baseline performance as a covariate into both within- and between-subjects deconstruction analyses. Firstly, two one-way repeated measures ANCOVAs were performed for each treatment group to assess individual treatment group within-subjects effects, and secondly, univariate ANCOVAs were performed on each individual test day to assess between-group treatment effects at each level of 'test day'/ 'withdrawal hour'/ 'withdrawal day'. Simple effects analysis revealing a significant main effect of 'test day'/ 'withdrawal hour'/ 'withdrawal day' were further analysed via post hoc sidak corrected multiple comparisons. P values of between-groups simple effects analysis were corrected via the Benjamini-Hochberg correction ($\alpha = (i/k)*0.05$) to control for increased false discovery rates following multiple between group comparisons (Benjamini and Hochbery, 1995).

In addition, in order to identify the specific initiation and longevity of treatment effects in line with exploring experimental aims to examine the direct effects of amphetamine treatment on behavioural inhibition, all main effects of 'treatment group' were further investigated via conducting multiple between-group comparisons on individual test days (Benjamini-Hochbery correction of $\alpha = (i/k)*0.05$)) along with one-way repeated measures ANCOVAs within each treatment group. This analysis aimed to specifically identify the development and extinction of between-group differences within the go/no-go task.

4.2.12 The effects of acute amphetamine challenges on behavioural disinhibition

After checks of normality, the following transformations were performed before conducting analysis of acute amphetamine challenges: $\log_{10}$ transformation (No-go trial response latency, Go trial magazine latency).

The effect of acute amphetamine challenges upon behavioural disinhibition was assessed via 2 x 5 repeated measures ANOVAs with 'amphetamine dose' entered as the within-subjects factor and 'drug treatment group' entered as the between-subjects factor. All main effects were followed up via post hoc Sidak corrected tests and significant interactions were deconstructed via simple effects analysis, whereby the pooled error term from the original ANOVA model was utilised to generate the post hoc F value, as described in section 4.2.11, page 85.

4.3 Results

Unless otherwise stated, for all of the following ANCOVAs homogeneity of regression was assumed ($p > 0.05$) and average baseline performance was found to significantly affect treatment groups ($p < 0.05$), reinforcing the need to control for within-subject variation. Due to data remaining abnormally distributed following transformations, the total percentage correct of Go trials, No-go trial response latencies and Go trial panel responses were re-expressed as a percentage of baseline performance and analysed in ANOVA. During baseline these variables were analysed using non-parametric tests Friedman's ANOVA and Mann Whitney U tests. All values within graphs represent means and error bars represent the standard error of the mean (SEM).

4.3.1 Experiment 2a: Pre-drug baseline performance

4.3.1.1 Performance accuracy:

No main effect of baseline day was revealed by the analysis of the total percentage correct of trials ($F(6, 102) = 1.777$, NS), the total percentage correct of Go trials ($\chi(6) = 6.559$, NS) or the total percentage correct of No-go trials ($F(6, 102) = 1.877$, NS). Accuracy of performance also did not differ between treatment groups within the total percentage correct of all trials ($F(1, 17) = 0.068$, NS), the total percentage correct of Go trials (all days $U > 36$, NS) and the total percentage correct of No-go trials ($F(1, 17) = 0.180$, NS). No significant baseline day x treatment group interactions were found within the total percentage correct of trials ($F(6, 102) = 1.033$, NS) or the total percentage correct of No-go trials ($F(6, 102) = 1.143$, NS) (Figs 4.1 - 4.2).

4.3.1.2 Speed of Responding:

No main effect of baseline day was revealed by the analysis of Go trial response latencies ($F(6, 102) = 0.623$, NS), No-go trial response latencies ($F(6, 102) = 1.383$, NS), Go trial magazine latencies ($F(1, 17) = 0.170$, NS) or No-go trial magazine latencies ($F(3.659, 62.211) = 0.705$, NS). No difference was found between drug treatment groups within the analysis of Go trial responses latencies ($F(1, 17) = 1.730$, NS), No-go trial response latencies ($F(1, 17) = 0.098$, NS), Go trial magazine latencies ($F(1, 17) = 0.170$, NS) and No-go trial magazine latencies ($F(1, 17) = 0.046$, NS). Furthermore, no significant baseline day x treatment group interactions were found by the analysis of Go trial response latencies ($F(6, 102) = 0.777$, NS), No-go trial response

latencies (F(6, 102) =1.370, NS), Go trial magazine latencies(F(3.220, 57.732) = 0.777, NS) and No-go trial magazine latencies (F(63.659, 62.211) = 0.705, NS) (Table 4.1).

4.3.1.3 Anticipatory Responding:

No significant difference was revealed by the analysis of Go trial early responses (F(6, 102) = 0.569, NS), No-go trial early responses (F(6, 102) = 0.595, NS), Go trial panel responses (χ(6) = 4.622, NS) and No-go trial panel responses (F(6, 102) = 0.618, NS). Similarly, no differences were detected between treatment groups in the analysis of Go trial early responses (F(1, 17) = 0.370, NS), No-go trial early responses (F(1, 17) = 0.829, NS), Go trial panel responses (all days U > 30, NS) and No-go trial panel responses (F(1, 17) = 0.031, NS). Furthermore, no significant baseline day x treatment group interactions were detected by the analysis of Go trial early responses (F(6, 102) = 2.042, NS), No-go trial early responses (F(6, 102) = 1.155, NS) and No-go trial panel responses (F(6, 102) = 0.937, NS) (Table 4.2).

Table 4.1: Baseline: Speed of responding during baseline week. Values represent means and the standard error or the mean (SEM).

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go Trial Response Latency	Saline	0.98 ± 0.16	0.91 ± 0.12	0.86 ± 0.14	0.96 ± 0.12	0.81 ± 0.13	1.05 ± 0.13	1.11 ± 0.17
	Amphetamine	0.77 ± 0.13	1.77 ± 0.09	0.70 ± 0.11	0.85 ± 0.14	0.75 ± 0.11	0.75 ± 0.14	0.73 ± 0.17
No-go Trial Incorrect Response Latency	Saline	1.95 ± 0.33	1.93 ± 0.25	1.80 ± 0.29	2.40 ± 0.36	2.81 ± 0.36	2.02 ± 0.27	1.88 ± 0.33
	Amphetamine	1.82 ± 0.22	1.97 ± 0.41	2.09 ± 0.29	1.78 ± 0.41	2.12 ± 0.23	2.34 ± 0.32	1.97 ± 0.38
Go Trial Magazine Latency	Saline	0.42 ± 0.08	0.40 ± 0.10	0.38 ± 0.06	0.40 ± 0.06	0.37 ± 0.05	0.38 ± 0.05	0.37 ± 0.04
	Amphetamine	0.42 ± 0.05	0.41 ± 0.04	0.40 ± 0.04	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.04	0.38 ± 0.04
No-go Trial Magazine Latency	Saline	0.60 ± 0.13	0.66 ± 0.15	0.71 ± 0.17	0.73 ± 0.16	0.71 ± 0.12	0.69 ± 0.12	0.64 ± 0.13
	Amphetamine	0.64 ± 0.07	0.65 ± 0.07	0.67 ± 0.08	0.63 ± 0.09	0.60 ± 0.06	0.68 ± 0.07	0.65 ± 0.06

Table 4.2:Baseline: Anticipatory responding during baseline week. Values represent means and the SEM.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go Trial Early Response	Saline	19.50 ± 2.54	20.30 ± 2.84	22.10 ± 3.01	19.60 ± 2.42	19.90 ± 2.06	18.00 ± 2.54	17.60 ± 1.86
	Amphetamine	22.22 ± 3.58	22.22 ± 2.90	20.78 ± 2.33	21.00 ± 2.95	20.67 ± 2.59	21.33 ± 2.88	23.78 ± 3.17
No-go Trial Early Response	Saline	10.10 ± 2.09	9.60 ± 2.07	9.80 ± 1.85	9.30 ± 1.63	8.10 ± 1.68	8.50 ± 1.68	9.40 ± 2.07
	Amphetamine	11.44 ± 1.89	11.89 ± 2.97	12.00 ± 1.50	11.89 ± 2.21	12.89 ± 1.87	11.11 ± 2.09	10.33 ± 2.15
Go Trial Panel Response	Saline	1.30 ± 0.45	1.90 ± 2.42	1.00 ± 0.30	1.00 ± 0.37	0.80 ± 0.20	0.90 ± 0.23	0.50 ± 0.17
	Amphetamine	1.11 ± 0.42	1.11 ± 0.48	0.44 ± 0.18	0.67 ± 0.33	0.78 ± 0.32	1.33 ± 0.75	0.67 ± 0.33
No-go Trial Panel Response	Saline	11.00 ± 2.31	10.30 ± 2.39	9.00 ± 2.23	9.50 ± 2.42	9.40 ± 2.27	8.00 ± 2.03	9.20 ± 2.32
	Amphetamine	10.22 ± 2.53	9.56 ± 2.64	10.56 ± 2.40	10.00 ± 1.97	9.22 ± 2.18	10.22 ± 3.02	10.56 ± 2.81

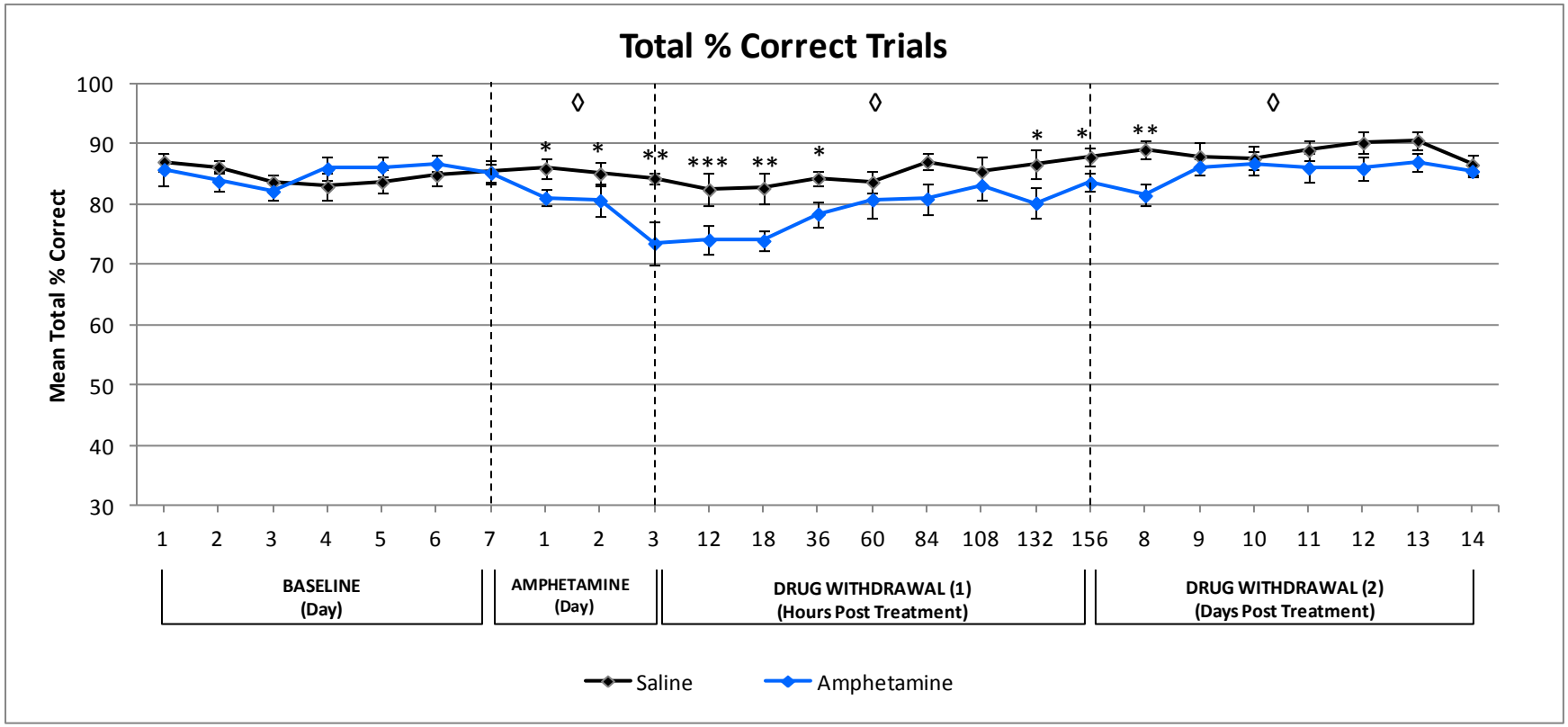


Figure 4.1. Performance within the total percentage correct of all trials during baseline week, drug administration, withdrawal week one and withdrawal week two. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and error bars represent the SEM.

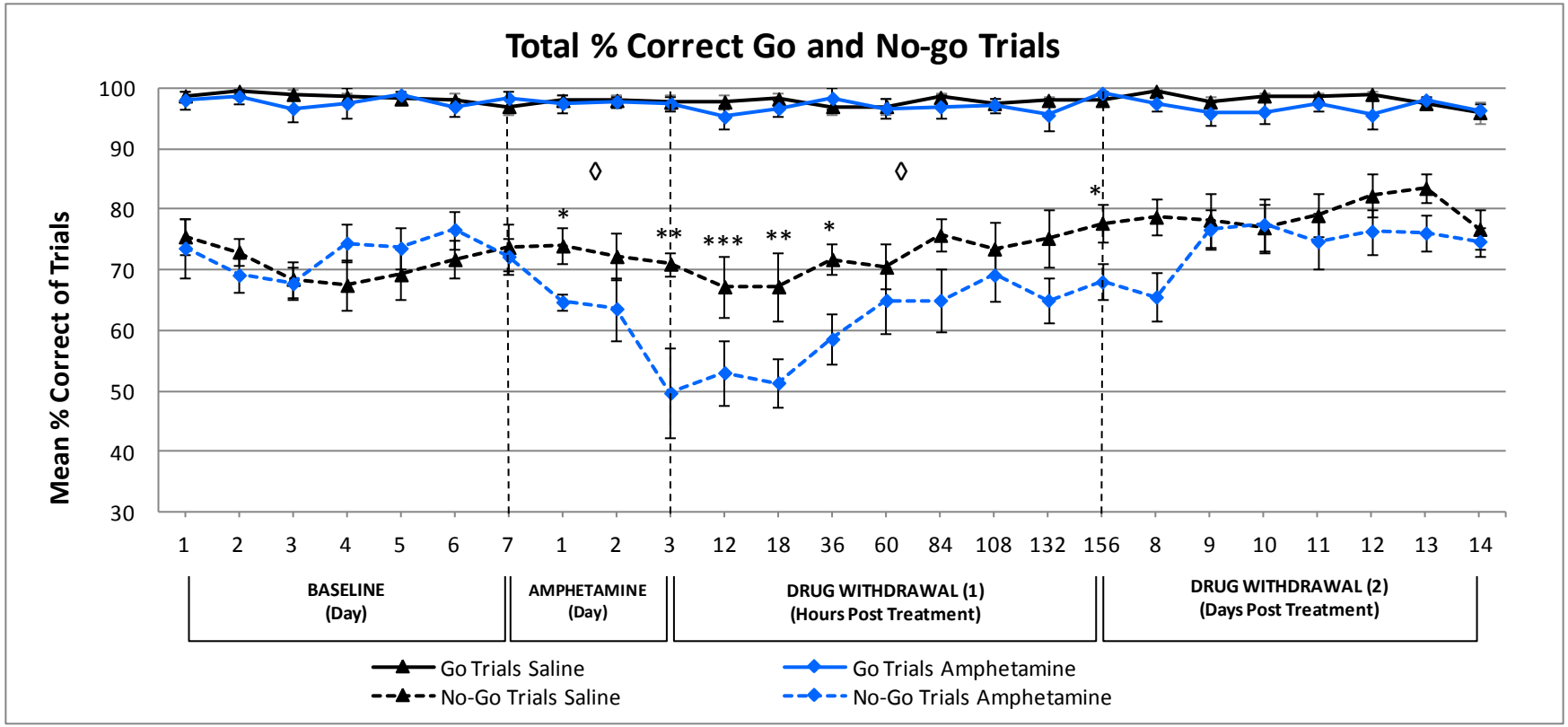


Figure 4.2. Performance within the total percentage correct of Go and No-go trials during baseline week, drug administration, withdrawal week one and withdrawal week two. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and error bars represent the SEM.

4.3.2 Experiment 2a: 4-day amphetamine treatment

4.3.2.1 Performance accuracy:

Analysis of the total percentage correct of trials revealed no main effect of drug day ($F(2, 32) = 2.398$, $p > 0.05$, NS), however, a main effect of treatment group revealed that amphetamine treated animals had lower accuracy during drug treatment in comparison to saline controls ($F(1, 16) = 23.117$, $p < 0.001$). Further within-subjects analysis of this effect revealed that the total percentage of correctly completed trials was not affected between drug days amongst saline treated animals ($F(1.237, 9.894) = 3.920$, NS), however, amphetamine treated animals had reduced accuracy across drug days ($F(2, 14) = 5.513$, $p < 0.05$), specifically on drug day 3 in comparison to drug day 2 ($p < 0.05$) (Fig. 4.1). Further analysis of between-subjects effects revealed that amphetamine treated animals had lower accuracy in comparison to saline controls specifically on drug day 1 ($F(1, 16) = 7.838$, $p < 0.05$), day 2 ($F(1, 16) = 6.205$, $p < 0.05$) and day 3 ($F(1, 16) = 14.965$, $p < 0.001$) (Fig. 4.1) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.016$, see Appendix 1, Table 1a for specific F- and corrected p-values). However, no drug day x treatment group interaction was detected within the total percentage correct of trials ($F(2, 32) = 1.807$, NS).

Analysis of Go trials independently revealed no main effect of drug day ($F(2, 34) = 0.039$, NS), drug treatment ($F(1, 17) = 0.096$, NS) or day x treatment group interaction ($F(2, 34) = 0.034$, NS) (Fig. 4.2). Analysis of No-go trials also revealed no main effect of drug day on No-go trial accuracy ($F(2, 32) = 1.957$, NS), however, amphetamine treated animals had significantly lower accuracy within No-go trials in comparison to saline controls ($F(1, 16) = 17.742$, $p < 0.001$). Further within-subjects analysis of this effect revealed that the total percentage of correctly completed No-go trials was not affected between drug days amongst saline ($F(1, 14) = 3.402$, NS) or amphetamine treated animals ($F(2, 16) = 3.228$, NS). Further analysis of between-subject effects revealed that amphetamine treated animals had lower accuracy within No-go trials in comparison to controls specifically on drug day 1 ($F(1, 16) = 10.848$, $p < 0.05$) and day 3 ($F(1, 16) = 11.253$, $p < 0.01$) (Fig. 4.2) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.016$, see Appendix 1, Table 1b for specific F- and corrected p-values). No drug day x treatment group interaction was detected within No-go trial accuracy ($F(2, 34) = 2.065$, NS).

4.3.2.2 Speed of Responding:

Analysis of Go trial response latencies revealed no main effect of drug days ($F(2, 32) = 2.570$, NS), drug treatment group ($F(1, 16) = 2.749$, NS) or drug day x drug treatment group

interaction (F(2, 32)= 0.017, NS) (Table 4.3). Analysis of No-go trial response latencies also revealed no main effect of drug day (F(2, 32) = 0.044, NS) and drug treatment group (F(1, 16) = 0.028, NS). A drug day x drug treatment group interaction was however detected within No-go-trial response latencies (F(2, 32) = 2.695, $p < 0.05$). Within-subjects simple effects analysis of this interaction revealed that neither saline (F(16, 32) = 0.507, $p > 0.05$) or amphetamine treatment (F(14, 32) = 1.362, $p > 0.05$) affected the speed to make an incorrect response during No-go trials across drug days (Table 4.3). In addition, between-subjects simple effects analysis revealed no difference in the speed to make an incorrect response during No-go trials on drug day 1, day 2, or day 3 (df= 1, 48, all $F \leq 2.845$, NS) (Table 4.3) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.016$, see Appendix 1, Table 1c. for specific F- and corrected p-values).

Go trial magazine latencies were unaffected by drug days (F(2, 32) = 0.463, NS), drug treatment group (F(1, 16) = 0.199, NS), and no drug day x drug treatment group interaction was found (F(2, 32) = 0.263, NS) (Table 4.3). A main effect of drug day was detected within No-go trial magazine latencies (F(2, 32) = 3.939, $p < 0.05$), however, post-hoc analysis of this main effect failed to identify a significant difference between individual drug days in the speed to collect the food reward during No-go trials (all $p > 0.05$) (Table 4.3). No-go trial magazine latencies were unaffected by drug treatment group (F(1, 16) = 3.412, NS) and no drug day x drug treatment group interaction was found (F(2, 32) = 2.529, NS) (Table 4.3).

4.3.2.3 Anticipatory Responding:

Go trial early responses were unaffected by drug day (F(2, 32) = 0.791, NS), drug treatment group (F(1, 16) = 0.109, NS) and no drug day x drug treatment group interaction was found (F(2, 32) = 1.623, NS) (Table 4.4). Similarly, No-go trial early responses were not affected by drug day (F(2, 32) = 2.095, NS), drug treatment group (F(1, 16) = 0.241, NS) and no drug day x drug treatment group interaction was found (F(2, 32) = 0.125, NS) (Table 4.4). Analysis of Go trial panel responses revealed no main effect of drug day (F(2, 34) = 2.120, NS), however, a main effect of drug treatment group revealed that amphetamine treated animals performed more Go trial panel responses in comparison to saline controls during drug treatment (F(1, 17) = 7.064, $p < 0.05$). Analysis of within-subject effects of amphetamine and saline groups independently revealed no main effect of amphetamine (F(2, 18) = 2.120, NS) or saline (F(2, 16) = 2.698, NS) on Go trial panel responses between drug days. Further analysis of between-group effects revealed that amphetamine treated animals made more Go trial panel responses specifically on drug day 3 (F(1, 17) = 12.140, $p < 0.01$) but not drug days 1 and 2 (df = 1, 17, all $F \leq 3.059$, NS) (Table 4.4) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.016$, see Appendix

1, Table 1d for specific F- and corrected p-values). No drug day x drug treatment group interaction was detected during Go trial panel responses (F(2, 34) = 2.698, NS) (Table 5). No-go trial panel responses were unaffected by drug day (F(1.475, 23.597) = 1.753, NS), drug treatment group (F(1, 16) = 2.152, NS) or drug day x drug treatment group interaction (F(1.475, 23.597) = 0.898, NS) (Table 4.4).

Table 4.3: 4-Day Amphetamine: Speed of Responding during 4-day amphetamine binge. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3
Go Trial Response Latency	Saline	0.88 ± 0.13	0.91 ± 0.10	1.06 ± 0.09
	Amphetamine	0.84 ± 0.15	1.04 ± 0.20	1.13 ± 0.12
No-go Trial Incorrect Response Latency	Saline	2.52 ± 0.39	1.74 ± 0.29	1.97 ± 0.35
	Amphetamine	1.79 ± 0.28	2.23 ± 0.23	2.08 ± 0.38
Go Trial Magazine Latency	Saline	0.40 ± 0.11	0.41 ± 0.09	0.44 ± 0.11
	Amphetamine	0.41 ± 0.05	0.41 ± 0.04	0.40 ± 0.03
No-go Trial Magazine Latency	Saline	0.65 ± 0.15	0.65 ± 0.11	0.73 ± 0.13
	Amphetamine	0.64 ± 0.06	0.85 ± 0.09	0.71 ± 0.07

Table 4.4: 4-Day Amphetamine: Anticipatory Responding during 4-day amphetamine binge. **p*<0.05 and ***p*<0.01, ****p*<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3
Go Trial Early Response	Saline	17.60 ± 2.39	21.3 ± 2.13	19.30 ± 1.76
	Amphetamine	21.89 ± 2.53	20.00 ± 2.93	19.00 ± 2.07
No-go Trial Early Response	Saline	8.70 ± 1.56	9.40 ± 1.92	11.2 ± 1.71
	Amphetamine	11.67 ± 2.07	12.33 ± 2.16	12.89 ± 2.49
Go Trial Panel Response	Saline	1.30 ± 0.40	1.00 ± 0.33	1.20 ± 0.42
	Amphetamine	1.44 ± 0.53	1.89 ± 0.59	** 2.77 ± 0.32
No-go Trial Panel Response	Saline	8.00 ± 2.23	8.90 ± 1.83	8.30 ± 2.27
	Amphetamine	11.44 ± 2.90	10.44 ± 2.17	9.44 ± 1.69

4.3.3 Experiment 2b: Amphetamine withdrawal week one

4.3.3.1 Performance Accuracy:

Analysis of the total percentage of correctly completed trials revealed no main effect withdrawal days ($F(4.082, 65.305) = 2.007$, NS), however, amphetamine treated animals were found to perform significantly less correct responses in comparison to controls ($F(, 1, 16) = 13.523$, $p < 0.01$). Further within-subjects analysis of this effect revealed that performance was not affected between withdrawal days within either saline ($F(1, 14) = 3.402$, NS) or amphetamine ($F(2, 16) = 3.228$, NS) treated animals when analysed independently (Fig. 4.1). Further analysis of between-group effects revealed that amphetamine treated animals performed less correct responses specifically at 12 ($F(1, 16) = 40.041$, $p < 0.001$), 18 ($F(1, 16) = 7.694$, $p < 0.01$), 36 ($F(1, 16) = 12.921$, $p < 0.05$), 132 ($F(1, 16) = 5.67$, $p < 0.05$) and 156 ($F(1, 16)$ 6.519, $p < 0.05$) hours of amphetamine withdrawal (Fig. 4.1) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.006$, see Appendix 1, 1e for all specific F- and corrected p-values). No significant withdrawal day x drug treatment group interaction was detected by the analysis of the total percentage correct of correctly completed trials during withdrawal week one ($F(4.082, 65.305) = 2.007$, NS).

Independent analysis of Go trials revealed no main effect of withdrawal day ($F(3.733, 63.464) = 0.677$, NS), drug treatment group ($F(1, 17) = 0.038$, NS) or withdrawal day x drug treatment group interaction ($F(3.733, 63.464) = 0.880$, NS) on Go trial accuracy during withdrawal week one (Fig. 4.2). Independent analysis of No-go trials also revealed that withdrawal days did not affect No-go trial accuracy ($F(7, 112) = 1.930$, NS), however, amphetamine treated animals were found to perform significantly less correct No-go trials during withdrawal week one in comparison to controls ($F(1, 16) = 12.397$, $p < 0.01$). Further within-subjects analysis of this effect revealed that No-go trial performance was not affected between withdrawal days within either saline ($F(2.959, 23.672) = 1.151$, NS) or amphetamine ($F(4, 49) = 1.708$, NS) treated animals when analysed independently. Further analysis of drug treatment group effects, however, revealed that amphetamine treated animals made less correct No-go trials than controls specifically at 12 ($F(1, 16) = 17.893$, $p < 0.001$), 18 ($F(1, 16) = 6.148$, $p < 0.01$), 36 ($F(1, 16) = 15.368$, $p < 0.05$) and 156 ($F(1, 16) = 8.762$, $p < 0.05$) hours of drug withdrawal (Fig. 4.2) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.006$, see Appendix 1, Table 1f for all specific F- and corrected p-values). However, no significant withdrawal hour x drug treatment group interaction was found during No-go trial accuracy during withdrawal week one ($F(7, 112) = 0.835$, NS).

4.3.3.2 Speed of responding:

Analysis of Go trial response latencies revealed no significant main effects of either withdrawal hour ($F(4.319, 60.104) = 1.174$, NS) or drug treatment group ($F(1, 16) = 3.967$, NS). However, a withdrawal hour x drug treatment group interaction was detected within the Go trial response latencies ($F(4,319, 69.104) = 3.920$, $p < 0.01$). Within-subjects simple effects analysis of this interaction revealed no effect of withdrawal hours on the speed of responding during Go trials within saline ($F(7, 56) = 1.910$, NS) or amphetamine treated animals ($F(7, 49) = 1.186$, NS), whilst between-subjects simple effects analysis revealed that amphetamine treated animals became significantly slower in their speed of responding during Go trials at 60 hours of drug withdrawal in comparison to controls ($F(1, 16) = 10.298$, $p < 0.01$) (Table 4.5). In addition, no significant between-group effects were detected at any other time points in withdrawal week one (df = 1, 16, all $F \leq 5.210$, NS) (Table 4.5) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.005$, see Appendix 1, Table 1g for specific F- and corrected p-values). No-go trial response latencies were unaffected by withdrawal hour ($F(4.241, 72.094) = 0.957$, NS), drug treatment group ($F(1, 17) = 0.970$, NS) and no withdrawal hour x drug treatment group interaction was found ($F(4.241, 72.094) = 1.476$, NS).

Go trial magazine latency was unaffected by withdrawal hour ($F(3.716, 59.451) = 0.501$, NS), drug treatment group ($F(1, 16) = 0.515$, NS), and no withdrawal hour x drug treatment interaction was detected ($F(3.716, 59.451) = 0.887$, NS) (Table 4.5). Analysis of No-go trial magazine latencies revealed no main effect of withdrawal hour ($F(7, 112) = 1.026$, NS) and by drug treatment group ($F(1, 16) = 0.749$, NS). However, a withdrawal hour x drug treatment interaction was detected in No-go trial magazine latency ($F(7, 112) = 3.848$, $p < 0.001$). Within-subjects simple effects analysis of this interaction revealed that withdrawal hour significantly affected the speed to collect food reward during No-go trials in amphetamine treated animals ($F(49, 122) = 1.475$, $p < 0.05$) whilst the speed to collect food reward during No-go trials was unaffected by withdrawal hour in saline treated animals ($F(56, 112) = 1.150$, NS). The main effect of withdrawal hour on No-go trial magazine latencies within amphetamine treated animals, however, did not reveal any specific significant differences between individual withdrawal hours when analysed with post-hoc tests (all $p > 0.05$). Between-groups simple effects analysis also did not produce any significant difference in No-go trial magazine latencies between drug treatment groups at specific withdrawal hours (df = 1, 16, all $F \leq 6.953$, NS), and only detected a trend towards a significant increase in No-go trial magazine latencies amongst amphetamine treated animals at 18 hours of drug withdrawal ($F(1, 16) = 6.953$, $p = 0.009$, NS)

(Table 4.5) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.006$, see Appendix 1, 1h for specific F- and corrected p-values).

4.3.3.3 Anticipatory Responding:

Go trial early responses were unaffected by withdrawal hour ($F(7, 112) = 0.549$, NS), drug treatment group ($F(1, 16) = 0.0035$, NS) and no withdrawal hour x drug treatment group interaction was found during withdrawal week one ($F(7, 112) = 1.637$, NS) (Table 4.6). No-go trial early responses were similarly unaffected by withdrawal hour ($F(7, 112) = 1.482$, NS) and drug treatment group ($F(1, 16) = 0.292$, NS). A withdrawal hour x drug treatment group interaction was, however, detected within No-go trial early responses ($F(7, 112) = 2.418$, $p < 0.05$). Within-subject simple effect analysis of this interaction identified a main effect of withdrawal hour on No-go trial early responses within amphetamine treated animals ($F(49, 112) = 1.475$, $p < 0.05$) but not within saline treated animals ($F(56, 112) = 1.150$, NS). Post hoc analysis of this withdrawal hour effect within amphetamine treated animals, however, failed to produce any significant difference in the number of early responses made during No-go trials between individual withdrawal hours (all $p > 0.05$). Between-subjects simple effects analysis also revealed no significant difference between drug treatment groups at individual withdrawal hours measured during withdrawal week one (df = 1, 128, all $F \leq 3.081$, NS) (Table 4.6) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.006$, see Appendix 1, 1i for specific F- and corrected p-values).

A main effect of withdrawal hour was detected on Go trial panel responses during withdrawal week one ($F(2.967, 50.433) = 5.079$, $p < 0.01$). Post-hoc Sidak analysis of this effect, however, only revealed a trend towards a significant increase in Go trial panel responses at 12 hours vs. 108 hrs of drug withdrawal ($p = 0.069$), and all remaining post-hoc comparisons were not significant (all $p > 0.05$) (Table 4.6). Go trial panel responses were unaffected by treatment group during withdrawal week one ($F(1, 16) = 3.086$, NS) and no withdrawal hour x drug treatment group interaction as detected ($F(2.967, 50.433) = 2.579$, NS) (Table). No-go trial panel responses were unaffected by withdrawal hour ($F(7, 112) = 0.217$, NS), drug treatment group ($F(1, 16) = 1.881$, NS) and no withdrawal hour x drug treatment group interaction was found ($F(7, 112) = 0.600$, NS) (Table 4.6).

Table 4.5: Withdrawal Week One: Speed of Responding during withdrawal week one. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		12 hrs	18 hrs	36 hrs	60 hrs	84 hrs	108 hrs	132 hrs	156 hrs
Go Trial Response Latency	Saline	0.97 ± 0.12	0.86 ± 0.16	1.10 ± 0.21	0.876 ± 0.15	1.12 ± 0.12	1.06 ± 0.19	0.99 ± 0.12	1.04 ± 0.12
	Amphet	1.18 ± 0.12	1.07 ± 0.14	1.11 ± 0.16	1.22 ± 0.17**	0.83 ± 0.09	1.06 ± 0.14	1.03 ± 0.13	0.74 ± 0.07
No-go Trial Incorrect Response Latency	Saline	1.87 ± 0.26	2.08 ± 0.41	2.50 ± 0.29	1.96 ± 0.234	1.89 ± 0.27	2.11 ± 0.26	1.83 ± 0.18	2.22 ± 0.36
	Amphet	2.51 ± 0.29	2.26 ± 0.32	2.19 ± 0.19	2.20 ± 0.27	2.33 ± 0.25	3.07 ± 0.37	1.92 ± 0.18	2.92 ± 0.41
Go Trial Magazine Latency	Saline	0.43 ± 0.09	0.45 ± 0.14	0.45 ± 0.12	0.42 ± 0.11	0.40 ± 0.10	0.40 ± 0.10	0.41 ± 0.10	0.38 ± 0.08
	Amphet	0.39 ± 0.03	0.36 ± 0.03	0.38 ± 0.04	0.40 ± 0.06	0.36 ± 0.04	0.39 ± 0.04	0.34 ± 0.02	0.38 ± 0.05
No-go Trial Magazine Latency	Saline	0.74 ± 0.17	0.64 ± 0.13	0.68 ± 0.15	0.69 ± 0.16	0.66 ± 0.11	0.61 ± 0.10	0.76 ± 0.13	0.78 ± 0.16
	Amphet	0.87 ± 0.09	0.88 ± 0.08	0.75 ± 0.07	0.83 ± 0.12	0.62 ± 0.07	0.71 ± 0.09	0.61 ± 0.05	0.59 ± 0.04

Table 4.6: Withdrawal Week One: Anticipatory Responding during withdrawal week one. Values represent the mean and SEM.

Behavioural Measure		12 hrs	18 hrs	36 hrs	60 hrs	84 hrs	108 hrs	132 hrs	156 hrs
Go Trial Early Response	Saline	18.60 ± 2.50	19.90 ± 2.61	17.80 ± 2.63	18.70 ± 2.13	20.40 ± 2.15	17.30 ± 2.38	19.00 ± 2.39	18.80 ± 2.40
	Amphet	17.22 ± 2.24	18.33 ± 2.13	18.33 ± 2.36	20.78 ± 2.23	20.89 ± 2.53	22.22 ± 2.26	21.44 ± 2.41	22.67 ± 1.89
No-go Trial Early Response	Saline	8.90 ± 1.70	10.10 ± 2.08	8.50 ± 1.19	10.80 ± 1.85	8.00 ± 1.29	10.30 ± 1.58	9.10 ± 2.07	8.50 ± 2.07
	Amphet	13.11 ± 1.86	14.00 ± 1.81	12.78 ± 1.76	10.11 ± 1.59	10.89 ± 1.99	9.89 ± 1.33	11.44 ± 1.83	10.78 ± 1.87
Go Trial Panel Response	Saline	1.30 ± 0.56	1.70 ± 0.62	1.10 ± 0.23	0.60 ± 0.22	0.50 ± 0.22	0.60 ± 0.22	0.70 ± 0.21	0.50 ± 0.22
	Amphet	3.44 ± 0.88	2.44 ± 0.80	1.11 ± 0.54	1.33 ± 0.55	0.67 ± 0.29	0.33 ± 0.17	0.67 ± 0.24	1.11 ± 0.42
No-go Trial Panel Response	Saline	11.70 ± 2.66	12.00 ± 2.86	10.00 ± 2.87	10.70 ± 2.37	8.80 ± 2.25	9.70 ± 2.30	10.10 ± 2.27	10.60 ± 2.38
	Amphet	9.56 ± 1.89	10.00 ± 2.54	8.44 ± 1.59	7.89 ± 1.84	8.44 ± 2.36	8.44 ± 1.81	9.11 ± 2.28	8.11 ± 2.12

4.3.4 Experiment 2c: Withdrawal week two

4.3.4.1 Performance Accuracy:

Analysis of the total percentage of correctly completed trials during withdrawal week two revealed no main effect of withdrawal day ($F(6, 96) = 1.379$, NS), however, a main effect of drug treatment group was detected ($F(1, 16) = 4.983$, $p < 0.05$) (Fig. 4.1). Further analysis of this main effect revealed no effect of withdrawal day on performance accuracy within saline ($F(6, 48) = 2.029$, NS) or amphetamine treated animals ($F(6, 42) = 3.402$, NS) when analysed independently, however, amphetamine treated animals performed less correct trials on withdrawal day 8 in comparison to saline controls ($F(1, 16) = 16.958$, $p < 0.001$) (Fig. 4.1). All remaining drug withdrawal days did not reveal any difference in performance accuracy between amphetamine and saline controls (df = 1, 16, all $F \leq 4.513$) (Fig. 4.1) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.007$, see Appendix 1, Table 1j for specific F- and corrected p-values). A withdrawal day x drug treatment group interaction was not detected within the total percentage correct of trials ($F(6, 96) = 1.379$, NS).

Independent analysis of Go trial accuracy revealed no main effect of withdrawal day ($F(6, 102) = 1.554$, NS), drug treatment group ($F(1, 17) = 2.279$, NS) or withdrawal day x drug treatment group interaction ($F(6, 102) = 1.557$, NS) (Fig. 4.2). Independent analysis of No-go trial accuracy also revealed no main effect of withdrawal day ($F(6, 96) = 2.107$, $p = 0.059$, NS), drug treatment group ($F(1, 16) = 3.247$, $p = 0.090$, NS) or withdrawal day x drug treatment group interaction ($F(6, 96) = 2.053$, NS) (Fig. 4.2).

4.3.4.2 Speed of Responding:

Analysis of Go trial response latencies were revealed no main effect of withdrawal day ($F(6, 96) = 0.763$, NS), drug treatment group ($F(1, 16) = 0.145$, NS), and no withdrawal day x drug treatment group interaction ($F(6, 96) = 0.533$, NS) (Table 4.7). Analysis of No-go trial response latencies were similarly unaffected by withdrawal day ($F(4.108, 69.835) = 1.947$, NS), drug treatment group ($F(1, 17) = 0.041$, NS), and no withdrawal day x drug treatment group interaction was detected ($F(4.108, 69.835) = 2.356$, NS) (Table 4.7).

Analysis of Go trial magazine latencies also revealed no main effect of withdrawal day ($F(6, 96) = 1.551$, NS), drug treatment group ($F(1, 16) = 0.904$, NS) and no withdrawal day x drug treatment group interaction ($F(6, 96) = 1.534$, NS) (Table 4.7). Analysis of No-go trial magazine

latencies produced a main effect of withdrawal day ($F(6, 96) = 2.766$, $p < 0.05$), however, post hoc comparisons failed to identify any difference in the speed to collect food reward on individual withdrawal days during drug withdrawal week two (all $p > 0.05$) (Table 4.7). No main effect of drug treatment group ($F(1, 16) = 0.904$, NS) or withdrawal day x drug treatment group interaction ($F(6, 96) = 1.242$, NS) were additionally detected by the analysis of No-go trial magazine latencies during drug withdrawal week two (Table 4.7).

4.3.4.3 Anticipatory Responding:

Analysis of Go trial early responses revealed no main effect of withdrawal day ($F(6, 96) = 0.585$, NS), drug treatment group ($F(1, 16) = 0.017$, NS) and no withdrawal day x drug treatment group interaction was detected ($F(6, 96) = 0.534$, NS) (Table 4.8). Similarly, analysis of No-go trial early responses revealed no main effect of withdrawal day ($F(6, 96) = 0.571$, NS), drug treatment group ($F(1, 16) = 0.946$, NS), and no withdrawal day x drug treatment group interaction ($F(6, 96) = 0.571$, NS) (Table 4.8).

Analysis of Go trial panel responses revealed no main effect of withdrawal day ($F(3.091, 52.541) = 2.731$, $p = 0.051$, NS) and drug treatment group ($F(1, 17) = 2.079$, NS), and no withdrawal day x treatment group interaction ($F(3.091, 52.541) = 2.532$, NS) (Table 4.8). Analysis of No-go trial panel responses revealed no main effect of withdrawal day ($F(6, 96) = 0.916$, NS) and drug treatment group ($F(1, 16) = 0.028$, NS), however, a significant withdrawal day x drug treatment group interaction was detected by the analysis of No-go trial panel responses during withdrawal week two ($F(6, 96) = 2.924$, $p < 0.05$). Within-subjects simple effects analysis of this interaction, however, failed to detect a significant effect of withdrawal day on No-go trial panel responses within saline ($F(48, 96) = 0.877$, NS) or amphetamine treated animals ($F(42, 96) = 0.873$, NS). Between-subjects simple effects analysis also failed to detect a difference in No-go trial panel responses between drug treatment groups at specific days during withdrawal week two (df = 1, 16, all $F \leq 2.265$, NS) (Table 4.8) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.007$, see Appendix 1, Table 1k for specific F- and corrected p-values).

Table 4.7: Withdrawal Week Two: Speed of Responding during withdrawal week two. Values represent the mean and SEM.

Behavioural Measure		WD8	WD9	WD10	WD11	WD12	WD13	WD14
Go Trial Response Latency	Saline	0.77 ± 0.09	0.91 ± 0.15	0.81 ± 0.11	0.89 ± 0.13	0.93 ± 0.10	1.07 ± 0.18	0.92 ± 0.15
	Amphetamine	0.70 ± 0.10	0.81 ± 0.10	0.72 ± 0.11	0.94 ± 0.18	1.01 ± 0.18	0.85 ± 0.15	0.94 ± 0.15
No-go Trial Incorrect Response Latency	Saline	2.09 ± 0.29	1.94 ± 0.18	2.54 ± 0.45	1.72 ± 0.22	3.23 ± 0.34	2.25 ± 0.45	1.79 ± 0.27
	Amphetamine	2.45 ± 0.38	2.36 ± 0.32	1.57 ± 0.26	2.15 ± 0.30	2.06 ± 0.36	2.35 ± 0.23	2.01 ± 0.29
Go Trial Magazine Latency	Saline	0.43 ± 0.12	0.38 ± 0.11	0.39 ± 0.09	0.41 ± 0.09	0.41 ± 0.11	0.42 ± 0.11	0.44 ± 0.15
	Amphetamine	0.38 ± 0.05	0.37 ± 0.05	0.04 ± 0.06	0.32 ± 0.03	0.37 ± 0.04	0.38 ± 0.05	0.39 ± 0.05
No-go Trial Magazine Latency	Saline	0.75 ± 0.17	0.80 ± 0.14	0.68 ± 0.14	0.70 ± 0.15	0.78 ± 0.14	0.85 ± 0.20	0.68 ± 0.11
	Amphetamine	0.55 ± 0.06	0.61 ± 0.06	0.57 ± 0.07	0.67± 0.06	0.65 ± 0.07	0.72 ± 0.09	0.72 ± 0.09

Table 4.8: Withdrawal Week Two: Anticipatory Responding during withdrawal week two. Values Represent the mean and SEM.

Behavioural Measure		WD8	WD9	WD10	WD11	WD12	WD13	WD14
Go Trial Early Responses	Saline	19.10 ± 2.16	20.40 ± 2.34	21.20 ± 2.78	20.60 ± 2.23	19.20 ± 3.12	17.60 ± 3.31	18.70 ± 2.24
	Amphetamine	23.44 ± 2.62	21.56 ± 2.96	20.89 ± 3.13	21.56 ± 2.80	21.89 ± 2.24	22.11 ± 1.83	19.78 ± 2.36
No-go Trial Early Responses	Saline	9.90 ± 2.06	9.00 ± 2.11	9.40 ± 1.98	8.50 ± 2.12	9.10 ± 2.16	8.50 ± 2.23	9.50 ± 1.88
	Amphetamine	14.33 ± 1.78	11.56 ± 1.62	12.00 ± 2.12	11.44 ± 2.04	12.56 ± 1.95	13.11 ± 1.93	12.67 ± 1.94
Go Trial Panel Responses	Saline	1.10 ± 0.41	0.90 ± 0.31	0.80 ± 0.33	0.20 ± 0.13	0.30 ± 0.15	1.10 ± 0.31	0.30 ± 0.15
	Amphetamine	0.56 ± 0.24	1.89 ± 0.86	1.11 ± 0.26	0.33 ± 0.17	1.11 ± 0.39	0.67 ± 0.44	0.67 ± 0.24
Trial Panel No-go responses	Saline	10.10 ± 2.12	10.50 ± 2.04	9.30 ± 1.77	7.70 ± 1.40	8.10 ± 2.18	7.20 ± 1.61	7.60 ± 1.42
	Amphetamine	7.78 ± 2.03	8.11 ± 2.16	8.11 ± 2.38	8.56 ± 1.89	9.22 ± 1.77	10.22 ± 2.42	8.78 ± 2.39

4.3.5 Experiment 2d: Acute amphetamine challenges

4.3.5.1 Performance Accuracy

Acute amphetamine had an effect on the total percentage of correctly completed trials in both amphetamine and saline treated animals ($F(4, 68) = 23.258$, $p = 0.001$). Post hoc analysis of this effect revealed that the total percentage of correctly completed trials decreased following 0.8 and 1.2 mg/kg in comparison to 0 and 0.2 mg/kg amphetamine ($p < 0.001$), and following 1.2 mg/kg in comparison to 0.4 mg/kg amphetamine (all $p < 0.001$) (Fig. 4.3). However, there was no main effect of previous drug histories (saline vs. amphetamine) ($F(1, 17) = 2.0101$, NS) and no interaction between amphetamine dose x treatment group was detected ($F(4, 68) = 0.278$, NS).

Analysis of the percentage of correctly completed Go trials revealed no main effect of amphetamine dose (df = 4, $X^2 = 3.123$, NS), however, animals that previously received amphetamine treatment were found to have higher Go trial accuracy than animals that previously received saline treatment following 1.2 mg/kg of amphetamine (MWU = 20, $p < 0.05$) (Fig. 4.4). Analysis of the percentage of correctly completed No-go trials revealed a main effect of amphetamine dose ($F(4, 68) = 25.462$, $p = 0.001$). Post hoc analysis of this effect revealed that the total percentage correct of No-go trials decreased following 0.8 and 1.2 mg/kg in comparison to 0 and 0.2 mg/kg amphetamine ($p < 0.001$), and following 1.2 mg/kg in comparison to 0.4 mg/kg amphetamine (all $p < 0.001$) (Fig. 4.5). However, there was no main effect of previous drug treatment on No-go trial accuracy $F(1, 17) = 1.545$, NS) and no amphetamine dose x drug treatment group interaction was detected ($F(4, 68) = 0.306$, NS).

4.3.5.2 Speed of Responding

Analysis of Go trial response latencies revealed a main effect of amphetamine dose ($F(2.796, 47.531) = 6.882$, $p = 0.001$). Post hoc analysis of this effect revealed that animals produced increased response latencies during G trials following 1.2 mg/kg in comparison to 0.2 and 0.4 mg/kg of amphetamine ($p < 0.01$) (Fig. 4.6). However, no main effect of previous drug history (amphetamine vs. saline) was found in the analysis of Go trial response latencies ($F(1, 17) = 0.780$, NS) and no amphetamine dose x drug pre-treatment group interaction was detected ($F(2.796, 47.531) = 1.969$, NS). Analysis of No-go trial response latencies revealed no main effect of amphetamine dose ($F(4, 68) = 0.559$, NS) and previous drug treatment group ($F(1, 17) = 0.007$, NS) or dose x treatment group interaction ($F(4, 68) = 1.298$, NS) (Fig. 4.7).

Post hoc analysis of a significant main effect of amphetamine dose on Go trial magazine latencies ($F(2.137, 36.333) = 10.113$, $p < 0.001$) revealed that animals became slower to collect the food reward during Go trials following 1.2 mg/kg in comparison to 0, 0.2 and 0.4 mg/kg amphetamine (all $p < 0.05$) (Fig. 4.8). However, analysis of Go trial magazine latencies revealed no main effect of drug treatment group ($F(1, 17) = 0.411$, NS) or dose x treatment group interaction ($F(2.137, 36.666) = 1.468$, NS). Analysis of No-go trial magazine latencies revealed no main effect of amphetamine dose ($F(2.396, 40.740) = 1.979$, NS), previous drug treatment group ($F(1, 17) = 0.125$, NS) and no dose x treatment group interaction ($F(2.137, 36.666) = 1.094$, NS) (Fig. 4.9).

4.3.5.3 Anticipatory Responding:

Acute amphetamine produced a treatment effect on Go trial early responses ($F(2.973, 50.546) = 7.081$, $p = 0.001$). Post hoc analysis of this effect revealed a decrease in Go trial early responses following 1.2 mg/kg in comparison to 0, 0.2 and 0.4 m/kg amphetamine in both amphetamine and saline treated animals (all $p < 0.05$) (Fig. 4.10). However, Go trial early responses did not differ between previous drug groups following acute amphetamine ($F(1, 17) = 1.296$, NS) and no amphetamine dose x treatment group interaction was detected ($F(2.973, 50.546) = 1.400$, NS) (Fig. 4.10). Acute amphetamine also produced a main effect on No-go trial early responses in animals ($F(4, 68) = 3.665$, $p < 0.01$). Post hoc analysis of this effect revealed a decrease in No-go trial early responses following 1.2 mg/kg in comparison to 0.2 mg/kg of amphetamine in both saline and amphetamine pre-treated animals ($p < 0.05$) (Fig. 4.11). However, no effect of previous drug treatment group was found on No-go trial early responses following acute amphetamine ($F(1, 17) = 0.126$, NS) and no dose x treatment group interaction was detected ($F(4, 68) = 1.4892$, NS) (Fig. 4.11).

Analysis of Go trial panel responses revealed no main effect of amphetamine dose ($F(4, 68) = 2.342$, $p = 0.064$, NS), previous drug treatment group ($F(1, 17) = 0.239$, NS) or dose x treatment group interaction ($F(4, 68) = 0.625$, NS) (Fig. 4.12). Similarly, the analysis of No-go trial panel responses revealed no main effect of amphetamine dose acute ($F(2.761, 46.948) = 1.691$, NS), previous drug treatment group ($F(1, 17) = 0.197$, NS) or dose x treatment group interaction ($F(2.761, 49.948) = 0.865$, NS) (Fig. 4.13).

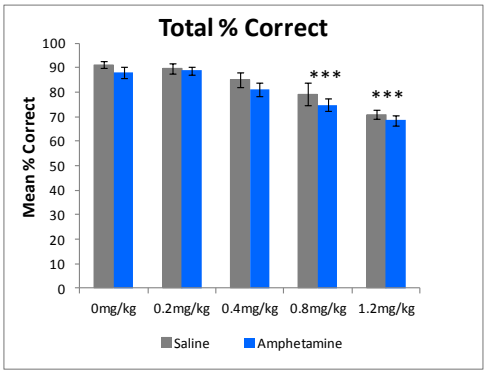


Fig. 4.3

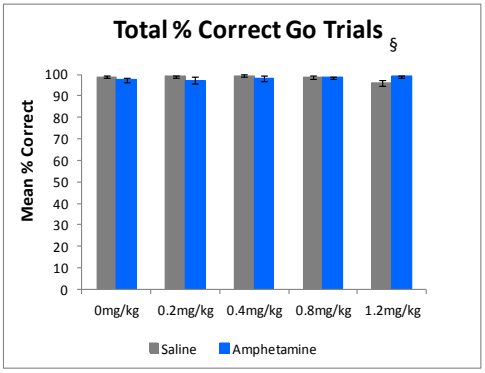


Fig. 4.4

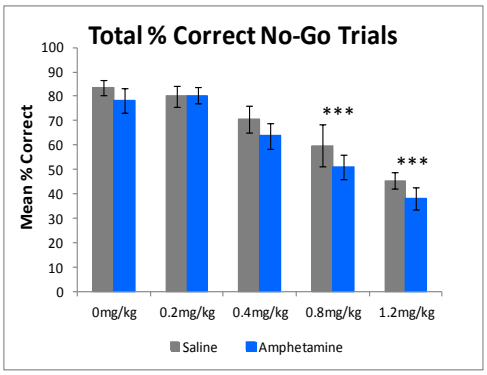


Fig. 4.5

Figures 4.3-4.5: Performance Following acute amphetamine challenges upon **(4.3)** the total percentage correct of all trials, **(4.4)** the total percentage correct of Go trials, **(4.5)** the total percentage correct of No-Go trials. **p*<0.05 and ***p*<0.01, ****p*<0.001 vs saline dose. *p*<0.05 vs saline group. Values represent means and error bars represent the SEM.

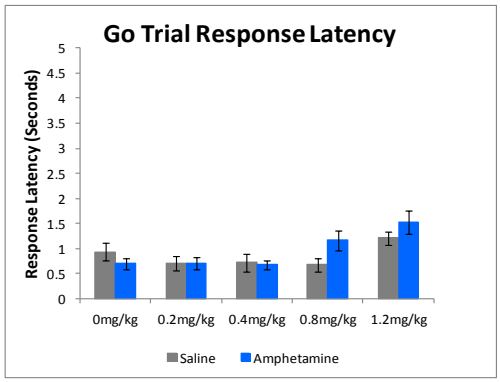
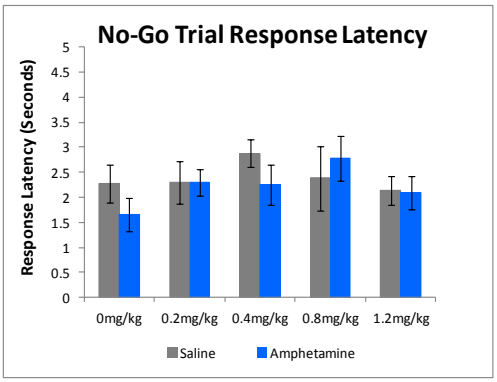


Fig. 4.6



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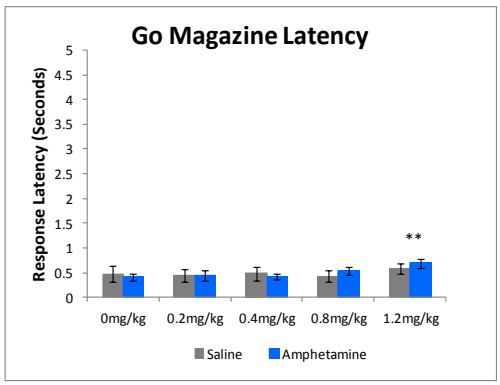


Fig. 4.8

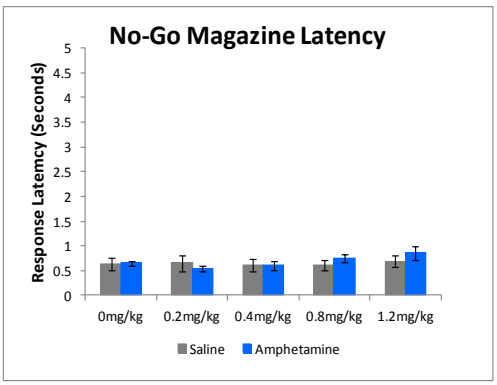


Fig. 4.9

Figures 4.6-4.9: Performance following acute amphetamine challenges on **(4.6)**Go trial response latency, **(4.7)**No-go trial incorrect response latency, **(4.8)** Go trial magazine latency, **(4.9)** No-go trial magazine latency. **p*<0.05 and ***p*<0.01, ****p*<0.001 vs saline dose. Values represent means and error bars represent the SEM.

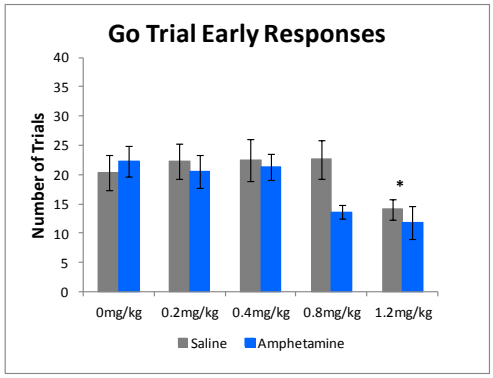


Fig. 4.10

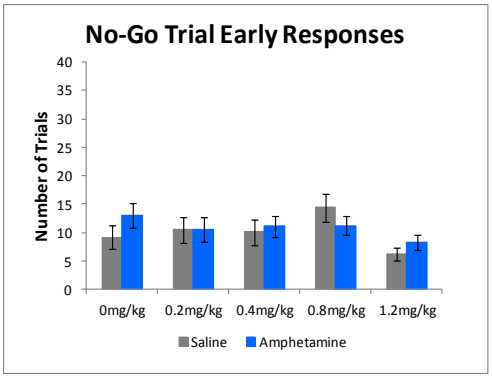


Fig. 4.11

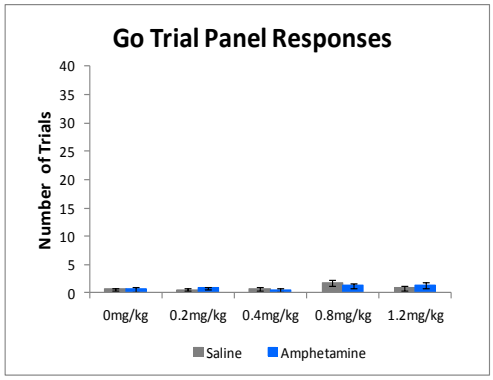


Fig. 4.12

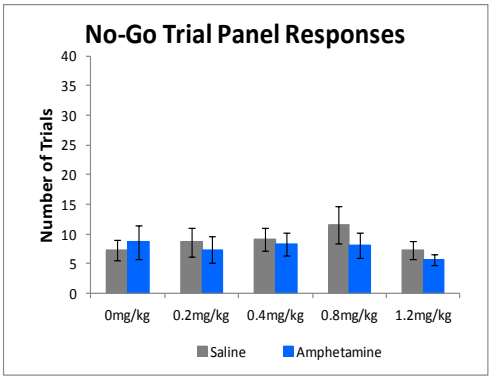


Fig. 4.13

Figures 4.10-13: Performance following acute amphetamine challenges on **(4.10)** Go trial early responses, **(4.11)** No-go trial early responses, **(4.12)** Go trial inappropriate panel responses, **(4.13)** No-go trial inappropriate panel responses. **p*<0.05 and ***p*<0.01, ****p*<0.001 vs saline dose. Values represent means and error bars represent the SEM.

4.4 Discussion

Amphetamine treatment decreased No-go trial accuracy during drug treatment with minimal effects detected on response latencies and anticipatory responding. Short-term withdrawal from amphetamine treatment also caused a reduction in No-go trial accuracy alongside slower correct response latencies and effects indicative of possible changes in motivation and anticipatory responding. Longer-term withdrawal from amphetamine treatment continued to cause a decrease in overall performance accuracy along with possible indications of changes in anticipatory responding during this phase. However, no change was observed in No-go accuracy amongst amphetamine treated animals in comparison to controls, suggesting that amphetamine induced elevations in impulsivity had recovered by this later stage of drug withdrawal. Subsequent amphetamine challenges revealed no changes in amphetamine induced impulsivity based on previous drug history. Collectively, these findings indicate that a 4-day amphetamine binge reduces behavioural inhibition in rats both during binge administration and through short-term drug withdrawal. However, this drug induced impairment in behavioural inhibition is transient and recoverable by long-term drug withdrawal and produces no lasting effects on sensitivity to drug induced effects on impulsivity following 2 weeks post drug exposure.

During amphetamine treatment deficits in No-go trial accuracy were observed alongside an increase in Go trial panel responses. It could be suggested that increased Go trial panel responses might represent amphetamine induced changes in attention, motor or motivational behaviour, given that amphetamine is known to disrupt these dimensions of behaviour in rats (Bardo, Valone, & Bevins, 1999; Barr & Markou, 2005; Dalley et al., 2007b; Dalley et al., 2005; Harrison et al., 1997; Pulvirenti & Koob, 1993; Schiørring, 1971; Segal & Mandell, 1974). However, in consideration of the fact no changes were observed in the accuracy of Go trials, the speed of responding during Go trials or the speed to collect food reward during all trials, it is unlikely that the observed increase in Go trial panel responses reflects drug induced changes in attention, motor and motivational behaviour, that would have noticeably affected these measures if such deficits were present. Furthermore, these null results indicate that reduced No-go trial accuracy during drug treatment is unlikely to be related to any drug induced deficit in attention, locomotor behaviour or motivation for food reward. Given that the most pronounced drug induced deficit during drug treatment was reduced No-go trial accuracy, indicting elevated impulsivity, the observed increase in Go trial panel responses might then represent drug induced increases in premature responding for food reward, in line with the clear deficits observed in behavioural inhibition. However, whilst this may be the case, given

that increased Go trial panel responses was the only measure of anticipatory responding to be affected by drug treatment, this suggests that any possible changes in premature responding for food reward was only a small change in anticipatory responding during drug treatment as otherwise this deficit would have been observed within more measures of anticipatory responding.

There was also a significant interaction detected in No-go trial response latencies during drug treatment that continued to be non-significant following simple effects analysis. From visual inspection of latency means during No-go trials (Table 4.3) it appears that amphetamine treated animals are faster to make incorrect responses during No-go trials than controls on drug day 1 and subsequently slower than controls to make incorrect responses on drug days 2 and 3. However, this pattern in the speed to make incorrect responses during No-go trials between amphetamine and saline treated animals is similar to that observed during the pre-drug baseline week (Table 4.1) suggesting that amphetamine treated animals were not behaving unusually in their speed to make incorrect responses during drug treatment. Alongside the lack of any additional latency effects during drug treatment, this pattern of behaviour between amphetamine treated animals and controls suggests that latencies were unaffected by drug treatment.

Withdrawal from the 4-day amphetamine binge lead to more latency effects than that observed during drug administration, consistent with the behavioural profile of amphetamine withdrawal in rats (Barr & Markou 2005). Amphetamine treated animals were slower to respond during Go trials at 60 hours of withdrawal, and there was a trend towards slower collection of food reward during No-go trials at 18 hours of withdrawal ($p = 0.009$, Benjamini-Hochberg correction $\alpha = 0.006$) (See Table 4.5 and Appendix 1, Table 1h). Reduced response speed during Go trials observed at 60 hours of amphetamine withdrawal is consistent with previous literature reporting a reduction in photobeam counts produced by rats at 56 hours of amphetamine withdrawal (Pulvirenti & Koob, 1993). In addition, the trend observed in reduced No-go trial magazine latencies at 18 hours of withdrawal is consistent with previous literature demonstrating that animals treated with the same 4-day amphetamine binge to that in the current experiment develop elevated reward thresholds for up to 108 hours of withdrawal (Harrison et al., 2001; Lin, Koob, & Markou, 1999), suggesting that animals might have been experiencing anhedonia during this phase of drug withdrawal making animals less motivated to gain food reward. In addition, binge amphetamine regimes similar to that employed in the current experiment have reported reduced motivation to earn food reward during acute amphetamine withdrawal (20 – 68 hours of withdrawal) (Barr & Phillips, 1999). Interestingly, from visual inspection of No-go trial magazine latencies (Table 4.5), the speed to collect food

reward amongst amphetamine treated animals appears to slow down during early drug withdrawal, and following 60 hours of withdrawal speed up to become slightly faster than controls. Although non-significant, this pattern in the speed to collect food reward during No-go trials is consistent with the duration of decreased motivation to received food reward observed by Barr & Phillips, (1999).

However, in consideration of the fact the trend in elevated No-go trial magazine latencies represents a possible reduction in motivation to gain food reward amongst amphetamine treated rats, it could be suggested that reduced No-go trial accuracy might be related to a loss of motivation to perform No-go trials correctly amongst these animals as opposed to increased impulsivity. However, given that amphetamine treated rats continued to produce a high level of performance during Go trials and no changes were observed between amphetamine and saline treated rats within Go trial magazine latencies or No-go trial latencies, this indicates that the trend in withdrawal induced effects on motivation was not substantial. In addition, from Table 4.5, it is clear that the effects detected within latency measures do not represent gross deficits in the speed of responding. It is therefore unlikely that increased response latencies, mainly increased No-go trial magazine latencies, would account for the substantial reduction observed within No-go trial accuracy.

Furthermore, given that increased Go trial latencies and trends in elevated No-go trial magazine latencies indicate slowing down of response behaviour during short-term withdrawal, it is interesting that amphetamine treated animals were also more impulsive during this phase of withdrawal. This suggests that despite withdrawal induced effects that reduce the speed of responding in amphetamine treated animals, these animals continue to remain more impulsive through short-term drug withdrawal. Interestingly, acute amphetamine induced behavioural disinhibition in rats is often accompanied by faster response latencies (Harrison et al., 1997; Pattij et al., 2007) and withdrawal from pharmacologically similar drugs to amphetamine, including MA, MDMA and cocaine, that is accompanied by increased premature responding has been found not to overlap with increased response latencies during the same phases of drug withdrawal (Dalley et al., 2007b) or not to overlap at all (Dalley et al., 2007b; Winstanley et al., 2009). The co-occurrence of both increased response latencies and behavioural disinhibition in the current experiment therefore demonstrates that greater behavioural activation is not always required for increased behavioural disinhibition.

Despite reduced No-go trial accuracy throughout withdrawal week one, anticipatory responding remained relatively normal during this withdrawal phase. An interaction was detected within No-go trial early responses and an effect of withdrawal day was detected

within Go trial panel responses, however, post hoc analyses failed to detect a significant difference between treatment groups in No-go early responding at any specific withdrawal hour or significant difference in Go trial panel responses across drug days. Furthermore, from Table 4.6 it is clear that amphetamine treatment did not produce any substantial changes in the number of No-go trials with early responses or the number of Go trials with panel responses that would be indicative of changes in anticipatory responding during short-term amphetamine withdrawal.

During withdrawal week two, amphetamine treated animals continued to show reduced overall performance on withdrawal day 8 relative to controls, however, no other significant effects were detected across all remaining variables during this withdrawal phase. This confirms that withdrawal induced reductions on behavioural inhibition and latencies observed during withdrawal week one had recovered by the second week of drug withdrawal. Possible changes in No-go trial magazine latencies and panel responses were indicated by an interaction and main effect of withdrawal day during this withdrawal phase, respectively. However, neither of these effects produced any significant post hoc effects, and from Table 4.8, it does not appear that either amphetamine or saline treated animals behaved unusually during this late phase of drug withdrawal. In following, the effects of a 4-day amphetamine binge on impulsivity had recovered by two weeks of drug withdrawal.

The increase in impulsivity observed during experiment 2a is the first to examine and identify reduced behavioural inhibition during the administration of an amphetamine binge in rats. Increased impulsivity observed during amphetamine treatment is consistent with previous studies that have reported elevated impulsivity during the administration phase with stimulant drugs (Winstanley et al., 2009; Richards, 1999; Paine et al., 2003; Gipson & Bardo 2009; Kolokotroni et al., 2012; Dallery and Locey, 2005). Chronic administration of stimulants that are pharmacologically similar to amphetamine, including, methamphetamine and cocaine increase impulsivity during adjusting delay and delayed reward procedures, respectively, (Richards et al., 1999; Paine et al., 2003) and long access (6 hrs) to amphetamine self-administration increases impulsivity during an adjusting delay procedure (Gipson et al., 2009). This is the first study, however, to report reduced behavioural inhibition during amphetamine administration. Reduced behavioural inhibition observed during a 4-day amphetamine binge therefore expands on these studies by suggesting that multiple dimensions of impulsivity may be disrupted during phases of stimulant, and specifically, amphetamine use.

Reductions in behavioural inhibition during chronic drug treatment have also been reported with other stimulant drugs including cocaine and nicotine (Winstanley et al. 2009; Kolokotroni

et al. 2012). Winstanley et al., (2009) found that rats became transiently more impulsive during the acquisition phase of cocaine self-administration with no simultaneous disruptions in accuracy or latency measures. Similarly, chronic nicotine administered through osmotic mini-pumps reduces No-go trial accuracy in rats throughout this phase of drug administration (Kolokotroni et al., 2012). These studies therefore support that chronic stimulant administration can reduce behavioural inhibition during the phase of drug administration itself. Such reductions in behavioural control might then increase vulnerability to repeated drug use during phases of drug use. In particular, increased impulsivity observed during a 4-day amphetamine binge suggests that reduced behavioural inhibition might play a role in fuelling the escalation of drug use during periods of binging.

The persistence of elevated impulsivity during short-term drug withdrawal observed in experiment 2b is the first report that spontaneous withdrawal from an amphetamine binge can transiently reduce behavioural inhibition in rats. This findings differs from previous observations that rats repeatedly withdrawn from cycles of 5 day amphetamine self-administration do not become impulsive on the 5CSRTT during amphetamine withdrawal (Dalley et al., 2005). Dalley et al., (2005) found that amphetamine withdrawal decreased accuracy, increased response latencies and increased omissions, indicative of a withdrawal induced deficit in attention, but did not differ in the number of premature responses made in comparison to controls in rats. In contrast, animals in experiment 2b did not demonstrate any obvious deficits in attention, as demonstrated by the high percentage correct observed within Go trial accuracy and a lack of gross deficits observed in either latencies or anticipatory responses (See Fig. 4.2 and Table 4.5 – 4.8), however, impulsive behaviour was found to increase. Differences between these results might relate to differences in the tasks employed to measure impulsivity. The 5CSRTT primarily measures accuracy of attention meaning that reward for impulsive behaviour is intertwined with attentional performance. In contrast, successful response inhibition during a No-go trial results in the direct gain of receiving food reward that is not dependent on a high level of attention, meaning the animal has an incentive to successfully inhibit behaviour coupled will relatively low attentional demands. Given that amphetamine withdrawal impairs accuracy of performance within the 5CSRTT (Dalley et al., 2005; 2007b), the previous lack of withdrawal induced effects on impulsivity in the 5CSRTT compared to those observed in experiment 2b might then relate to differences in task sensitivity to attention and subsequent parameters to measure behavioural inhibition. Consequently, the withdrawal induced deficit in behavioural inhibition observed in experiment 2b might then reflect a more fundamental loss of inhibitory control that is distinguishable from

deficits in attentional processes, of which premature responses within the 5CSRTT are much more related to.

Interestingly, early responses within the Go/No-go symmetrically reinforced paradigm, that might be more comparable to premature responses on the 5CSRTT were not affected during withdrawal week one. Early responses within the Go/No-go symmetrically reinforced paradigm represent anticipatory responding in animals before gaining trial information from light cues whilst premature responses on the 5CSRTT represent anticipatory responses made before the illumination of a light stimulus to guide response behaviour. These measures might then be more comparable to premature responses on the 5CSRTT than No-go trial accuracy. Consistency between the lack of withdrawal induced effects on early responses within experiment 2b and premature responses within the 5CSRTT (Dalley et al., 2005) therefore supports that impulsivity measured by anticipatory responding that is more closely under the control of attentional processes than No-go trials are not disrupted during short or long term amphetamine withdrawal.

Reduced impulsivity during drug withdrawal may also have occurred as a consequence of the dose and duration of amphetamine employed. Animals in experiment 2b were treated with higher amphetamine doses (up to 15 mg/kg per day) in a shorter period of time (4 days) than animals in the Dalley et al., (2005) study that could self administer up to 3.75 mg/kg during extended access self-administration over 5 days. This indicates that animals in experiment 2b received a more intense binge of amphetamine administration than animals treated with amphetamine in the study by Dalley et al., 2005. The intensity of amphetamine withdrawal measured though ICSS thresholds has been shown to change in relation to the duration (days) and severity (dose) of amphetamine pre-treatment, with the greatest increase in reward thresholds occurring following longer and more intense phases of amphetamine treatment (Lin et al., 1999). Animals treated with a 4-day amphetamine binge in experiment 2b might then have entered a more intense withdrawal period following spontaneous amphetamine termination, in line with a more intense phase of amphetamine treatment, than animals receiving a shorter duration and smaller amount of amphetamine (Dalley et al., 2005). If this was the case then impulsivity may have also been more substantially disrupted in rats in experiment 2b in comparison to rats withdrawal from less amphetamine self-administered over a longer period of time in Dalley et al., (2005).

Withdrawal from chronic amphetamine treatment (21 days self-administration) has previously been found to increase MAD in rats during acute drug withdrawal (Gipson & Bardo, 2009). Reduced behavioural inhibition observed during short-term amphetamine withdrawal

therefore suggests that both impulsive choice and action might be transiently disrupted following chronic amphetamine use. However, rats treated with repeated amphetamine administration do not differ to saline controls in the acquisition of, or long-term performance, on a DRT (Slezak et al., 2012; Stanis et al., 2008). This suggests that transient reductions in reward discounting, but not delay discounting, alongside loss of behavioural inhibition might be residual short-term consequences of amphetamine use.

Reduced behavioural inhibition observed through short-term amphetamine withdrawal also expands on previous literature similarly identifying that impulsivity increases following withdrawal from chronic drug administration. Chronic cocaine and nicotine administration have both been shown to produce elevations in impulsive action and choice procedures during drug withdrawal (Anderson & Diller, 2010; Dallery & Locey, 2005; Dalley et al., 2005; Mendez et al., 2010; Simon, Mendez, & Setlow, 2007; Winstanley et al., 2009). Reduced behavioural inhibition observed during a 4-day amphetamine binge therefore contributes towards this literature area by supporting that elevated impulsivity persists following sub-chronic drug use and can be a consequence of drug per se following drug termination.

These results also expand upon previous literature that has identified specific periods of anhedonia during amphetamine withdrawal. Amphetamine induced anhedonia has been shown to last for up to 60- 108 hours of amphetamine withdrawal (Lin et al. 1999; Harrison et al. 2001; Barr & Phillips 1999; Barr & Phillips 2002). Animals in experiment 2b became transiently more impulsive for up to 36 hours of withdrawal and again at 156 hours of withdrawal (Fig. 4.2). Animals became the most impulsive during acute amphetamine withdrawal, as demonstrated by the greatest significance between groups occurring at 12 and 18 hours of drug withdrawal. These findings therefore expand on this previous literature by additionally identifying that animals are also more impulsive through the same period of drug withdrawal that they are also experiencing anhedonia. The combination of these observations of during the same phase of acute amphetamine withdrawal, suggest that the combinations of that symptoms of withdrawal symptoms may persist into periods of heightened vulnerability to relapse.

No long-term changes were observed in impulsivity amongst animals treated with amphetamine relative to controls following acute amphetamine challenges following two weeks of drug withdrawal. The only difference observed between pre-treatment drug groups at the stage was during Go trial accuracy, whereby amphetamine pre-treated animal displayed a slight greater tolerance to amphetamine induced disruptions of performance at 1.2 mg/kg (Fig. 4.4). However, from Figs. 4.3 – 4.13, it is clear that this tolerance is only a small difference

between drug treatment groups and given that there were no differences in performance across any other variables between pre-treatment groups, it can be concluded that treatment with a 4-day amphetamine binge did not produced any substantial long-term changes in performance of the Go/No-go task in comparison to controls. These findings are consistent with previous studies reporting no difference in impulsivity following acute amphetamine challenges tested at 6 weeks and 10 weeks post drug on DRT and DRL30 tasks, respectively (Hankosky & Gulley, 2012; Stanis et al., 2008). This finding supports literature indicating that prior amphetamine treatment does not alter amphetamine induced impulsivity following a protracted abstinence period. However, Dalley et al. (2005) did observe that rats repeatedly withdrawn from amphetamine self-administration displayed reduced premature responding in comparison to controls following an acute of 0.8 mg/kg at two months post amphetamine treatment (Dalley et al,. 2005). Differences in these findings might relate to differences in the duration of drug administration between studies. Animals in the study by Dalley et al. (2005) self-administered amphetamine for five days followed by nine days of withdrawal over the course of ten weeks, whereas Hankosky & Gulley (2012) and Stanis et al. (2008) administered amphetamine over 18 and 20 days, respectively. Differences in the duration of these administration regimes might suggest that longer exposure to amphetamine might produced greater long-term changes in amphetamine induced impulsivity.

4.4.1 Key Findings

The main findings from this study are that a 4-day amphetamine binge reduces response inhibition in rats both during phases of drug treatment and short-term withdrawal. These findings suggest that subchronic amphetamine exposure might directly impair response inhibition during phases of drug use where the propensity to escalate (drug treatment) and relapse (short-term withdrawal) are high. However, amphetamine induced reductions in response inhibition did not persist into withdrawal week two, and there was no indication that amphetamine treatment caused any changes in sensitivity to drug induced response disinhibition in rats. These additional findings suggest that a subchronic level of amphetamine exposure might not cause any enduring changes in neural systems mediating response inhibition. The main objective of this study was to further disentangle the relationship between amphetamine and impulsivity through assessing the effects of subchronic amphetamine on the Go/No-go symmetrically reinforced task. Observations that 4-day amphetamine can elevate impulsivity in rats helps to disentangle this relationship through, firstly, demonstrating that amphetamine use might increase impulsivity, and secondly,

indicating that the effects of amphetamine on impulsivity might occur at specific time points that facilitate the development and maintenance of addiction.

4.4.2 Limitations

Whilst this study has helped to establish the effects of subchronic amphetamine on response inhibition in rats, there are caveats in the study design that limit interpretations from these findings. One main limitation of this study is in the scalability of the main findings to human populations. The drug regime employed was non-contingent, meaning that rats had no control over drug administration. Contingent drug regimes (self-administration) are more comparable to human drug use since animals have control over administering the drug provided to them, more closely matching the control that humans have over drug use. Whilst non-contingent regimes can control dose exposure more closely than contingent regimes, experimental designs employing non-contingent drug regimes are not as reliable as contingent regimes for modelling human drug use. As a result of employing a non-contingent amphetamine regime in the current study, observations that a 4-day amphetamine binge reduces response inhibition in rats might not necessarily translate to how an amphetamine binge might affect response inhibition within a human population.

Animals in this study were not screened for differences in trait impulsivity at baseline. The effects of amphetamine and ATS on impulsivity are known to relate to baseline levels of impulse control (de Wit, 2000; Eagle et al., 2007; Hand, Fox & Reilly, 2009) and highly impulsive rats show greater sensitivity to the effects of amphetamine than less impulsive rats (Yates et al., 2012). Increased impulsive behaviour is also associated with increased risk of engaging with drug use amongst humans and highly impulsive rats show increased vulnerability to key stages of addiction including drug acquisition, escalation and relapse (Broos et al., 2012b; Dalley et al., 2007; Diergaarde et al., 2009; Kollins, 2003; Perry, 2005; 2008). These studies demonstrate a relationship, firstly, between trait impulsivity and the effects of amphetamine, and secondly, between trait impulsivity and drug addiction. As the current study did not screen rats for high and low levels of trait impulsivity at baseline, there are limitations to the main findings of this study in relation to how individual differences in impulsivity at baseline might have affected amphetamine induced effects on response inhibition and also the time points at which effects occurred.

There were no neuroanatomical or neurochemical controls in this study, limiting interpretations into the possible neural mediators of amphetamine induced impulsivity observed. In order to consider the possible neuroanatomical regions mediating the effects of

amphetamine on impulsivity, it would have been useful to employ an additional group of rats with lesions to neural sites hypothesised to mediate impulsivity. The ventral striatum has been associated with impulsivity in drug addicts and lesions to the nucelus accumbens disrupt amphetamine induced impulsivity in rats when measured by the 5CSRTT (Lee et al., 2009; Murphy et al., 2008). Including an additional group of rats with accumbal lesions might have been useful in order to establish a possible anatomical location that amphetamine induced impulsivity is mediated. In order to address possible neurochemical mediation of amphetamine induced impulsivity that was observed, it would have been useful to employ an l-amphetamine group in addition to the d-amphetamine and saline control groups. As d-amphetamine has a greater binding affinity for dopamine than l-amphetamine, this additional group would have created a neurochemical control to test the involvement of amphetamine induced changes in dopamine on impulsivity.

A final limitation of this study is that rats were not assigned to drug treatment groups at random. Rats were assigned to treatment groups based on their baseline performance on the task. Assigning groups in this manner was conducted in attempt to counterbalance individual differences in performance at baseline between treatment groups. However, as a consequence of assigning groups in this manner it is possible that bias might have been introduced into the drug groups before experimental procedures began.

4.4.3 Future Research

Having established that subchronic amphetamine can reduce response inhibition in rats on the Go/No-go symmetrically reinforced task, future research investigating the effects of chronic amphetamine on response inhibition measured by this task will be useful for understanding how different levels of amphetamine exposure affects impulsivity. Alternatively, research exploring the affects of multiple binge regimes on response inhibition measured by the Go/No-go task would be useful for establishing how chronic amphetamine exposure in an intermittent pattern affects impulsivity. These research directions will help to address how different patterns and levels of amphetamine exposure might affect impulsivity, and more broadly, contribute towards understanding the relationship between patterns of human drug use and impulsivity.

Investigations into the neurobiological changes mediating subchronic amphetamine induced impulsivity will also be a useful future research direction. More specifically, it would be useful to investigate the involvement of the ventral striatum and the D_2 receptors in mediating the effects of subchronic amphetamine on response inhibition that was observed. Previous

research has linked changes in D_2 receptor function within the ventral striatum to impulsivity in drug addicts and in acute amphetamine induced impulsivity on the 5CSRTT in rats (Lee et al., 2009; Pattij et al., 2007). It would therefore be useful to assess whether the D_2 receptors within the ventral striatum are involved in mediating more chronic drug induced changes in impulsivity. Future research focused in this direction will be essential for establishing the contribution of drug use per se to neurochemical changes related to impulsivity observed amongst addicts.

4.4.4 Conclusions

In conclusion, these findings provide further insight into the complex and paradoxical relationship of amphetamine use and impulsivity. Whilst amphetamine is a highly addictive drug that has a relationship with impulsivity, evidenced through therapeutic benefits of amphetamine for alleviating ADHD symptoms, the effects of repeated and chronic amphetamine use per se on impulsivity remain elusive. Given recent conceptualisations of addiction proposing that the binge and withdrawal phases fuelling the addiction cycle become increasingly more intense and severe over the course of dependence (Koob & Le Moal, 2008), better understanding of how impulsivity may persist during phases of binging and withdrawal will give a wider understanding of psychological factors that may be treatable at these stages of the addiction cycle to reduce chronic relapse. Furthermore, given that amphetamine is a drug commonly administered in an intense binge pattern, observations of that behavioural inhibition is reduced during a 4-day binge and through short-term binge withdrawal in rats are useful for considering how behavioural control may change throughout phases of binging and drug withdrawal during amphetamine dependence.

Chapter 5 The effects of 11-day chronic amphetamine on behavioural inhibition in rats

5.1 Introduction

Drug use can occur through a variety of different settings and administration patterns (Perry et al., 2008). Amphetamine is a drug that is abused in a number of different settings for different demands. For example, the performance enhancing effects of amphetamine are desirable for sustained alertness, energy and concentration for long periods of time, whilst the stimulant and euphoric effects of amphetamine are desirable for the recreational drug use (Davey, Richards, & Freeman, 2007; Degenhardt et al., 2007; Hall, Darke, Ross, & Wodak, 1993; Low & Gendaszek, 2002; McCabe et al., 2006; Riley et al., 2001; Teter et al., 2006).

Different patterns of drug use are also associated with different levels of impulsivity. For example, MDMA users that report greater weekly use of cocaine and more years of cocaine and amphetamine use show greater reflection impulsivity than MDMA users with reports of lower weekly and yearly use of cocaine and amphetamine (Quednow et al., 2007). 'Heavy' (weekly) MDMA users also make more errors on the IMT/DMT in comparison to 'light' MDMA (less than weekly use) and non-drug using controls (Moeller et al., 2002), suggesting that patterns of MDMA use leading to greater frequency and consumption of MDMA are associated increased levels of behavioural disinhibition than patterns of less frequent and no MDMA use. Greater levels of cocaine consumption have also been shown to correlate with an increase in SSRT amongst recreational cocaine users (Colzato et al., 2007), suggesting that patterns of drug use leading to greater quantity of cocaine consumption might lead to higher behavioural disinhibition. In addition, subjects that smoke between 11-20 cigarettes per day show significantly longer SSRT than subjects that smoke below 10 cigarettes per day (Billieux et al., 2010), again suggesting that patterns of drug use associated with greater consumption are associated with poorer behavioural inhibition capacity.

In addition, current smokers (20 cigarettes plus per day) show greater discounting of hypothetical money rewards than ex-smokers and controls (Bickel et al., 1999) and 'heavy' smokers (over 40 cigarettes per day) also show steeper discounting of actual monetary reward in comparison to 'chippers' (less than 40 cigarettes per day) and controls (Heyman & Gibb, 2006). This suggests that smoking patterns that are associated with greater nicotine consumption are associated with greater levels of impulsive choice. Similarly, amongst subjects that report illicit drug use, occasions of stimulant use over the previous month, year and

lifetime stimulant use has been found to correlate with disadvantageous decision making within the IGT (Hanson et al., 2008). Severity of dependence amongst alcoholics, crack cocaine and methamphetamine dependent subjects also correlates with disadvantageous decisions on the IGT, whereby subjects with greater severity scores for dependence make more bad decision on the IGT relative subjects lower severity of dependence scores (Bechara et al. 2001). This suggests that patterns of drug use that employ a greater frequency and greater quantity of drug use, might be associated with both increased levels of impulsive action and choice.

Different patterns of repeated drug administration have also been shown to produce different effects on behavioural inhibition in rats. For example, rats that self-administer 0.5 mg/kg of cocaine (2 hr access, unlimited) over the course of four weeks become transiently more impulsive during cocaine withdrawal on the 5CSRTT (Winstanley et al., 2009), whilst rats self-administering 0.25 mg/kg of cocaine (8 hr access, limit of 75 infusions) intermittently over the course of 8 weeks do not become more impulsive on the 5CSRTT during intermittent drug withdrawal from cocaine self-administration (Dalley et al., 2005b). This might suggest that animals receiving a more maintained and higher drug dose regime are more vulnerable to deficits in behavioural inhibition during drug withdrawal. However, rats intermittently withdrawn from long-access (8 hrs) amphetamine self-administration over 2-3 months and rats withdrawn following 21 days of long-access (8 hrs) amphetamine self-administration do not display any changes in behavioural inhibition during amphetamine withdrawal (Dalley et al., 2005; Dalley et al., 2007b). This indicates that despite extending the duration of amphetamine exposure before animals entered drug withdrawal, there were no changes in behavioural inhibition in rats. This alternatively suggests that impulsivity might not be differentially affected by different patterns of amphetamine use. However, as previously discussed (Chapter 4, section 4.1), the attentional parameters of the 5CSRTT might suggests that this lack of withdrawal induced effect on impulsivity following different patterns of amphetamine administration might only reflect a distinct dimension of attentional impulsivity.

Interestingly, rats treated with a long-access (6 hrs) amphetamine self-administration regime for 21 days, similar therefore to the methodology employed by Dalley et al (2007b), have found that rats do become more impulsive during drug administration and acute drug withdrawal on an adjusting delay procedure (Gipson & Bardo, 2009). This further suggests that the effects of different patterns of drug use on impulsivity might relate to the specific aspect of impulsive behaviour being studied.

In consideration of the results observed in experiments 2a and b (See Chapter 4, section 4.3) demonstrating that subchronic amphetamine treatment can lead to a transient reduction in

behavioural inhibition on the symmetrically reinforced Go/No-go task, the current chapter subsequently sought to investigate the effects of a longer term and more maintained pattern of amphetamine administration on impulsivity within this Go/No-go task.

5.1.1 Objectives

In order to investigate the effects of a long-term chronic amphetamine regime on behavioural inhibition measured by the symmetrically reinforced Go/No-go task, the objectives of Chapter 5 were:

i) To determine the effects of 11 day chronic amphetamine on behavioural inhibition during drug treatment within the symmetrically reinforced Go/No-go task (Experiment 3a)

ii) To determine the effects of 11 day chronic amphetamine on behavioural inhibition throughout short term amphetamine withdrawal within the symmetrically reinforced Go/No-go task (Experiment 3b)

iii) To determine the effects of 11 day chronic amphetamine on behavioural inhibition throughout long-term amphetamine withdrawal within the symmetrically reinforced Go/No-go task (Experiment 3c)

iv) To determine the effect of acute amphetamine challenges upon behavioural inhibition following prolonged withdrawal period from chronic (11 day) amphetamine within the symmetrically reinforced Go/No-go task (Experiment 3d)

5.2 Methods

5.2.1 Subjects

19 male Lister Hooded rats (Charles River, UK) were housed in pairs and maintained under a 12 hour light/ dark cycle (lights on 0700), in a temperature (21°C ± 2°C) and humidity (50% ± 5%) controlled environment. Throughout experimentation animals were maintained on a food restriction schedule of 18.6g per day (inclusive of food received during testing), maintaining animals at 85% of their adult free feeding body weight. Water was available ad libitum and feeding took place in the morning after testing. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996. All procedures were covered by Home Office Project Licence No. PIL 40/2711 and 40/3606, and Home Office Personal Licence No. 40/9836.

5.2.2 Drugs

d-Amphetamine sulphate (Sigma Aldrich, UK) was dissolved in 0.9% physiological saline solution and was administered intraperioneally (i.p.) in a volume of 1 ml/kg body weight.

5.2.3 Apparatus

Behavioural testing took place in eight aluminium operant chambers (30.5 x 24.1 x 21 cm, Med Associates Inc., USA) placed inside sound attenuating and ventilated cubicles (63.5 x 49.1 x 39.4 cm, Med Associates Inc., USA). Apparatus was controlled, and all data was recorded from chambers, using MED-PC IV software (Med Associates Inc., USA). For a more detailed description of all apparatus refer to Chapter 2, section 2.1.3, page 60.

5.2.4 Behavioural Testing

Behavioural inhibition was assessed via the symmetrically reinforced go/no-go visual discrimination paradigm. Behavioural training took place for approximately 8 weeks until animals reached a total of 85% correct or more over seven consecutive training sessions. Upon stable baseline performance, animals were subsequently entered into the experiment. All behavioural training and testing took place at the same time each day, between 0700-0930, and prior to morning drug administrations. For a detailed outline of the task refer to Chapter 2, section 2.1.4, page 61.

5.2.5 Design and Procedure

A mixed design was employed to assess the effects of 11-day chronic amphetamine upon behavioural disinhibition, with 'drug treatment group' (saline and amphetamine) acting as the between-subjects factor and 'test day' acting as the within-subjects factor. Following 7 days of stable performance, constituting the baseline week, animals were assigned to groups receiving either systemic amphetamine or saline treatment according to their baseline performance accuracy, programmed levers, and fast/ slow- Go/ No-go contingencies to ensure matched baseline performance of both groups prior to drug administration.

5.2.6 Experiment 3a: The effects of 11-day chronic amphetamine on behavioural disinhibition

Animals were entered into two groups receiving amphetamine (n=9) or saline (n=10) treatment. Amphetamine was administered i.p. twice a day (0800 hrs/2000 hrs) over the course of eleven days in an escalating dose regime. Dosages started at 1 mg/kg increasing up to and stabilising at 5mg/kg. A total of 100 mg/kg of amphetamine was administered per animal in the following daily regimes; Day 1 (1, 2 mg/kg), Day 2 (3, 4 mg/kg), Day 3 (5, 5 mg/kg), Day 4 (5, 5 mg/kg), Day 5 (5, 5 mg/kg), Day 6 (5, 5 mg/kg), Day 7 (5, 5 mg/kg), Day 8 (6, 5 mg/kg), Day 9 (5, 5 mg/kg), Day 10 (5, 5 mg/kg), Day 11 (5, 5 mg/kg). Day 1 of drug treatment began immediately after behavioural testing on baseline day 7, therefore impulsivity measured throughout the subsequent 10 drug days constituted the drug administration phase labelled on Figure and Figure. Saline treated animals were administered with physiological saline i.p. twice a day (0800 hrs/2000 hrs) over the course of eleven days, mimicking the administration regime of amphetamine treated animals. Dosages were based on the principle of doubling the total amount of amphetamine received in experiment 2a and extending the administration of drug over a more continuous, rather than binge, administration regime. Subsequently, amphetamine was administered twice daily instead of three times daily, in line with amphetamine regimes employed over a long administration period (Hankosky & Gulley, 2012; Slezak, Krebs, & Anderson, 2012; Stanis et al., 2008).

5.2.7 Experiment 3b-c: The effects of short- and long-term spontaneous amphetamine withdrawal on behavioural disinhibition

Spontaneous amphetamine withdrawal was initiated after the eleventh day of chronic amphetamine treatment. Animals were subsequently tested for the following 14 days to assess the effects of short- and long-term amphetamine withdrawal upon behavioural

disinhibition. In order to more thoroughly examine behavioural disinhibition throughout acute amphetamine withdrawal animals were tested at 12, 18, 36 and 42 hours post drug termination; constituting two test sessions on day 1 and day 2 of drug withdrawal. Animals were tested once per day across all remaining withdrawal days. Specifically, animals were tested at the following hours post drug treatment; 12, 18, 36, 42, 60, 84, 108, 132, 156 hrs (withdrawal week 1), and on the following days post drug treatment; 8, 9, 10, 11, 12, 13, 14 (withdrawal week 2).

5.2.8 Experiment 3d: The effects of acute amphetamine challenges on behavioural disinhibition

Upon return to stable baseline performance on the Go/No-go task, both amphetamine and saline treated animals received five acute amphetamine challenges (0, 0.2, 0.4, 0.8 and 1.2 mg/kg) in a latin square design. The acute amphetamine dose range was chosen based upon findings by Harrison et al. (unpublished data) demonstrating that 0.8 mg/kg of amphetamine reliably induces a decrease in behavioural inhibition as measured by the go/no-go task. Smaller and larger dosages of amphetamine relative to 0.8 mg/kg (0.2, 0.4 and 1.2 mg/kg) were incorporated into the dose range in order to detect any subtle changes in the sensitivity to this effect of amphetamine following chronic drug treatment. Animals were required to return to their baseline performance on the total percent correct of trials (+/- 5%) across two consecutive days between acute injections in order to ensure that task performance was stable before receiving the following acute amphetamine challenge. A wash out period of a minimum of 72 hours was employed between each drug challenge.

5.2.9 Statistical Analysis

All data was initially checked for normality through Shaprio-Wilk tests and was appropriately transformed via arcsine, log10, square root and reciprocal transformations following any violations to normality. Homogeneity of variance was checked via Mauchley's test of Sphericity and following any significant violation of equal variances, the GLM degrees of freedom were adjusted using the Greenhouse- Geisser correction. For all employed ANCOVAs, homogeneity of the regression slope was assessed via testing the interaction between baseline performance and independent variables. Following a significant baseline x treatment group or baseline x test day interaction, the use of ANCOVA was considered no longer appropriate, and subsequently data was re-expressed a percentage of baseline performance and entered into 2 x x mixed ANOVA (Field, 2005). The validity of using average baseline performance as a covariate was also assessed through checking that baseline performance yielded a main effect

upon treatment groups within the ANCOVA model (Field et al., 2005). Upon the rare occasion that baseline performance was found to not significantly affect treatment groups, data was re-expressed a percentage of baseline performance and entered into a 2 x x mixed ANOVA (Field, 2005).

Unless otherwise stated $\alpha < 0.05$ was employed. Subsequently, the following four sets of data analyses were performed:

5.2.10 Assessment of baseline performance

After checks of normality, the following transformations were performed before conducting baseline analysis: $\log_{10}$ transformation (No-go trial incorrect response, Go trial magazine latency, No-go trial magazine latency, Go trials early responses).

Baseline performance was analysed via 2 x 7 mixed ANOVAs with 'drug treatment group' entered as the between- subjects factor, and 'baseline day' entered as the within-subjects factor. Main effects of 'baseline day' were followed up via Sidak corrected post hoc tests, and significant treatment group x baseline day interactions were deconstructed via simple effects analysis. Simple effects analysis was conducted in two steps. Firstly, two one-way repeated measures ANOVAs were performed for each treatment group to assess individual group within-subject effects, with subsequent significant main effects of 'baseline day' followed up via Sidak corrected post hoc tests. Secondly, seven independent t-tests were performed to examine between-treatment group effects within individual baseline days (Benjamini-Hochberg correction of $\alpha = (i/k)*0.05$).

5.2.11 The effects of 11-day chronic amphetamine, short- and long-term spontaneous amphetamine withdrawal on behavioural inhibition

After checks of normality, the following transformations were applied across chronic drug and withdrawal data: $\log_{10}$ transformation (Go trial response latency, No-go trial response latency, Go trial magazine latency, No-go trial magazine latency, Go trial early responses).

Performance across 11-day chronic amphetamine, short- and long-term spontaneous amphetamine withdrawal was analysed via mixed ANCOVAs, with 'drug treatment group' entered as the between-subjects factor and 'test day', 'withdrawal hour', or 'withdrawal day' entered as the within-subjects factor. In summary, the following ANCOVAs were performed across all behavioural parameters with subjects average baseline performance entered as the covariate; 2 x 10 mixed ANCOVA (chronic drug), 2 x 9 mixed ANCOVA (withdrawal week one),

and 2 x 7 mixed ANCOVA (withdrawal week two). Further analysis of within-subjects main effects was conducted via post hoc Sidak corrected tests and significant interaction effects were deconstructed via post hoc simple effects analysis. Simple effects were performed according to that described in Chapter 4, section 4.2.11.

In addition, in order to identify the specific initiation and duration of treatment effects, all main effects of 'treatment group' were further investigated via conducting multiple between-group comparisons on individual test days (Benjamini-Hochbery correction of $\alpha = (i/k)*0.05$)) along with one-way repeated measures ANCOVAs within each treatment group.

5.2.12 The effects of acute amphetamine challenges on behavioural disinhibition

After checks of normality, the following transformations were performed before conducting analysis of acute amphetamine challenges: $\log_{10}$ transformation (Go trial response latency, Go trial magazine latency, No-go trial magazine latency, No-go trials panel responses).

The effect of acute amphetamine challenges upon behavioural disinhibition was assessed via 2 x 5 repeated measures ANOVAs with 'amphetamine dose' entered as the within-subjects factor and 'drug treatment group' entered as the between-subjects factor. All main effects were followed up via post hoc Sidak corrected tests and significant interactions were deconstructed via simple effects analysis, whereby the pooled error term from the original ANOVA model was utilised to generate the post hoc F value, as described in Chapter 4, Section 4.2.11.

5.3 Results

Unless otherwise stated, for all of the following ANCOVAs homogeneity of regression was assumed ($p > 0.05$) and average baseline performance was found to significantly affect treatment groups ($p < 0.05$), reinforcing the need to control for within-subjects variation in behaviour. Due to data remaining abnormally distributed following transformations, the total percentage correct of Go trials, Go trial magazine latencies and Go trial panel responses were re-expressed as a percentage of average baseline performance and analysed in an ANOVA. This data was analysed using the non-parametric tests Friedman's ANOVA and Mann Whitney U Tests during baseline. Non-parametric tests were also run on Go trial magazine latencies during withdrawal week one and two due to data remaining abnormally distributed after re-expression as a percentage of baseline performance.

5.3.1 Experiment 3a: Pre-drug baseline performance

5.3.1.1 Performance accuracy:

No main effect of baseline day was found within the analysis of total percentage of correctly completed trials ($F(6, 120) = 0.686$, NS), the total percentage of correct Go trials ($\chi^2(6) = 10.696$, NS), or the total percentage of correct No-Go trials ($F(6, 120) = 1.268$, NS). Analysis of the accuracy of performance also produced no main effect of drug treatment group within the total percentage of correctly completed trials ($F(1, 17) = 0.334$, NS), the total percentage of correct Go trials (all $U \geq 36$, NS) and the total percentage of correct No-go trials ($F(1,17) = 0.501$, NS). No significant baseline day x treatment group interactions were found within the analysis of the total percentage of correctly completed trials ($F(6, 120) = 1.941$, NS), or the total percentage of correct No-go trials ($F(6, 120) = 0.476$, NS) (Figs. 5.1 and 5.2).

5.3.1.2 Speed of Responding:

No main effect of baseline day was found within the analysis of Go trial response latencies ($F(6, 102) = 1.416$, NS), Go trial magazine latencies ($\chi^2(6) = 3.284$, NS), No-go trial incorrect response latencies ($F(6, 102) = 0.307$, NS) or No-go trial magazine latencies ($F(6, 102) = 2.172$, NS). In addition, no main effect of drug treatment group was found in the analysis of Go trial response latencies ($F(1, 17) = 0.091$, NS), No-go trial incorrect response latencies ($F(1, 17) = 0.010$, NS), Go trial magazine latencies (all $U \geq 38$, NS) and No-go trial magazine latencies ($F(1, 17) = 0.105$, NS). Furthermore, no significant baseline day x treatment group interactions were found in the analysis of Go trial response latencies ($F(6, 102) = 0.424$, NS), No-go trial incorrect

response latencies ($F(6, 102) = 0.803$, NS) and No-go trial magazine latencies ($F(6, 102) = 1.312$, NS) (Table 5.1).

5.3.1.3 Anticipatory Responding:

No significant difference was observed between baseline days within the analysis of Go trial early responses ($F(6, 102) = 1.262$, NS), Go trial panel responses ($\chi^2(6) = 8.735$, NS), No-go trial early responses ($F(6, 102) = 0.547$, NS), No-go trial panel responses ($F(6, 102) = 0.999$, NS). In addition, no difference was detected between treatment groups in the analysis of Go trial early responses ($F(1, 17) = 0.757$, NS), No-go trial early responses ($F(1, 17) = 0.195$, NS), Go trial panel responses (all $U \geq 34$, NS) or No-go trial panel responses ($F(1, 17) = 1.539$, NS). Furthermore, no significant baseline day x treatment group interactions were detected in the analysis of Go trial early responses ($F(6, 102) = 0.930$, NS), No-go trial early responses ($F(6, 102) = 1.261$, NS) or No-go trial panel responses ($F(6, 102) = 0.860$, NS) (Table 5.2)

Table 5.1: Baseline Week: Speed of responding during baseline week. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go Trial Response Latency	Saline	0.90 ± 0.32	0.95 ± 0.49	0.82 ± 0.33	0.94 ± 0.57	0.85 ± 0.37	0.86 ± 0.31	0.89 ± 0.42
	Amphetamine	0.98 ± 0.55	1.12 ± 0.67	0.82 ± 0.46	0.94 ± 0.50	0.88 ± 0.43	0.79 ± 0.43	1.04 ± 0.58
No-go Trial Incorrect Response Latency	Saline	2.42 ± 1.08	2.05 ± 1.10	2.43 ± 1.32	1.89 ± 1.08	2.21 ± 1.08	1.72 ± 0.97	1.68 ± 0.93
	Amphetamine	2.01 ± 1.63	2.55 ± 1.39	2.26 ± 2.33	2.67 ± 2.54	2.63 ± 2.22	2.49 ± 1.57	2.17 ± 1.62
Go Trial Magazine Latency	Saline	0.44 ± 0.44	0.50 ± 0.55	0.45 ± 0.48	0.47 ± 0.55	0.45 ± 0.51	0.41 ± 0.34	0.41 ± 0.34
	Amphetamine	0.35 ± 0.12	0.35 ± 0.17	0.32 ± 0.09	0.34 ± 0.11	0.34 ± 0.12	0.31 ± 0.10	0.35 ± 0.12
No-go Trial Magazine Latency	Saline	0.62 ± 0.30	0.58 ± 0.25	0.63 ± 0.27	0.66 ± 0.29	0.70 ± 0.25	0.66 ± 0.23	0.69 ± 0.26
	Amphetamine	0.74 ± 0.37	0.71 ± 0.46	0.67 ± 0.38	0.71 ± 0.32	0.69 ± 0.46	0.75 ± 0.41	0.78 ± 0.46

Table 5.2: Baseline Week: Anticipatory responding during baseline week. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go Trial Early Response	Saline	13.10 ± 1.29	15.60 ± 1.99	14.80 ± 1.68	15.90 ± 2.32	16.40 ± 2.10	15.30 ± 1.40	17.60 ± 2.10
	Amphetamine	16.67 ± 2.59	16.89± 3.15	19.11 ± 2.56	18.00 ± 2.32	19.56 ± 2.84	18.78 ± 2.13	16.11 ± 2.12
No-go Trial Early Response	Saline	5.00 ± 1.18	4.7 ± 1.14	5.10 ± 1.03	5.40 ± 1.39	6.50 ± 1.19	6.60 ± 1.73	6.60 ± 1.53
	Amphetamine	6.11 ± 1.52	6.22 ± 1.66	6.89 ± 1.56	7.89 ± 2.19	5.33 ± 1.68	6.33 ± 1.22	6.11 ± 1.16
Go Trial Panel Response	Saline	1.30 ± 0.70	1.30 ± 0.40	1.20 ± 0.33	0.90 ± 0.31	1.50 ± 0.58	0.80 ± 0.33	0.40 ± 0.22
	Amphetamine	1.44 ± 0.44	1.33 ± 0.44	1.22 ± 0.36	1.56 ± 0.73	0.56 ± 0.24	0.67 ± 0.55	0.67 ± 0.33
No-go Trial Panel Response	Saline	12.20 ± 2.15	10.90 ± 2.34	11.60 ± 2.19	9.10 ± 2.06	11.70 ± 2.65	10.70 ± 2.27	9.60 ± 1.89
	Amphetamine	7.22 ± 2.27	8.22 ± 2.27	7.22 ± 2.48	7.56 ± 2.07	7.11 ± 1.87	7.22 ± 2.09	6.44 ± 1.75

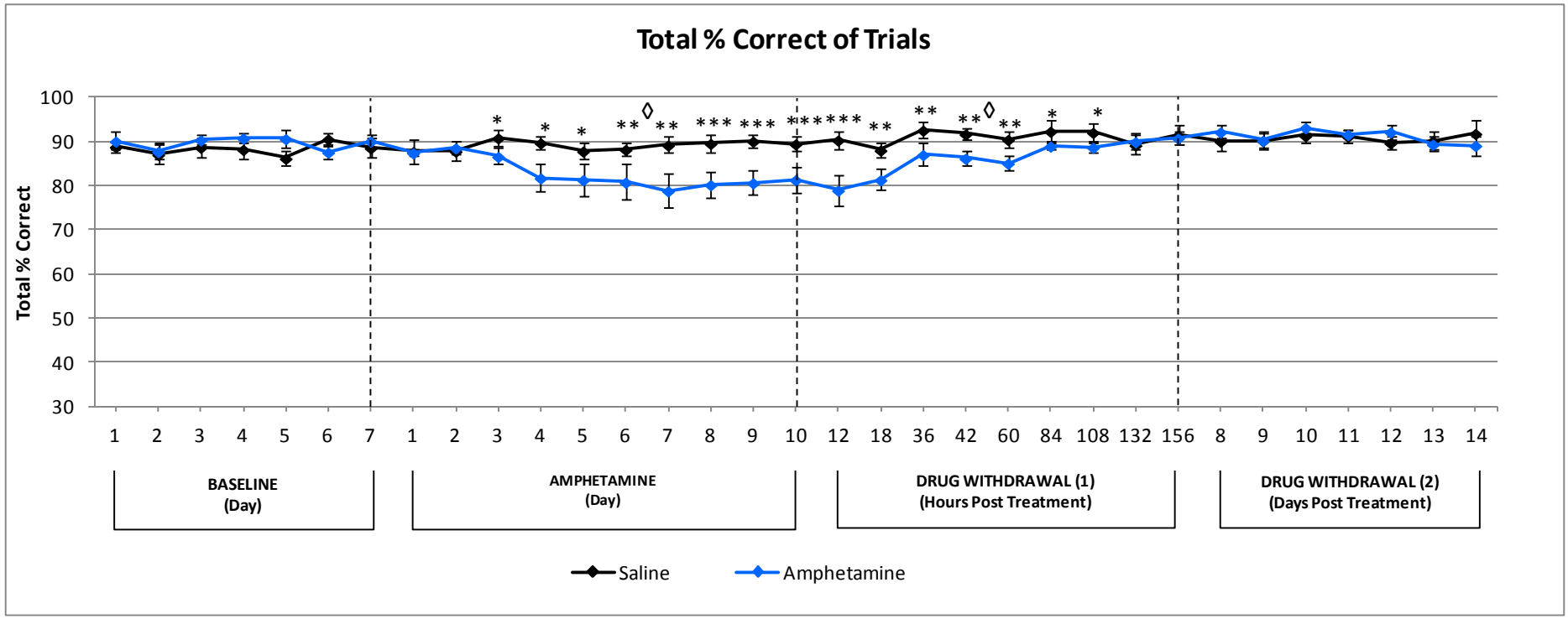


Figure5.1: Performance within the total percentage correct of all trials during baseline week, drug administration, withdrawal week one and withdrawal week two. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and errors represent SEM.

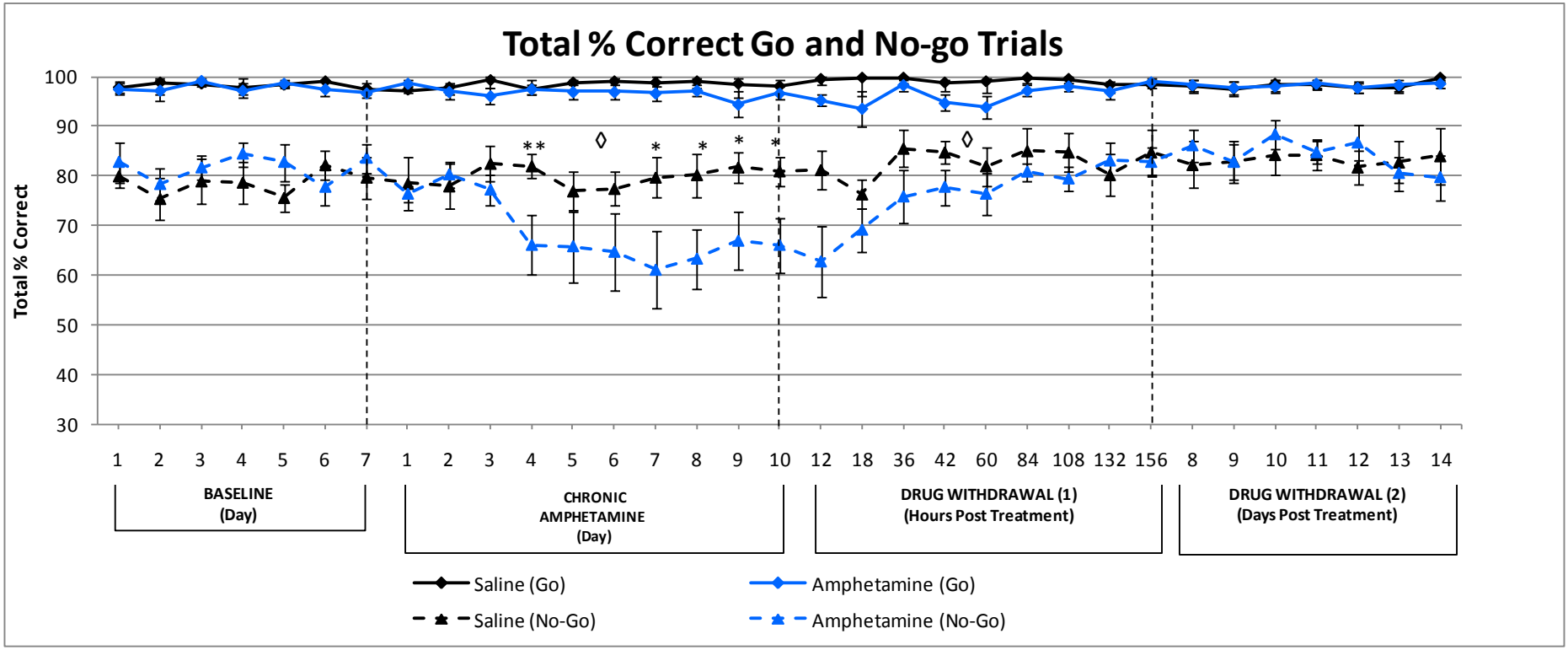


Figure 5.2: Performance within the total percentage correct of Go and No-go trials during baseline week, drug administration, withdrawal week one and withdrawal week two. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and errors represent SEM.

5.3.2 11-Day amphetamine treatment

5.3.2.1 Performance accuracy:

Analysis of the total percentage of correctly completed trials revealed no main effect of drug day ($F(9, 144) = 0.494$, NS), however, a main effect of drug treatment group ($F(1, 16)= 14.676$, $p < 0.001$) and drug day x drug treatment group interaction were detected ($F(9, 144) = 2.071$, $p < 0.05$). Within-subjects simple effects revealed no significant main effect of drug day within either saline ($F(72, 144) = 0.01$, NS) or amphetamine treated animals ($F(63, 144) = 0.03$, NS) when analysed independently. Between-subjects simple effects, however, revealed that amphetamine treated animals had lower accuracy in comparison to controls during drug day 3 ($F(1,16) = 4.343$, $p < 0.05$), day 4 ($F(1,16) = 8.045$, $p < 0.01$), day 5 ($F(1, 16) = 5.140$, $p < 0.05$), day 6 ($F(1, 16) = 6.420$, $P < 0.05$), day 7 ($F(1, 16) = 15.605$, $p < 0.001$), day 8 ($F(1, 16) = 11.733$, $p < 0.001$), day 9 ($F(1, 16) = 11.847$, $p < 0.001$) and day 10 ($F(1, 16) = 8.873$, $p < 0.01$) (Fig. 5.1) (Benjamini-Hochberg correction, $\alpha = p < 0.04 - p < 0.005$, see Appendix 2, Table 2a for specific corrected p-values).

Analysis of the total percentage of correct Go trials revealed no significant main effect of drug day ($F(4.027, 68.452) = 0.542$, NS) or drug treatment group ($F(1, 17) = 0.920$, NS), and no significant drug day x drug treatment group interaction ($F(4.027, 68.452) = 1.436$, NS) (Fig. 5.2). Analysis of the total percentage of correct No-go trials also failed to a significant main effect of drug day ($F(3.985, 63.275) = 0.485) = 0.485$, NS), however, amphetamine treated animals were found to have significantly lower No-go performance in comparison to controls ($F(1, 16) = 9.041$, $p < 0.01$). Further within-subjects analysis revealed that neither saline ($F(3.650, 29.201) = 1.427$, NS) or amphetamine treated animals ($F(1.913, 13.388) = 1.409$, NS) performance within No-go trials altered across drug days when analysed independently, however, further between-subjects analysis revealed that amphetamine treated animals had lower No-go trial accuracy in comparison to controls on drug day 4 ($F(1, 16) = 8.398$, $p < 0.01$), day 7 ($F(1,16) = 6.127$, $p < 0.05$), day 8 ($F(1,16) = 8.297$, $p < 0.05$), day 9 ($F(1,16) = 6.845$, $p < 0.0$) and day 10 ($F(1,16) = 8.214$, $p < 0.05$) (Fig. 5.2) (Benjamini-Hochberg correction, $\alpha = p < 0.025 – p < 0.005$, see Appendix 2, Table 2b for specific corrected p-values). No significant drug day x drug treatment group interaction was found ($F(3.985, 63.275) = 1.492$, NS).

5.3.2.2 Speed of Responding:

Analysis of Go trial response latencies revealed no main effect of drug day ($F(5.234, 83.751) = 0.355$, NS), however, amphetamine treated animals were slower in responding during Go trials in comparison to saline controls during drug treatment ($F(1, 16) = 8.166$, $p < 0.05$). Further with-subjects analysis revealed that neither saline ($F(3.239, 25.909) = 0.451$, NS) or amphetamine treated animals ($F(3.871, 27.095) = 0.383$, NS) differed in speed of responding during Go trials when analysed independently. Further between-subjects analysis also failed to produce any significant differences between saline and amphetamine treated animals on individual drug days (Table 5.3) (df = 1, 16, all $F \geq 0.076$, Benjamini-Hochberg correction applied, see Appendix 2, Table 2c for specific F- and corrected p-values). In addition, no significant drug day x drug treatment group interaction was found within Go trial response latencies ($F(5.234, 83.751) = 2.198$, NS). Analysis of No-go trial incorrect response latencies also revealed no main effect of drug day ($F(9, 144) = 1.280$, NS), drug treatment group ($F(1, 16) = 0.590$, NS) or significant drug day x treatment group interaction ($F(9, 144) = 0.881$, NS) (Table 5.3).

Analysis of Go trial magazine latencies revealed no main effect of drug day ($F(2.817, 48.894) = 0.291$, NS), drug treatment group ($F(1, 17) = 0.001$, NS) or drug day x drug treatment group interaction was detected ($F(2.817, 48.894) = 0.590$, NS) (Table 5.3). Analysis of No-go trial magazine latencies also revealed no main effect of drug day ($F(4.848, 77.596) = 1.280$, NS), however, amphetamine treated animals were slower to collect of the food reward within No-go trials in comparison to controls during drug treatment ($F(1, 16) = 6.638$, $p < 0.05$). Further within-subjects analysis revealed that neither saline ($F(4.593, 36.741) = 1.037$, NS) nor amphetamine ($F(3.275, 22.927) = 0.821$, NS) treated animals differed across drug days in their speed to collect food following a correct No-go trial when analysed independently. However, further between-subjects analysis revealed that amphetamine treated animals were significantly slower to collect food reward within No-go trials relative to controls specifically on drug day 7 ($F(1, 16) = 13.928$, $p < 0.01$) and day 9 ($F(1, 16) = 15.649$, $p < 0.01$) (Table 5.3) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.005$, see Appendix 2, Table 2d for specific F- and corrected p-values).

5.3.2.3 Anticipatory Responding:

Analysis of Go trial early responses revealed no main effect of drug day ($F(9, 144) = 0.802$, NS), however, amphetamine treated animals were found to perform less Go trial early response in

comparison to saline treated animals during drug treatment ($F(1, 16) = 6.951$, $p < 0.05$). Further within-subjects analysis revealed that neither saline ($F(3.536, 28.291) = 0.523$, NS) or amphetamine $F(3.536, 28.291) = 0.523$, NS) treated animals significantly differed in the number of Go trial early responses across drug days, however, further between-subjects analysis revealed that amphetamine treated animals performed less Go trial early responses in comparison to controls on drug day 7 ($F(1, 16) = 11.341$, $p < 0.01$) and drug day 9 ($F(1, 16) = 13.979$, $p < 0.01$) (Table 5.4) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.005$, see Appendix 2, Table 2e for specific F- and corrected p-values)**.** No significant drug day x drug treatment group interaction was however detected with Go trial early responses ($F(9, 144) = 1.717$, NS). Analysis of No-go trial early responses revealed no main effect of drug day ($F(4.640, 74.247) = 0.644$, NS), drug treatment group ($F(1, 16) = 0.028$, NS) and no significant drug day x treatment group interaction ($F(4.640, 74.247) = 0.945$, NS) (Table 5.4).

Analysis of Go trial panel responses revealed no main effect of drug day ($F(9, 153) = 1.591$, NS), however, amphetamine treated animals performed significantly more Go trial panel responses during drug administration in comparison to controls ($F(1, 17) = 5.152$, $p < 0.05$). Further within-subjects analysis demonstrated that neither amphetamine ($F(4.380, 35.039) = 1.128$, NS) nor saline ($F(3.693, 33.234) = 0.835$, NS) treated animals differed in the number of Go trials performed with panel responses during drug administration when analysed independently. Further between-subjects analysis also failed to produce any significant differences between drug treatment groups on individual drug days (df= 1, 16, all $F \geq 0.120$, NS) (Table 5.4) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p < 0.005$, see Appendix 2, Table 2f for specific F- and corrected p-values). Additionally, no significant drug day x drug treatment group interaction was found in the analysis of Go trial panel responses ($F(9, 153) = 0.576$, NS). No main effect of drug day ($F(3.967, 63.469) = 0.340$, NS), drug treatment group ($F(1, 16) = 0.164$, NS) and no significant drug day x drug treatment group interaction was found in the analysis of No-go trial panel responses during drug treatment ($F(3.967, 63.469)$ (Table 5.4).

Table 5.3: Chronic Amphetamine: Speed of responding during amphetamine treatment. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Go Trial Response Latency ◊	Saline	0.89 ± 0.09	0.70 ± 0.12	0.75 ± 0.11	0.88 ± 0.14	0.86 ± 0.14	0.91 ± 0.12	0.70 ± 0.15	0.67 ± 0.08	0.76 ± 0.12	0.75 ± 0.13
	Amphet	0.95 ± 0.17	1.00 ± 0.24	1.10 ± 0.23	1.15 ± 0.24	1.18 ± 0.24	1.09 ± 0.22	1.32 ± 0.22	1.16 ± 0.26	1.55 ± 0.34	1.30 ± 0.23
No-go Trial Incorrect Response Latency	Saline	2.24 ± 0.29	1.68 ± 0.44	2.22 ± 0.35	1.84 ± 0.44	1.70 ± 0.23	1.63 ± 0.35	1.62 ± 0.59	2.25 ± 0.43	2.23 ± 0.50	1.53 ± 0.32
	Amphet	2.02 ± 0.50	2.51 ± 0.50	1.90 ± 0.27	2.34 ± 0.29	1.93 ± 0.39	2.29 ± 0.38	2.22 ± 0.25	2.35 ± 0.32	2.23 ± 0.32	2.53 ± 0.39
Go Trial Magazine Latency	Saline	0.38 ± 0.09	0.36 ± 0.08	0.37 ± 0.10	0.40 ± 0.12	0.41 ± 0.14	0.43 ± 0.15	0.45 ± 0.17	0.44 ± 0.17	0.44 ± 0.18	0.42 ± 0.16
	Amphet	0.34 ± 0.04	0.34 ± 0.04	0.35 ± 0.03	0.33 ± 0.0.	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	0.36 ± 0.02
No-go Trial Magazine Latency ◊	Saline	0.60 ± 0.07	0.62 ± 0.09	0.65 ± 0.09	0.71 ± 0.07	0.66 ± 0.10	0.67 ± 0.12	0.54 ± 0.06	0.63 ± 0.08	0.59 ± 0.06	0.62 ± 0.07
	Amphet	0.76 ± 0.14	0.78 ± 0.16	0.82 ± 0.15	0.79 ± 0.13	0.78 ± 0.12	0.78 ± 0.14	0.86 ± 0.14**	0.81 ± 0.14	0.92 ± 0.16**	0.88 ± 0.13

Table 5.4: Chronic Amphetamine: Anticipatory responding during amphetamine treatment. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Go Trial Early Response ◊	Saline	18.70 ± 2.31	18.50 ± 2.52	18.30 ± 2.85	16.50 ± 2.53	17.10 ± 2.07	16.90 ± 1.83	17.90 ± 1.90	17.10 ± 2.08	17.30 ± 2.13	17.20 ± 2.25
	Amphet	18.79 ± 2.23	18.44 ± 2.21	14.78 ± 2.32	17.22 ± 2.44	16.44 ± 2.33	17.56 ± 2.29	14.11 ± 2.19**	16.67 ± 2.25	14.11 ± 3.04**	15.22 ± 2.48
No-go Trial Early Response	Saline	5.90 ± 1.86	8.20 ± 2.02	6.60 ± 1.45	4.70 ± 1.41	6.80 ± 1.65	6.50 ± 1.42	15.90 ± 1.52	6.30 ± 1.34	6.30 ± 1.44	6.30 ± 1.51
	Amphet	6.78 ± 1.16	7.33 ± 1.71	5.67 ± 1.29	6.67 ± 1.01	7.22 ± 1.36	8.00 ± 1.40	7.67 ± 1.40	7.67 ± 1.49	6.00 ± 1.34	7.11 ± 1.56
Go Trial Panel Response ◊	Saline	0.70 ± 0.26	0.30 ± 0.21	0.60 ± 0.22	1.10 ± 0.67	1.10 ± 0.43	0.80 ± 0.20	0.50 ± 0.31	1.16 ± 0.58	1.16 ± 0.52	1.00 ± 0.37
	Amphet	1.22 ± 0.66	1.11 ± 0.48	1.00 ± 0.33	0.56 ± 0.47.	2.20 ± 0.85	0.89 ± 0.26	0.33 ± 0.50	1.78 ± 0.55	3.00 ± 0.85	1.11 ± 0.26
No-go Trial Panel Response	Saline	9.40 ± 1.48	11.30 ± 2.73	10.80 ± 2.56	11.30 ± 2.80	10.20 ± 2.76	10.70 ± 2.40	10.90 ± 2.42	12.20 ± 2.43	10.90 ± 2.28	10.30 ± 2.49
	Amphet	7.78 ± 2.09	7.22 ± 2.25	7.28 ± 2.31	6.33 ± 1.99	8.11 ± 1.84	9.00 ± 2.32	7.11 ± 2.00	8.11 ± 2.04	8.67 ± 2.07	6.78 ± 2.90

5.3.3 Experiment 3b: Withdrawal week one

5.3.3.1 Performance accuracy:

Analysis of the total percentage of correctly completed trials revealed no main effect of withdrawal hour ($F(4.580) = 0.455$, NS). However, amphetamine treated animals were found to have significantly lower overall accuracy in comparison to controls during withdrawal week one ($F(1, 16) = 18.516$, $p < 0.001$). Further analysis of this effect revealed that saline ($F(8, 64) = 0.621$, NS) and amphetamine treated animals ($F(2{,}832, 19{,}763) = 0.440$, NS) did not differ in performance between withdrawal hours during withdrawal week one when analysed independently, however, further between-subjects comparisons at specific hours of withdrawal demonstrated that amphetamine treated animals had lower overall accuracy in comparison to controls at 12 hrs ($F(1, 16) = 14.694$, $p < 0.001$), 18 hrs ($F(1, 16) = 8.868$, $p < 0.01$), 36 hrs ($F(1, 16) = 8.716$, $p < 0.01$), 42 hrs ($F(1, 16) = 12.217$, $p < 0.01$), 60 hrs ($F(1, 16) = 9.793$, $p < 0.01$), 84 hrs ($F(1, 16) = 5.123$, $p < 0.05$) and 108 hrs of drug withdrawal ($F(1, 16) = 6.880$, $p < 0.05$) (Fig. 5.1) (Benjamini-Hochberg correction, $\alpha. = p < 0.039 – p < 0.005$, see Appendix 2, Table 2g for specific corrected p-values).

Analysis of Go trial performance during withdrawal week one revealed no main effect of withdrawal hour ($F(2.826, 48.039) = 2.123$, NS), drug treatment group ($F(1, 17) = 2.953$, NS) or withdrawal day x drug treatment group interaction ($F(2.826, 48.039) = 2.503$, NS) (Fig. 5.2). Analysis of No-go trial performance during withdrawal week one revealed no significant main effect of withdrawal hour ($F(4.320, 69.113) = 0.536$, NS), however, amphetamine treated animals did have lower No-go trial accuracy during withdrawal week one ($F(1, 16) = 12.352$, $p < 0.01$). Further analysis of this treatment group effect revealed no significant difference in No-go trial performance between withdrawal hours amongst either saline ($F(8, 64) = 0.612$, NS) or amphetamine treated animals ($F(2.664, 18.668) = 0.698$, NS) when analysed independently. And further analysis of between-subjects effects also revealed no significant difference between saline and amphetamine treated animals performance within No-go trials throughout withdrawal week one ($df = 1, 16$, all $F \geq 0.021$. NS) (Fig. 5.2) (Benjamini-Hochberg correction, see Appendix 2, Table 2h for specific F- and corrected p-values). No significant withdrawal hour x treatment group effect was found ($F(4.320, 69.112) = 0.536$, NS).

5.3.3.2 Speed of Responding:

Analysis of Go trial response latencies revealed no main effect of withdrawal hour ($F(8, 128) = 1.313$, NS), however, amphetamine treated animals were significantly slower to respond during Go trials in comparison to controls ($F(1, 16) = 5.491$, $p < 0.05$). Further within-subjects analysis revealed that neither saline ($F(2.807, 22.459) = 0.926$, NS) nor amphetamine ($F(3.826, 26.780) = 0.702$, NS) treated animals differed across withdrawal hours in their speed to respond within Go trials when analysed independently. Further between-groups analysis also revealed no significant differences between saline and amphetamine treated animals in the speed of responding within Go trials at specific hours of withdrawal (df = 1, 16, all $F \geq 0.127$, NS) (Table 5.5) (Benjamini-Hochberg correction, see Appendix 2, Table 2i for specific F- and corrected p-values). Analysis of No-go trial incorrect response latencies revealed no main effect of withdrawal hour ($F(4.779, 76.780) = 0.970$, NS), drug treatment group ($F(1, 16) = 0.018$, NS) or significant withdrawal hour x drug treatment group interaction ($F(4.779, 76.780) = 0.1644$, NS) (Table 5.5).

Analysis of Go trial magazine latencies revealed that the speed to collect the food reward did not change across withdrawal hours ($\chi^2(8) = 6.517$, NS) and between-subjects analysis of Go trial magazine latencies also revealed that amphetamine and saline treated animals did not differ in the speed to collect food reward within Go trials during withdrawal week one (all $U \geq 28$, NS) (Table 5.5). Analysis No-go trial magazine latencies also revealed no main effect of withdrawal hour ($F(8, 128) = 0.593$, NS), however, amphetamine treated animals were slower to collect the food reward during No-go trial trials in comparison to controls during withdrawal week one ($F(1, 16) = 8.767$, $p < 0.01$). Further within-subjects analysis revealed that neither saline ($F(4.367, 34.934) = 0.681$, NS) nor amphetamine ($F(3.029, 21.200) = 1.483$, NS) treated animals significantly differed in the speed to collect food reward following successful No-go trials when analysed independently, however, multiple comparisons of between-subjects effects revealed that amphetamine treated animals were significantly slower to collect food reward during No-go trials at 18 hours of withdrawal ($F(1, 16) = 13.718$, $p < 0.01$) (Table 5.5) (Benjamini-Hochberg correction, $\alpha = p < 0.05 – p< 0.006$, see Appendix 2. Table 2j for specific F- and corrected p-values). No significant withdrawal hour x drug treatment group interaction was detected in the analysis of No-go trial magazine latencies ($F(8, 128) = 1.659$, NS).

5.3.3.3 Anticipatory Responding:

Analysis of Go trial early responses revealed no main effect of withdrawal hour ($F(3.629, 58.062) = 0.976$, NS), however, amphetamine treated animals performed significantly less Go

trial early responses during withdrawal week one in comparison to controls ($F(1, 16) = 8.258$, $p < 0.05$). Further within-subjects analysis revealed that there was no significant difference between withdrawal hours within either saline ($F(8, 64) = 1.357$, NS) or amphetamine treated animals ($F(2.588, 18.113) = 1.615$, NS) during withdrawal week one when analysed independently. Further between-subjects analysis also failed to identify a significant difference between treatment groups at specific hours of withdrawal ($df = 1, 16$, all $F \geq 0.015$, NS) (Table 5.6) (Benjamini-Hochberg correction, see Appendix 2, Table 2k for specific F- and corrected p-values). In addition, no significant withdrawal hour x drug treatment group interaction was found ($F(3.629, 58.062) = 0.967$, NS). Analysis of No-go trial early responses revealed no main effect of withdrawal hour $(4.029, 64.463) = 1.138$, NS), drug treatment group ($F(1, 16) = 0.024$, NS) and no withdrawal hour x drug treatment group interaction was detected during withdrawal week one ($F(4.029, 64.463) = 0.793$, NS) (Table 5.6).

Analysis of Go trial panel responses revealed no main effect of withdrawal hour ($F(4.221, 71.756) = 1.214$, NS), drug treatment group ($F(1, 17) = 0.540$, NS) and no significant withdrawal hour x drug treatment group interaction ($F(4.221, 71.756) = 0.835$, NS) (Fig. 5.6). Similarly, the analysis of No-go trial panel responses revealed no main effect of withdrawal hour ($F(4.729, 75.662)$), drug treatment group ($F(1, 16) = 1.121$, NS) and no withdrawal hour x drug treatment group interaction ($F(4.729, 75.662) = 0.603$, NS) (Table 5.6).

Table 5.5: **Withdrawal Week One:** Speed of responding during withdrawal week one. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		12 hrs	18 hrs	36 hrs	42 hrs	60 hrs	84 hrs	108 hrs	132 hrs	156 hrs
Go Trial Response Latency ◊	Saline	0.79 ± 0.12	0.71 ± 0.08	0.69 ± 0.09	0.86 ± 0.10	0.77 ± 0.11	0.71 ± 0.09	0.78 ± 0.18	0.68 ± 0.08	0.80 ± 0.16
	Amphet	1.40 ± 0.28	1.11 ± 0.15	1.02 ± 0.21	1.12 ± 0.24	1.23 ± 0.28	0.93 ± 0.17	0.93 ± 0.17	0.86 ± 0.13	0.86 ± 0.19
No-go Trial Incorrect Response Latency	Saline	2.09 ± 0.31	1.89 ± 0.24	1.59 ± 0.38	2.16 ± 0.35	1.78 ± 0.35	1.59 ± 0.30	1.75 ± 0.39	1.53 ± 0.26	1.15 ± 0.26
	Amphet	2.63 ± 0.35	2.10 ± 0.37	2.12 ± 0.41	1.41 ± 0.36	1.43 ± 0.30	1.65 ± 0.27	1.70 ± 0.37	2.00 ± 0.43	2.19 ± 0.63
Go Trial Magazine Latency	Saline	0.42 ± 0.16	0.43 ± 0.16	0.50 ± 0.24	0.46 ± 0.19	0.51 ± 0.20	0.46 ± 0.20	0.43 ± 0.15	0.44 ± 0.17	0.49 ± 0.18
	Amphet	0.38 ± 0.04	0.38 ± 0.67	0.37 ± 0.07	0.37 ± 0.07	0.37 ± 0.07	0.43 ± 0.13	0.44 ± 0.14	0.43 ± 0.51	0.37 ± 0.07
No-go Trial Magazine Latency ◊	Saline	0.68 ± 0.11	0.58 ± 0.08 **	0.63 ± 0.09	0.62 ± 0.08	0.64 ± 0.08	0.63 ± 0.09	0.58 ± 0.07	0.67 ± 0.08	0.74 ± 0.09
	Amphet	0.95 ± 0.17	1.02 ± 0.20	0.77 ± 0.14	0.87 ± 0.20	0.87 ± 0.19	0.80 ± 0.12	0.82 ± 0.17	0.83 ± 0.17	0.82 ± 0.21

Table 5.6: Withdrawal Week One: Anticipatory responding during withdrawal week one. ◊ = main effect of group. *p<0.05 and **p<0.01, ***p<0.001 = significant difference between amphetamine and saline group. Values represent means and SEM.

Behavioural Measure		12 hrs	18 hrs	36 hrs	42 hrs	60 hrs	84 hrs	108 hrs	132 hrs	156 hrs
Go Trial Early Response ◊	Saline	18.60 ± 2.84	17.70 ± 2.37	17.90 ± 1.96	15.80 ± 2.17	19.80 ± 2.18	19.50 ± 2.53	20.00 ± 2.87	18.60 ± 2.33	18.90 ± 2.18
	Amphet	14.22 ± 2.53	16.22 ± 1.96	14.44 ± 2.13	14.67 ± 1.98	16.33 ± 2.97	18.00 ± 2.70	19.78 ± 2.18	18.11 ± 2.81	19.22 ± 2.49
No-go Trial Early Response	Saline	5.10 ± 0.96	7.90 ± 2.00	6.10 ± 1.73	5.70 ± 1.35	5.00 ± 1.67	4.70 ± 1.67	5.50 ± 2.09	6.00 ± 1.45	6.40 ± 2.00
	Amphet	7.78 ± 1.42	7.67 ± 1.47	6.33 ± 1.68	6.00 ± 1.11	5.56 ± 1.29	5.78 ± 1.06	7.11 ± 1.34	5.67 ± 1.54	6.56 ± 1.42
Go Trial Panel Response	Saline	1.30 ± 0.40	1.30 ± 0.45	0.70 ± 0.30	1.11 ± 0.46	0.90 ± 0.53	1.20 ± 0.39	1.00 ± 0.37	0.80 ± 0.36	1.30 ± 0.42
	Amphet	1.00 ± 0.29	1.78 ± 0.64	0.89 ± 0.77	2.44 ± 1.26	1.33 ± 0.75	1.11 ± 0.75	1.44 ± 0.87	1.44 ± 0.58	1.11 ± 0.75
No-go Trial Panel Response	Saline	9.10 ± 1.77	10.90 ± 2.01	10.60 ± 2.60	10.60 ± 2.78	9.70 ± 2.21	10.30 ± 2.23	10.30 ± 2.74	9.30 ± 2.78	9.90 ± 2.54
	Amphet	10.00 ± 2.89	8.44 ± 2.58	7.78 ± 2.74	8.67 ± 2.51	7.67 ± 2.13	9.00 ± 2.64	8.00 ± 2.33	6.78 ± 2.07	7.44 ± 3.06

5.3.4 Experiment 3c: Withdrawal week two

5.3.4.1 Performance accuracy:

Analysis of the total percentage of correctly completed trials during withdrawal week two revealed no main effect of withdrawal day ($F(6, 96) = 0.558$, NS), drug treatment group ($F(1, 16) = 0.203$, NS) or significant withdrawal day x treatment group interaction ($F(6, 96) = 0.558$, NS) (Fig. 5.1). Analysis of the total percentage of correct Go trials also revealed no main effect of withdrawal day ($F(3.450, 58.654) = 1.060$, NS), drug treatment group ($F(1, 17) = 0.782$, NS) or withdrawal day x treatment group interaction ($F(3.450, 58.654) = 0.391$, NS) (Fig. 5.2). Analysis of the total percentage of correct No-go trial further revealed no main effect of withdrawal day ($F(6, 96) = 0.834$, NS), drug treatment group ($F(1, 16) = 0.162$, NS) or withdrawal day x treatment group interaction ($F(6, 96) = 1.280$, NS) (Fig. 5.2).

5.3.4.2 Speed of Responding:

Analysis of Go trial response latencies revealed no main effect of withdrawal day($F(6, 96) = 0.547$, NS), drug treatment group ($F(1, 16) = 1.725$, NS) or withdrawal day x drug treatment group interaction during withdrawal week one ($F(6, 96) = 0.802$, NS) (Table 5.7). Analysis of No-go trial incorrect response latencies also revealed no main effect of withdrawal day ($F(6, 96) = 1.520$, NS), drug treatment group ($F(1, 16) = 0.175$, NS) or withdrawal day x drug treatment group interaction during withdrawal week two ($F(6, 96) = 0.670$, NS) (Table 5.7). Analysis of Go trial magazine latencies also revealed that the speed to collect the food reward during correct Go trials did not differ across days during withdrawal week two ($\chi^2(6) = 4.99$, NS) and did not differ between drug treatment groups (all $U \geq 40$, NS) (Table 5.7). Analysis of No-go trial magazine latencies further revealed no main effect of withdrawal day ($F(3.431, 54.899) = 2.069$, NS), drug treatment group ($F(1, 16) = 0.589$, NS) or withdrawal day x drug treatment group interaction ($F3.431, 54.899) = 2.250$, NS) (Table 5.7).

5.3.4.3 Anticipatory Responding:

Analysis of Go trial early response revealed no main effect of withdrawal day ($F(2.927, 46.832) = 0.748$, NS), drug treatment group ($F(1, 16) = 0.333$), NS) or withdrawal day x drug treatment group interaction during withdrawal week two ($F(2.927, 46.832) = 0.520$, NS) (Table 5.8). Analysis of No-go trial early responses also revealed no main effect of withdrawal day ($F(6, 96) = 0.210$, NS), drug treatment group ($F(1, 16) = 1.406$, NS) and no withdrawal day x drug treatment group interaction (6, 96) = 0.539, NS) (Table 5.8). Analysis of Go trial panel

responses revealed no main effect of withdrawal day ($F(6, 102) = 1.131$, NS), drug treatment group($F(1, 17) = 1.113$, NS) or withdrawal day x drug treatment group interaction during withdrawal week two ($F(6, 102) = 1.028$, NS) (Table 5.8). Analysis of No-go trial panel responses also revealed no main effect of withdrawal day ($F(6, 96) = 0.182$, NS), drug treatment group ($F(1, 16) = 0.325$, NS or withdrawal day x drug treatment group interaction during withdrawal week two ($F(6, 96) = 0.229$, NS) (Table 5.8).

Table 5.7: Withdrawal Week Two: Speed of responding during withdrawal week two during withdrawal week one. Values represent means and SEM.

Behavioural Measure		WD8	WD9	WD10	WD11	WD12	WD13	WD14
Go Trial Response Latency	Saline	0.83 ± 0.13	0.68 ± 0.11	0.65 ± 0.10	0.66 ± 0.10	0.67 ± 0.13	0.80 ± 0.13	0.82 ± 0.11
	Amphet	0.96 ± 0.16	1.00 ± 0.23	0.83 ± 0.17	0.97 ± 0.23	1.01 ± 0.24	0.93 ± 0.21	0.83 ± 0.17
No-go Trial Incorrect Response Latency	Saline	1.74 ± 0.33	1.73 ± 0.31	1.41 ± 0.27	1.54 ± 0.31	1.96 ± 0.51	1.09 ± 0.20	1.85 ± 0.37
	Amphet	1.36 ± 0.24	1.60 ± 0.21	1.43 ± 0.35	1.75 ± 0.38	1.58 ± 0.57	2.02 ± 0.55	1.80 ± 0.46
Go Trial Magazine Latency	Saline	0.49 ± 0.19	0.55 ± 0.23	0.43 ± 0.16	0.41 ± 0.12	0.48 ± 0.11	0.40 ± 0.12	0.40 ± 0.10
	Amphet	0.47 ± 0.18	0.46 ± 0.17	0.45 ± 0.15	0.45 ± 0.15	0.37 ± 0.10	0.40 ± 0.13	0.41 ± 0.12
No-go Trial Magazine Latency	Saline	0.69 ± 0.09	0.66 ± 0.09	0.62 ± 0.07	0.74 ± 0.12	0.65 ± 0.08	0.70 ± 0.14	0.45 ± 0.08
	Amphet	0.83 ± 0.19	0.92 ± 0.21	0.84 ± 0.16	0.70 ± 0.13	0.82 ± 0.17	0.72 ± 0.17	0.72 ± 0.13

Table 5.8: Withdrawal Week Two: Anticipatory responding during withdrawal week two during withdrawal week one. Values represent means and SEM.

Behavioural Measure		WD8	WD9	WD10	WD11	WD12	WD13	WD14
Go Trial Early Response	Saline	18.50 ± 2.37	20.0 ± 3.35	18.60 ± 2.99	19.00 ± 2.93	19.20 ± 3.15	19.80 ± 3.00	16.80 ± 2.86
	Amphet	18.56 ± 1.65	19.67 ± 2.22	20.89 ± 2.68	19.56 ± 2.36	19.44 ± 2.04	17.67 ± 2.63	17.33 ± 2.72
No-go Trial Early Response	Saline	6.50 ± 2.03	6.90 ± 21.95	7.20 ± 1.99	6.40 ± 2.00	7.60 ± 2.49	7.30 ± 2.22	7.44 ± 1.88
	Amphet	4.22 ± 1.22	5.22 ± 1.39	5.11 ± 1.09	5.22 ± 1.32	5.11 ± 1.51	6.44 ± 1.34	7.00 ± 1.62
Go Trial Panel Response	Saline	1.80 ± 0.51	0.90 ± 0.31	0.70 ± 0.30	0.80 ± 0.29	0.10 ± 0.26	0.70 ± 0.21	0.80 ± 0.29
	Amphet	1.78 ± 1.42	1.56 ± 0.96	1.67 ± 1.08	1.22 ± 0.80	1.33 ± 0.75	1.33 ± 0.58	1.33 ± 0.65
No-go Trial Panel Response	Saline	10.50 ± 2.38	11.00 ± 2.84	9.90 ± 2.47	11.00 ± 2.37	10.90 ± 2.35	10.00 ± 2.11	9.80 ± 2.17
	Amphet	6.22 ± 2.25	7.11 ± 2.34	5.56 ± 1.58	6.44 ± 2.00	6.56 ± 2.27	5.33 ± 1.62	6.56 ± 2.57

5.3.5 Experiment 3d: Acute amphetamine challenges

5.3.5.1 Performance accuracy:

Acute amphetamine challenges produced a treatment effect on the total percentage of correctly completed trials ($F(2.444, 41.554) = 18.504$, $p < 0.001$). Post hoc analysis revealed that the total percentage of correctly completed trials decreased in both amphetamine and saline pre-treated animals following administration of 0.8 and 1.2 mg/kg amphetamine in comparison to the saline control treatment, 0.2 ($p < 0.001$) and 0.4 mg/kg amphetamine ($p < 0.05$) (Fig. 5.3). However, there was no main effect of previous drug history (saline vs. amphetamine) ($F(1, 17) = 0.762$, NS) and no amphetamine dose x drug treatment group interaction was detected ($F(2.444, 41.554) = 0.007$, NS) (Fig. 5.3).

Independent analysis of the percentage of correctly completed Go trials revealed no main effect of amphetamine dose ($\chi^2(4) = 2.169$, NS), however, animals with a previous history of chronic amphetamine treatment were found to have reduced Go trial accuracy in comparison to animals previously treated with saline following 1.2 mg/kg amphetamine ($U = 20$, $p < 0.01$), however, smaller dose challenges of amphetamine did not differentially effect the performance of animals with previous exposure to saline or amphetamine (all $U \geq 40$, NS) (Fig. 5.4). Independent analysis of No-go trial accuracy revealed a main effect of amphetamine dose ($F(2.382, 40.486) = 17.628$, $p < 0.001$). Post hoc analysis of this effect revealed that the total percentage of correctly completed No-go trials decreased following 0.8 and 1.2 mg/kg amphetamine in comparison to saline control treatment and 0.2 mg/kg amphetamine ($p < 0.001$), and 1.2 mg/kg also reduced No-go trial accuracy in comparison to 0.4 mg/kg ($p < 0.05$) (Fig. 5.5). However, there was no main effect of previous drug treatment group ($F(1, 17) = 0.001$, NS) and no dose x drug treatment group interaction was found within the analysis of No-go trial accuracy ($F(2.382, 40.486) = 0.685$, NS) (Fig. 5.5).

5.3.5.2 Speed of Responding:

Analysis of Go trial response latencies revealed no main effect of amphetamine dose ($F(4, 68) = 0.225$, NS), previous drug treatment group ($F(1, 17) = 1.941$, NS) and no significant dose x drug treatment group interaction ($F(4, 68) = 0.616$. NS) (Fig. 5.6). Analysis of No-go trial response latencies revealed a trend towards an increase in speed to make incorrect responses during No-go trials ($F(4, 68) = 2.2400$, $p = 0.057$, NS), however, previous drug treatment group had no effect on No-go trial incorrect response latencies ($F(1, 17) = 0.254$, NS), and no significant dose x drug treatment group interaction was detected ($F(4, 68) = 1.489$, NS) (Fig.

5.7). Analysis of Go trial magazine latencies revealed no main effect of amphetamine dose ($F(4.937, 32.924) = 0.724$, NS), previous drug treatment group ($F(1, 17) = 0.05$, NS) or significant dose x drug treatment group interaction ($F(4.937, 32.924) = 0.622$, NS) (Fig. 5.8). In addition, no main effect of amphetamine dose ($F(2.252, 38.277) = 0.336$, NS), previous drug treatment group ($F(1, 17) = 0.332$, NS) and no significant dose x drug treatment group interaction was detected in the analysis of No-go trial magazine latencies ($F(2.252, 38.277) = 0.185$, NS) (Fig. 5.9).

5.3.5.3 Anticipatory Responding:

Analysis of Go trial early responses revealed no main effect of amphetamine dose ($F(2.584, 43.933) = 0.571$, NS), previous drug treatment group ($F(1, 17) = 0.064$, NS) and no dose x drug treatment group was detected ($F(2.584, 43.933) = 0.264$, NS) (Fig. 5.10). In addition, analysis of No-go trial early responses revealed no main effect of amphetamine dose ($F(1.955, 33.235) = 2.706$, NS), previous drug treatment group ($F(1, 17) = 0.168$, NS) and no significant dose x drug treatment group interaction ($F(2.584, 43.933) = 0.295$, NS) (Fig. 5.11). Analysis of Go trial panel responses also revealed no main effect of amphetamine dose ($\chi^2(4) = 3.233$, NS) or previous drug treatment group (all $U \geq 28.5$, NS) (Fig. 5.12). No main effect of amphetamine dose was detected in the analysis of No-go trial panel responses ($F(1.745, 29.663) = 1.442$, NS), however, animals previously treated with amphetamine performed significantly less No-go trials with a panel response following all acute amphetamine challenges in comparison to animals with a previous history of saline treatment ($F(1, 17) = 6.404$, $p < 0.05$) (Fig. 5.13). However, no dose x previous drug treatment group interaction was found in the analysis of No-go trial panel responses ($F(1.754, 26.663) = 0.457$, NS) (Fig. 5.13).

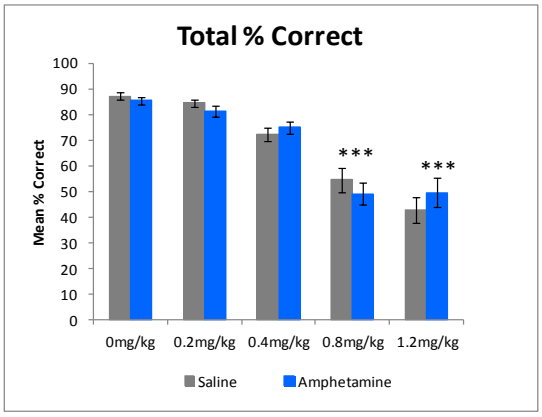


Fig. 5.3

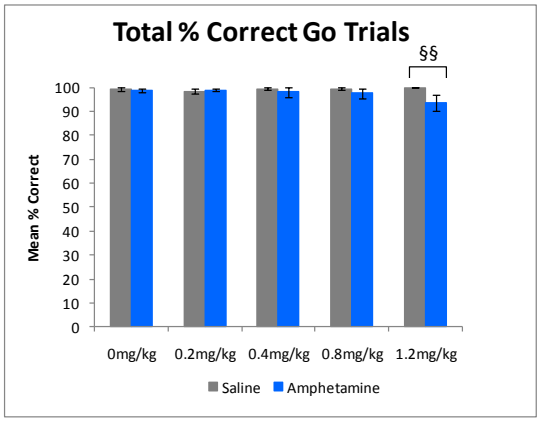


Fig.5.4

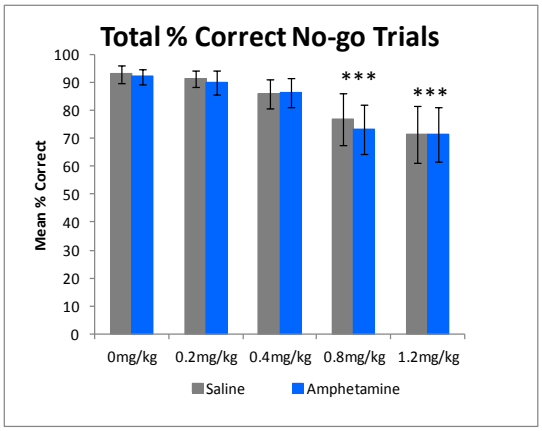


Fig. 5.5

Figures 5.3-5.13: Performance following acute amphetamine challenges within **(5.3)** the total percent correct of all trials, **(5.4)** the total percent correct Go trials, **(5.5)** the total percent correct No-go trials,**(5.6**) Go trial latency, **(5.7)** No-go trial response latency,**(5.8)** Go trial magazine response latency, **(5.9)** No-go trial magazine latency, **(5.10)** Go trial early responses, **(5.11)** No-go trial early response, **(5.12)** Go trial panel response and **(5.13)** No-go trial panel response. ◊ = main effect of group. **p*<0.05, ***p*<0.01 and ****p*<0.001 vs saline dose. § *p*<0.05, §§ *p*<0.01 and §§§<0.001 vs saline group. Values represent means and error bars represent SEM.

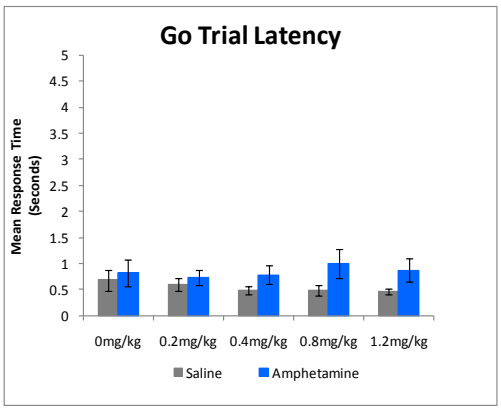
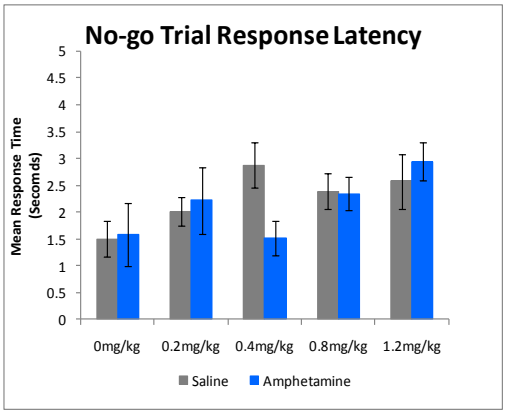


Fig. 5.6



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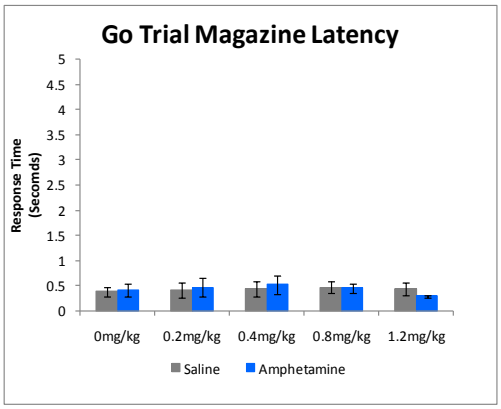

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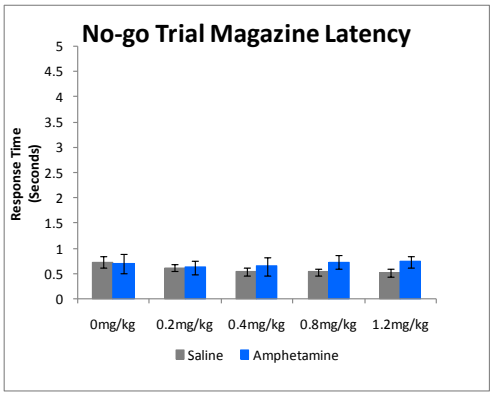

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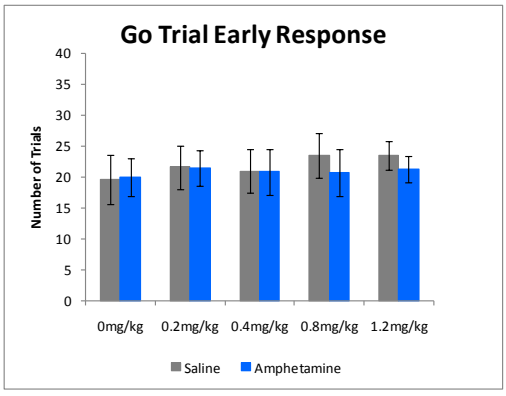


Fig. 5.10

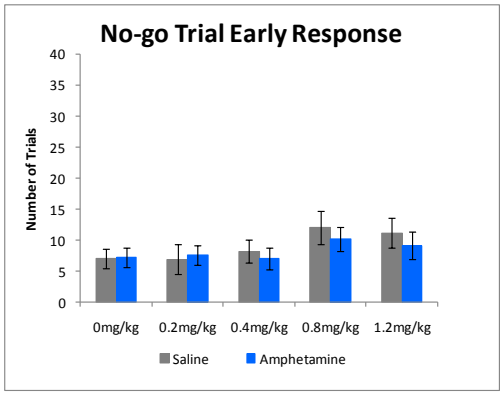


Fig. 5.11

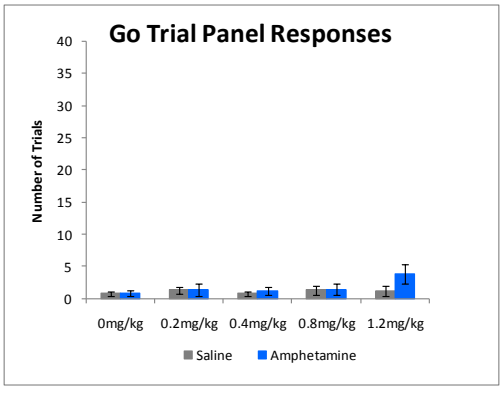


Fig. 5.12

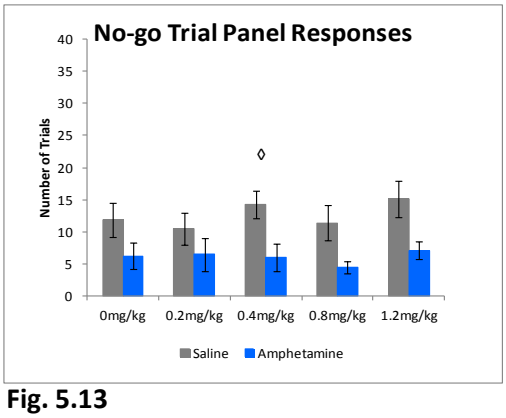


Fig. 5.13

5.4 Discussion

Results revealed that treatment with 11 day chronic amphetamine caused an increase in impulsivity during drug treatment that was coupled with slower response speed during Go trials and in the speed to collect food reward during No-go trials. In addition, increased impulsivity was coupled with less early but more panel responses during Go trials during drug treatment. This pattern of behavioural deficits continued into withdrawal week one, where amphetamine treated animals remained more impulsive than controls and displayed slower response speed during Go trials and in the speed collect food reward during No-go trials. In addition, amphetamine treated animals continued to perform less early responses during Go trials relative to controls. This pattern of behaviour terminated by withdrawal week two where there was no difference observed between amphetamine treated animals and controls on all behavioural measures, indicating that drug induced changes in behavioural inhibition, speed of responding and anticipatory responding had recovered by two weeks following drug termination. Acute drug challenges revealed that animals with a history of chronic amphetamine treatment displayed an increase in sensitivity to amphetamine induced performance effects during Go trials at the highest dose of acute amphetamine in comparison to rats with a history of saline treatment. However, no differences were observed in impulsivity on the Go/No-go task in relation to previous drug history, indicating that 11 day chronic amphetamine did not cause any long term changes in drug induced impulsivity.

Increased Go trial responses latencies amongst amphetamine treated animals during drug treatment indicates that animals became slower in their speed of responding during drug treatment. However, the speed with which amphetamine treated animals collected food reward following correct Go trials was similar to the saline treated animals. Interestingly, amphetamine treated animals also became slower to collect the food reward during No-go trials during drug treatment. Given that the first active response during a correct No-go trial is the collection of the food reward, this change in behaviour may not reflect a change in the motivation for the food reward, but rather a general slowing of responding in amphetamine treated rats during drug treatment. This is supported by results indicating that amphetamine treated animals performed less Go trial early responses at the same time as increased No-go trial magazine latencies were observed (days 7 and 9 of treatment, see Table 5.3 and 5.4). Furthermore, although analysis of Go trial panel responses revealed a significant main effect of treatment group during this phase of the study, with amphetamine treated animals performing more panel responses, closer examination of Table 5.4 indicates that on days 4 and 7 amphetamine treated animals actually performed less panel responses than the saline

treated animals. Therefore, eleven day amphetamine treatment appears to produce a robust increase in impulsive behaviour as indicated by reduced no-go trial performance accuracy, and transiently slows response behaviour, at the same time as reducing anticipatory responding. These data are interesting because increases in impulsive behaviour are often accompanied by faster response speeds and increased response frequency (Harrison et al., 1997; Pattij et al., 1997). The findings in the data therefore indicate that the inability to withhold responding is not directly related to speeding of responding.

General slowing of response behaviour observed during the drug treatment phase of this experiment is consistent with reduced locomotor activity is observed during spontaneous amphetamine withdrawal following contingent and non-contingent chronic (10 days – 6 weeks, respectively) amphetamine administration (Paulson & Robinson, 1996; Pulvirenti & Koob, 1993) and is considered a marker of amphetamine withdrawal (Barr & Markou, 2005). A possible interpretation of slower response behaviour observed during drug treatment might then be that amphetamine treated animals were entering an early phase of drug withdrawal when tested at 12 hours following the preceding amphetamine injection. Interestingly, however, rats tested at 16 hours following 6 hour amphetamine self-administration do not differ in latencies to respond during forced choice trials on an adjusting delay procedure, although variance in latencies was high in this study (Gipson & Bardo 2009).

During withdrawal week one the pattern of behavioural deficits was consistent with that during drug administration. Amphetamine treated animals continued to display lower No-go trial accuracy relative to controls, indicative of a continuation in reduced behavioural inhibition relative into short-term drug withdrawal. Similarly, amphetamine treated animals continued to display slower response speed during Go trials and in collection of food reward during No-go trials. As discussed above, this profile of behaviour coupled with unaffected Go trial magazine latencies suggests that animals were slower in response behaviour whilst also continuing to be motivated to gain food reward. The combination of these results again indicates that despite slowing down in response behaviour and maintained motivation to gain food reward, amphetamine treated animals remained more impulsive relative to controls during withdrawal week one. Furthermore, the similarity in the behavioural profile of amphetamine treated animals during amphetamine treatment and withdrawal week one support the possibility that animals might have entered early drug withdrawal during chronic amphetamine treatment.

Slower speed of responding during amphetamine withdrawal coincides with previous literature reporting slower speed of responding to visual targets by rats in amphetamine withdrawal on the 5CSRTT (Dalley et al., 2005; Dalley et al., 2007b) and literature

demonstrating that rats display reduced locomotor activity during amphetamine withdrawal (Paulson & Robinson, 1996; Pulvirenti & Koob, 1993). Slower response speed during stimulant withdrawal has previously been suggested to represent withdrawal induced deficits on attentional processes (Dalley et al., 2005), which could be suggested to underlay observations of reduced response speed in this study. However, stable performance during Go trials indicates that amphetamine treated animals sustained accuracy in response selection and discrimination of visual targets throughout short term drug withdrawal. During this period the Go trial magazine latencies of amphetamine and saline treated animals were similar indicating that motivational processes were matched between treatment groups. It is unlikely, therefore, that increased Go trial response latencies and No-go trial magazine latencies represent a gross deficit in attention, and further, that such deficits may underlay decreased No-go trial performance.

Amphetamine treated animals also continued to performed less early responses during Go trials relative to controls during withdrawal week one. However, as previously discussed, given that animals became slower to respond during the same phase as these reductions in early responses, it is likely that this reduction is related to the slower response behaviour during Go trials rather than withdrawal induced changes in anticipatory responding relating to impulsivity. Interestingly, the only drug effect that did not continue into withdrawal week one was amphetamine induced increases in Go trial panel responses. The prevalence of this effect solely to drug administration and not withdrawal further supports that the drug induced effect on Go trial panel responses was both mild and transient during drug administration.

Although during most of week one of withdrawal amphetamine treated animals performed significantly less accurately than saline treated animals, as indicated by reduced total percentage of correctly completed trials, analysis of the significant main effect of group identified in the analysis of No-go trial accuracy failed to identify any individual day when the performance of amphetamine treated animals was less than that of saline treated animals. This additional analysis run on drug treatment effects is therefore useful for elucidating the specific time course over which impulsivity was affected by 11-day chronic amphetamine treatment. The significant group effect detected alongside non-significant post hoc tests therefore indicates that whilst impulsivity was transiently elevated amongst amphetamine treated animals relative to controls during amphetamine withdrawal, the difference in impulsivity relative to controls was only slight throughout drug withdrawal in comparison to during drug administration, as demonstrated by the lack of significant between group differences at any specific withdrawal day. Consequently, these findings indicate that the most severe amphetamine induced deficits on impulsivity following 11 day chronic amphetamine

occur during amphetamine administration rather than short-term drug withdrawal. Interestingly, this pattern of results is similar to that reported by (Gipson & Bardo, 2009) that identified greater levels of impulsive choice throughout 21 days of amphetamine self-administration and for three days into amphetamine withdrawal.

The observed elevations in impulsive behaviour during drug treatment are the first to identify that chronic amphetamine can reduce behavioural inhibition in rats during drug administration. These findings therefore provide a new insight into the relationship between chronic amphetamine use and impulsivity. The only other study to measure impulsivity during a long-term amphetamine regime is Gipson & Bardo (2009) that assessed MAD during 21 days of amphetamine self-administration. Within this study animals that both escalated and did not escalate amphetamine self-administration became more impulsive during drug administration Gipson & Bardo (2009). Interestingly this suggests that escalation of amphetamine is not required to affect impulsivity, and in fact maintained self-administration can disrupt impulsivity to a comparable level of animals that escalate drug use. Animal within the Gipson & Bardo (2009) study that displayed a maintained pattern of self-administration also self-administered on average 8- 13 mg/kg amphetamine daily, indicating that the range of daily amphetamine administration was not dissimilar between Gipson & Bardo (2009) and experiment 3a. Whilst the route of administration between Gipson & Bardo (2009) and that employed within experiment 3a are different (contingent vs. non-contingent), and may in itself contribute towards the effects observed in both experiments, the fact that both sets of animals became more impulsive during long-term maintained amphetamine treatment supports that this pattern of chronic amphetamine use can increase impulsive behaviour, and furthermore, that this elevation can occur within distinct dimensions of impulsivity.

Elevated impulsivity during amphetamine administration also relates to previous literature demonstrating increased reflection impulsivity amongst current amphetamine users (Clarke et al., 2006), further supporting a relationship between chronic amphetamine exposure and altered impulse control. However, amphetamine users have also been shown to exhibit comparable levels of perseverative responding to healthy controls, suggesting that some forms of impulse control remain normal following chronic amphetamine exposure (Ersche et al., 2008). The decrease in behavioural inhibition observed in experiment 3a and b therefore expands upon previous literature by identifying alternative dimensions of impulsivity that may be disrupted as a consequence of repeated amphetamine exposure. Furthermore, in consideration of elevated reflection impulsivity amongst amphetamine users (Clark et al., 2006) the reported reduction in behavioural inhibition again suggests that multiple systems mediating inhibitory control may be altered during chronic amphetamine use.

Increased impulsivity during chronic amphetamine treatment also relates to literature reporting reduced behavioural inhibition amongst users of pharmacologically similar drugs such as cocaine (Colzato et al., 2007; Fillmore & Rush, 2002) and MDMA (Morgan, 1998; Quednow et al., 2007), and contributes towards substance abuse literature by clarifying that repeated amphetamine use alone can lead to significant alterations in behavioural control. These findings are useful in consideration of factors underlying the transition from recreational to maintained use of amphetamine, both alone or in combination with other psychostimulant use, by demonstrating that repeated exposure to high dosages of amphetamine per se can reduce behavioural control that may lower inhibitory control over repeated and maintained drug use.

The observed elevation in impulsivity that continued into drug withdrawal are the first to identify that withdrawal from chronic amphetamine can reduce behavioural inhibition in rats. Studies that have previously administered chronic non-contingent amphetamine have found that chronic amphetamine produces no long term effects on impulsive choice (Slezak et al., 2012; Stanis et al., 2008). However, both of these studies employed lower doses to that in experiment 3a and Slezak et al. (2012) did not measure impulsivity through short-term drug withdrawal. In contrast, sub-chronic amphetamine regimes consisting of 5 mg/kg for 5 days have been shown to cause an increase in premature responding within DRL procedures (Peterson, Wolf, & White, 2003) supporting that withdrawal from non-contingent daily administration of amphetamine can reduce behavioural inhibition. In addition (Gipson & Bardo, 2009) also found that amphetamine treated animals remain more impulsive than controls for three days following amphetamine cessation, supporting the prevalence of residual amphetamine induced effects on impulsivity during drug withdrawal.

However, other studies employing long-term (21 day) amphetamine self-administration in rats have found that no changes in impulsivity measured by premature responding during amphetamine withdrawal (Dalley et al., 2007b). Differences in doses administered by animals and the route of drug administration (contingent vs. non-contingent) may account for the observed differences in amphetamine withdrawal effects on impulsivity, however, given that (Gipson & Bardo, 2009) employed a similar self-administration methodology to (Dalley et al., 2007b) and observed increased impulsive choice through acute drug withdrawal, this suggests that differences in withdrawal induced impulsivity observed between (Dalley et al., 2007b) and experiment 3a might relate to differences between the 5CSRTT and Go/No-go symmetrically reinforced paradigm. As previously discussed the 5CSRTT is sensitive to changes in attention capacity. Differences in the effects of amphetamine withdrawal on impulsivity might then be likely to reflect differences in task sensitivity to behavioural inhibition vs. attentional

processes. However, what is consistent between studies that have identified amphetamine withdrawal induced effects on cognitive processes is that these effects are transient during short-term amphetamine withdrawal (Dalley et al., 2005; Dalley et al., 2007b; Gipson & Bardo, 2009).

Further confirmation that amphetamine induced effects on impulsivity were transient was observed by a lack of effect of previous drug history on acute amphetamine challenges within performance measures of behavioural inhibition. Amphetamine treated animals did show a slight increase in sensitivity to amphetamine induced reductions of Go trial performance accuracy at 1.2 mg/kg amphetamine relative to controls, suggesting that chronic amphetamine leads to some residual changes in sensitivity to the disruptive performance effects by amphetamine. Although analysis of No-go trial panel responses revealed a main effect of previous drug history, there was no interaction with amphetamine dose. Thus animals previously treated with saline performed more no-go trial with panel responses than amphetamine treated animals at all doses including 0 mg/kg amphetamine. Therefore this result does not indicate differential sensitivity to amphetamine challenges between the two groups of animals. In addition, due to the fact no other between group differences were observed within any other variable, and no difference in No-go trial accuracy was observed in relation to previous amphetamine exposure, this indicates that previous amphetamine exposure did not lead to any substantial change in response to amphetamine induced effects on the Go/No-go symmetrically reinforced paradigm. Furthermore, the lack of difference between drug treatment groups during No-go trials suggests that no long term neural adaptations occurred in amphetamine treated animals that might render these animals more or less sensitive to the disinhibitory effects of amphetamine. These effects are consistent with previous literature reporting that chronic amphetamine treatment does not produce any long-term sensitivity or tolerance to amphetamine induced impulsivity (Slezak et al., 2012; Stanis et al., 2008).

5.4.1 Key Findings

The main findings from this study are that 11-day amphetamine treatment reduces response inhibition in rats during drug treatment and short-term drug withdrawal. These findings suggest that a chronic level of amphetamine exposure might directly reduce response inhibition during phases where drug use is maintained (chronic treatment) and vulnerability to relapse is high (short-term withdrawal). However, response inhibition returned to comparable levels in withdrawal week two and no changes in sensitivity to drug induced response disinhibition was observed in rats previously exposed to chronic amphetamine relative to

saline treated animals. This suggests that chronic amphetamine treatment did not lead to any longer term changes in response inhibition or neural systems mediating response inhibition. The main objective of this study was to assess how chronic amphetamine treatment affects response inhibition in rats, with a broader objective of disentangling the relationship between amphetamine use and impulsivity. Evidence that chronic amphetamine can impinge on response inhibition consequently helps to disentangle this relationship by, firstly, confirming that chronic amphetamine exposure can directly increases impulsive responding, and secondly, indicating that these drug induced effects on impulsivity may occur at specific time periods during which heightened impulsivity can contribute towards maintaining drug use and relapse.

5.4.2 Limitations

As discussed in Chapter 4, section 4.4.2, page 121, a limitation of using non-contingent drug administration is that there is less scalability to human drug use. The effects of non-contingent chronic amphetamine in rats on response inhibition may not reflect the effects of chronic voluntary administration of amphetamine in human drug users on response inhibition. In addition, amphetamine was administered in a high and frequent dose pattern. Amphetamine is typically binged amongst human users and is unlikely to be abused in a frequent pattern similar to that employed in the current study. The main findings from this study suggesting that chronic amphetamine use affects response inhibition should therefore be treated with caution when considering what the effects of human voluntary drug use and patterns of human drug use might be on impulsivity.

Similar to that discussed in Chapter 4, section 4.4.2, page 121, this study did not screen rats for high and low levels of trait impulsivity at baseline. This means that any possible effects of individual differences on amphetamine on response inhibition, and the time points at which this effect was prevalent, cannot be inferred. Consequently, this study does not account for how individual differences in rats might have been involved in the effects of amphetamine observed on impulsivity. In addition, this study did not employ neuroanatomical or neurochemical control groups that would have helped elucidate the possible neurobiological changes involved in chronic amphetamine induced impulsivity observed. It would have been useful to employ an additional lesion group or drug group to account for possible neural sites and neurochemical changes mediating chronic amphetamine induced impulsivity (see Chapter 4, section 4.4.2, page 121).

Finally, it should also be noted that rats were not randomly assigned to drug treatment groups. Rats were assigned to treatment groups based on their overall accuracy of performance on the Go/No-go task at baseline. This was done in order to counterbalance baseline differences in the accuracy of performance between drug treatment groups. However, through assigning rats in this manner, bias may have been introduced to drug treatment groups.

5.4.3 Future Research

In order to address the limitation of the scalability to human drug use, future research investigating the effects of chronic amphetamine self-administration on impulsivity measured by the Go/No-go symmetrically task would be useful. This would help determine whether there is a difference in the effects of contingent and non-contingent chronic amphetamine administration on impulsivity measured by the Go/No-go symmetrically reinforced task. Such investigations would help elucidate whether high dose and frequency amphetamine exposure per se affects response inhibition, regardless of whether this is voluntary or not, and would subsequently help elucidate what the consequence of chronic amphetamine use might be on impulsivity amongst human populations.

It would also be useful to determine whether the effects of chronic amphetamine on response inhibition are related to individual differences in impulsive behaviour. In order to do this a screening method that identifies high and low levels of trait impulsivity on the Go/No-go symmetrically reinforced task would need to be developed. Splitting animals into these groups would help to establish whether particular groups within the general population (high and low impulsivity) are differentially affected by chronic drug exposure. In addition, future research aimed at elucidating the neurochemical changes involved in chronic amphetamine induced impulsivity on the Go/No-go task will be useful for establishing the neurochemical pathology of impulsivity that is a direct consequence of long-term drug exposure. As there are indications that changes in ventral striatal D_2 receptors are linked to impulsivity observed in drug addicts, it would be useful to explore D_2 receptor mediation of chronic amphetamine induced impulsivity on the Go/No-go symmetrically reinforced task via intra-nucleus accumbens infusions of D_2 receptor ligands alongside chronic amphetamine treatment. This would help to elucidate how possible drug induced changes in key sub-receptors and neural sites associated with impulsivity amongst addicts might develop as a consequence of chronic drug use.

5.4.4 Conclusions

In conclusion, the observed elevations in impulsivity during chronic amphetamine treatment and withdrawal extend upon previous animal and human literature investigating the relationship between amphetamine use and impulsivity by identifying that chronic amphetamine use can cause a transient reduction in behavioural inhibition both during drug use and short-term drug withdrawal. Interestingly, elevated impulsivity was most pronounced during chronic amphetamine treatment, consistent with previous studies identifying chronic amphetamine induced impulsivity (Gipson & Bardo, 2009). This suggests that along with phases of drug withdrawal following complete drug cessation that act as periods of heightened vulnerability to relapse, drug induced changes in inhibitory control during drug use, possibly during phases of early withdrawal, might also constitute a period of significant vulnerability to repeated drug use as a consequence of drug induced reductions in behavioural control. Such elevations in behavioural disinhibition during drug use might then increase the chance of escalating drug use during maintained use, or alternatively might contribute towards maintaining drug use through lowering behavioural control during chronic maintained drug use.

Chapter 6 The effects of Dopamine D2 receptor antagonism within the NAcb core on behavioural inhibition and amphetamine induced behavioural disinhibition in rats

6.1 Introduction

Stimulant users display higher levels of impulsivity than controls (Fillmore & Rush, 2002; Hoffman et al., 2006; Lee et al., 2009; Monterosso et al., 2005). Alongside this elevation in impulsivity they also show changes in the structure of fronto-striatal regions associated with impulsivity (Ersche et al., 2012) and changes in dopamine function and D_2 receptor availability in the striatum(Martinez et al., 2004, 2009; Nora D Volkow et al., 1993; Nora D Volkow, Chang, Wang, Fowler, Ding, et al., 2001). Changes in D_2 receptor availability in the ventral striatum also correlates with trait impulsivity in methamphetamine users (Lee et al., 2009) linking ventral striatal D_2 receptors directly with elevated impulsivity in observed amongst stimulant users. Collectively these findings have led to the recent proposition that the D_2 receptors within the striatum are a biomarker of impulsivity in drug addiction (Trifilieff & Martinez, 2013).

Investigations into the role of the dopamine sub-receptors in impulsivity and stimulant induced impulsivity in animal models have found contrasting involvement of the D_1 and D_2 sub-receptors that appear to depend on the dimension of impulsivity measured. Systemic administration of the $D_{1/2}$ receptor antagonist *cis*-flupenthixol does not affect modafinil or MPH induced reductions in SSRT (Eagle et al., 2007) suggesting that D_1 and D_2 receptors are not involved in stimulant induced changes in behavioural inhibition in the form of action cancellation ('stopping'). However, the D_1 and D_2 receptors have been associated with behavioural inhibition in the form of 'waiting' behaviour. Systemic administration of the D_1 receptor antagonist SCH 23390 reduces premature responding on the 5CSRTT and increases delay discounting within a DRT when administered alone, but does not reverse amphetamine induced premature responding or reductions in delay discounting when administered in combination with amphetamine (van Gaalen, et al., 2006; van Gaalen et al., 2006b). In contrast, systemic administration of the D_2 receptor antagonist eticlopride does not produce any changes in premature responses when administered alone, but does reverse amphetamine, cocaine and nicotine induced premature responding on the 5CSRTT when administered in combination with these stimulants (van Gaalen et al., 2006) and attenuated amphetamine induced reductions in impulsive choice on a DRT (van Gaalen et al., 2006b).

These findings therefore suggest a specific role for the D_1 receptors in the mediation of impulsivity, and the D_2 receptors in the mediation of stimulant induced changes in impulsivity on the 5CSRTT and DRT.

However, an opposing pattern of results has been observed when impulsivity is measured using a symmetrically reinforced Go/No-go task. Acute amphetamine and nicotine both reduce No-go trial accuracy on the Go/No-go task indicating increased behavioural disinhibition similar to that observed in the 5CSRTT (Kolokotroni et al., 2011; Harrison et al., In prep). However, in contrast to the results observed on the 5CSRTT, systemic SCH 23390 does not affect No-go trial accuracy when administered alone, but does reverse amphetamine and nicotine reductions in No-go trial accuracy (Harrison et al., In prep). In contrast, systemic administration of eticlopride impairs active responding on the Go/No-go task and consequently produces unclear effects on impulsivity when combined with amphetamine (Harrison et al., In prep).

The differences in results between the 5CSRTT and the Go/No-go symmetrically reinforced task present a contrasting picture of D_1 vs D_2 involvement in behavioural inhibition considering that both of these tasks attempt to measure the same 'waiting' dimension of behavioural inhibition. The differences in D_1 and D_2 receptor mediation of impulsivity and drug induced impulsivity may relate to differences in the reinforcement of behavioural inhibition between the 5CSRTT and the symmetrically reinforced Go/No-go task, and in following, the motivational significance of behavioural inhibition between these two tasks. Rats that receive systemic SCH 23390 before performing the 5CSRTT produce reductions in premature responses alongside increases in omissions on this task (van Gaalen et al., 2006) indicating more extensive disruption to performance of the task that might be indicative of motivational changes following D_1 receptor antagonism. In contrast, administration of SCH 23390 on the symmetrically reinforced Go/No-go task produces no change in magazine latencies or omissions, indicating that no changes in motivation occur following D_1 antagonism on this task (Harrison et al., In prep). This suggests that SCH 23390 induced reductions in premature responding on the 5CSRTT might reflect motivational changes in rats that were only detected on the 5CSRTT, and not the Go/No-go task, possibly because the outcome of behavioural inhibition is less motivationally significant on the 5CSRTT than the Go/No-go task. In following, SCH 23390 reversal of amphetamine induced impulsivity on the Go/No-go task suggests that the D_1 receptors are involved in stimulant induced behavioural inhibition when this behaviour is more directly associated with reinforcement.

In contrast, systemic eticlopride produces general performance and motivation deficits on the Go/No-go task that are not observed following systemic eticlopride on the 5CSRTT (Harrison et al., In prep; van Gaalen et al., 2006; Pattij et al., 2007), suggesting the converse to that observed with the D_1 receptors, that D_2 meditated reversal of amphetamine induced impulsivity on the 5CSRTT might only be prevalent when there is less motivational significance to behavioural inhibition. Given that systemic administration of D_1 and D_2 antagonists, including SCH 23390 and eticlopride, can lower motivation to earn food reward (Salamone et al., 2002; Sink et al., 2008), such differences in the reinforcement value of behavioural inhibition between the 5CSRTT and Go/No-go task might be particularly important in dictating the effects of D_1 and D_2 receptor antagonism on behavioural inhibition in these tasks. Collectively, systemic investigations into the involvement of dopamine sub-receptors in impulsivity within animal models have identified distinct roles for the D_1 and D_2 receptors in impulsivity and drug induced impulsivity, however the specific role of these sub-receptors might relate to differences in the performance and motivational parameters of behavioural inhibition set out by the task employed.

Central investigations into the role of the D_1 and D_2 receptors in impulsivity and drug induced impulsivity have also been conducted through infusions of SCH 23390 and eticlopride into the NAcb core and shell alone and in combination with amphetamine. Similar to systemic SCH 23390 administration on the 5CSRTT, infusions of SCH 23390 into the NAcb core and shell reduces premature responding when administered alone, but does not fully reverse amphetamine induced premature responding when infused into either sites in combination with systemic amphetamine (Pattij et al., 2007).

In contrast, infusions of eticlopride alone into the NAcb core and shell do not affect premature responding on the 5CSRTT, but eticlopride infusions into the NAcb core in combination with systemic amphetamine produces full reversal of amphetamine induced premature responding, an effect that is not observed following eticlopride infusions into the shell in combination with systemic amphetamine (Pattij et al., 2007). However, eticlopride mediated reversal of amphetamine induced premature responding on the 5CSRRT was also reported alongside eticlopride mediated reversal of amphetamine induced deficits in accuracy and faster response latencies (Pattij et al., 2007) indicating extensive changes in performance on 5CSRTT that might contribute towards the observation of eticlopride mediated reversal of amphetamine induced impulsivity on this task. In addition, when eticlopride is infused into the NAcb core alone, a significant increase in omissions and response latencies has been reported (Pattij et al., 2007), suggesting the possibility of additive changes in motivation that might account for eticlopride induced reversal of amphetamine induced impulsivity on the 5CSRTT.

Central investigations into the role of the dopamine sub-receptors in mediating impulsivity and drug induced impulsivity has therefore, similar to systemic studies, indicated distinct roles for the D_1 and D_2 receptors, with a specific role for the D_2 receptors within the NAcb core in mediating amphetamine induced impulsivity. However, there also continues to be indications from central investigations that treatment induced changes in premature responding observed on the 5CSRTT might relate to changes in attention or motivation processes. This further indicates that differences in the reinforcement parameters of the behavioural inhibition might be significant to the effects of D_1 and D_2 antagonists on impulsivity observed.

In addition to evidence that the D_1 and D_2 receptors have distinct roles in relation to impulsivity, the NAcb core and shell have also been shown to have distinct functions in relation to drug induced impulsivity. Lesions to the NAcb core and shell have been shown to produce opponent effects on amphetamine induced impulsivity, with core lesions potentiating and shell lesions attenuating amphetamine induced premature responding in rats (Murphy et al., 2008). These findings, along with the evidence that lesions to the NAcb core lesions disrupt processing of effortful and adaptive behavioural output (Galtress & Kirkpatrick, 2010; Ghods-Sharifi & Floresco, 2010) whilst NAcb shell lesions disrupt the processing of acute drug reinforcement and hyperactivity (Parkinson et al., 1999), support a role for the NAcb core in processing inhibited behaviour and the NAcb shell in processing disinhibited behaviour.

In summary, a large amount of evidence has implicated changes in D_2 receptor availability in the striatum with stimulant use and impulsivity (Lee et al., 2009; Martinez et al., 2004, 2009; Volkow et al., 1993; Volkow et al., 2001). Evidence from animal models to date have also indicated a specific role for the D_2 receptors within the NAcb core in mediating amphetamine induced impulsivity (Pattij et al., 2007) and the NAcb core in processing inhibitory control over amphetamine induced impulsivity (Murphy et al., 2008). However, these effects have only been observed in the 5CSRTT, and given the differences observed between systemic D_1 and D_2 receptor antagonism on impulsivity measured by the 5CSRTT and Go/No-go task discussed, it is possible that the involvement of the D_2 receptor within the NAcb core in impulsivity and amphetamine induced impulsivity might relate to the reinforcement parameters of behavioural inhibition employed. In following, the current chapter sought to investigate the role of the D_2 receptors within the NAcb core on behavioural inhibition and amphetamine induced behavioural inhibition using a symmetrically reinforced Go/No-go task.

6.1.1 Objectives

In order to investigate the role of the D_2 receptors within the NAcb core in impulsivity and amphetamine induced impulsivity on the Go/No-go symmetrically reinforced task, the objectives were:

i) To examine the effects of D_2 receptor antagonism within the NAcb core on behavioural inhibition measured by the Go/No-go symmetrically reinforced task (Experiment 4)

ii) To examine the effects of D_2 receptor antagonism within the NAcb core on amphetamine induced behavioural disinhibition measured by the Go/No-go symmetrically reinforced paradigm (Experiment 5)

6.2 Methods

6.2.1 Subjects

17 male Lister Hooded rats (Charles River, UK) were housed in pairs upon arrival into Leeds Behavioural Neuroscience Laboratory and maintained under a 12 hour light/dark cycle (lights on 0700 hours), in a temperature (21°C ± 2°C) and humidity (50% ± 5%) controlled environment. Throughout behavioural experimentation animals were placed on a food restriction schedule of 18.6g per day, maintaining animals above 85% of their adult free feeding body weight. Water was available ad libitum in home cages and feeding took place in the morning after behavioural testing. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996. All procedures were covered by Home Office Project Licence No. PIL 40/3606 and Home Office Personal Licence No. 40/1989.

6.2.2 Drugs

D-Amphetamine sulphate (Sigma Aldrich, UK) was dissolved in 0.9% physiological saline solution and was administered intraperioneally (i.p.) in a volume of 1 ml/kg body weight. S-(-)-Eticlopride hydrochloride (Sigma Aldrich, UK) was dissolved in a vehicle of 0.9% NaCl and was administered via intracerebral infusion into the NAcb core in a volume of 0.5 µl per hemisphere. Both amphetamine and eticlopride were weighed out and prepared in solution on the morning of test sessions. Eticlopride was occasionally stored over night at a temperature of 4°c. Stored eticlopride was used within 3 days (Sigma-Aldrich UK guidelines). The dosages employed in experiments 4 and 5 were selected from previous literature eg. (Pattij et al., 2007).

6.2.3 Apparatus

Behavioural testing took place in eight aluminium operant chambers (30.5 x 24.1 x 21 cm, Med Associates Inc., USA) placed inside sound attenuating and ventilated cubicles (63.5 x 49.1 x 39.4 cm, Med Associates Inc., USA). Chambers were controlled, and all data was recorded from chambers, using MED-PC IV software (Med Associates Inc., USA). Administration of intracerebral drug infusions was performed using an infusion pump (KDS101 Infusion pump, KD Scientific Inc., USA). For a more detailed description of test chambers refer to Chapter 2 section 2.1.3, page 60.

6.2.4 Behavioural Testing

Behavioural disinhibition was assessed via the symmetrically reinforced go/no-go visual discrimination paradigm. Behavioural training took place for approximately 8 weeks until animals reached stable performance of 85% total percent correct (+/-5%). Following stable baseline performance animals were entered into the experiment. All behavioural training and testing took place between 0700-1030 hours during the light phase of animals Light/Dark cycle. For a detailed outline of the task refer to Chapter 2 section 2.1.4, page 61.

6.2.5 Surgery

Once behavioural performance was stable at the minimum criteria of 85% total percent correct (+/-5%), animals receiving surgery were removed from daily test sessions and placed on a free-feeding schedule for a minimum of 3 days prior to receiving surgery and approximately 12 hours before surgery all food was removed. Rats were anaesthetised using a combination of 4% isoflurane gas and oxygen (flow rate 3 l/min) before being placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) continuing to deliver 4% isoflurane gas and oxygen. Rats were secured into flat skull position via ear bars and a nose clamp with the incisor bar set to -3.3 - -5.0, depending on flat skull measurements from each rat. 22 gauge bilateral guide cannulae (centre-to-centre distance between each cannula: 3.8 mm; pedestal height: 10.41 mm; stainless steel length below pedestal: 7 mm; pedestal diameter: 6.12 mm; Plastics One Inc., USA) were positioned 2 mm above the NAcb core via the following stereotaxic coordinates: +1.5 mm anterior to bregma (AP), +/- 1.9 mm lateral to midline (ML), -5.2 mm ventral to skull surface (DV) calculated from Paxinos and Watson (1998). Co-ordinates were selected via a literature review identifying studies targeting the NAcb core in the administration of intracerebral drug infusions. Cannulas were fixed to the skull via dental cement (Kemdent Dental Cement, Associated Dental Products Ltd, UK) and three stainless steel screws (Plastics One Inc., USA) drilled posterior to the cannula. 29 gauge stylets extending 1mm below the guide cannula and sitting 1 mm above the NAcb core were inserted following secure placement of the guide cannula to the skull. Animals subsequently remained in their home cages on a free-feeding schedule for a minimum of 7 days post surgery. Following the demonstration of surgical recovery via weight gain and healing of the scalp wound surrounding the guide cannulae, animals were reintroduced to their original cage mate and were re-housed in pairs. On occasion animals continued to be singly housed following surgery due to failure to re-habituate to their cage mates. Following surgical recovery, animals were placed back on a food restriction schedule of 18.6g per day and behavioural training recommenced.

6.2.6 Microinfusion Procedure

Eticlopride dosages were infused in a volume of 0.5 µl/ side over a period of 30s (rate 1µl/min) via a 25µl Hamilton syringe driven by an infusion pump. To infuse, stylets were removed from bilateral guide cannulae and a 28 gauge bilateral internal injector (centre-to-centre distance, 3.8 mm; injector length from top of pedestal, 11 mm; Plastics One Inc., Roanoke, VA, USA) extending 2mm ventral to the tip of the guide cannulae was inserted delivering eticlopride into the NAcb core. Injectors were inserted 1 minute pre-drug infusion and were left in place for a further 1 minute post-drug infusion to allow for the diffusion of drug solutions. After drug infusions, stylets were replaced into the guide cannula. This infusion procedure was based on previous literature administering dopamine antagonists into the NAcb core (Besson et al., 2009; Pattij et al., 2007).

6.2.7 Design and Procedure

Following recovery from surgery and the return of stable baseline task performance, animals were assigned to either experiment 4 or 5. These groups were counterbalanced according to their baseline performance accuracy, programmed levers, and stimulus-response contingencies. A within subject design was employed in both experiment 4 and 5.

6.2.7.1 Experiment 4: The effects of eticlopride infusions into the NAcb core on behavioural disinhibition

Animals (N=9) received intracerebral (i.c.) infusions of (0 µg, 0.1 µg, 0.3 µg and 1.0 µg/ 0.5 µl/ side) eticlopride into the NAcb core 10 minutes before behavioural testing according to a Latin square design, with a minimum of 72 hours between each drug infusion to encourage site preservation between intracerebral injections. Prior to all drug treatments animal's task performance had returned to post-surgery baseline levels.

6.2.7.2 Experiment 5: The effects of eticlopride pre-treatment into the NAcb core on amphetamine induced behavioural inhibition

Animals (N=9) received the following combination treatments of eticlopride (i.c., 10 minutes prior to testing) immediately followed by amphetamine (i.p.); 0 µg + 0 mg/kg, 0 µg + 0.8 mg/kg, 0.1 µg + 0.8 mg/kg, 0.3 µg + 0.8 mg/kg, 1.0 µg/ 0.5 µl + 0.8 mg/kg. A minimum of 72 hours was employed between each drug infusion to encourage site preservation between intracerebral injections and to allow for a 3 day wash out period of amphetamine between

subsequent amphetamine administrations. Animals were also required to re-stabilise behavioural performance between intracerebral infusions to a criteria of +/-10% of their average baseline performance following surgery. All drug treatments were administered in a latin-square design.

6.2.8 Assessment of Cannulae Placement

Following completion of experimental procedures animals were administered i.p. with a lethal dose of pentobaribital sodium solution (Animal Health Ltd, Essex, UK). Conscious responsiveness was checked via assessment of the pedal withdrawal and corneal reflex. Upon termination of respiration, the heart was perfused via injection of 100ml of 0.9% NaCl into the left ventricle of the heart, followed by 400ml of 4% paraformaldehyde (Sigma Aldrich, UK) in a 0.2M phosphate buffer. The brain was immediately removed and stored in 4% paraformaldehyde solution at 4°C. Brains were transferred into 25% sucrose solution 48 hours before slicing. Rat brains were sliced in 40 µm coronal sections using a cryostat (Bright OTF 5000; Bright Instruments, Cambridge, UK). Slices were subsequently mounted onto microscope slides and stained using working cresyl violet solution to determine cannula placements.

6.2.9 Statistical Analysis

All data was initially checked for normality through Shaprio-Wilk tests and was appropriately transformed via arcsine and $\log_{10}$ transformations following violations to normality. Homogeneity of variance was checked via Mauchley's test of Sphericity and following any significant violation of equal variances, degrees of freedom in the GLM were adjusted using the Greenhouse- Geisser correction. All data were analysed via one-way repeated measures ANOVAs with 'drug dose' entered as the within-subjects factor. The effects of eticlopride infusions into the NAcb core on behavioural inhibition was assessed via 1 X 4 repeated measures ANOVAs across all behavioural parameters. The effects of eticlopride pre-treatment within the NAcb core on amphetamine induced behavioural disinhibition were assessed via 1 X 5 repeated measures ANOVAs. Significant main effects of drug treatment were further analysed using Sidak corrected post-hoc analyses. Unless otherwise stated $\alpha < 0.05$ was employed across all statistical analysis.

6.3 Results

After checks of normality, the following transformations were performed*: Experiment 4*: Log_{10} (No-go trial incorrect latency, No-go magazine latency; Go trial panel responses); *Experiment 5*: Log_{10} (No-go trial incorrect latency, No-go trial magazine latency, No-go trial early responses; Go trial panel responses). Due to Go trial accuracy remaining abnormally distributed following transformations within experiment 5, the total percentage correct of Go trials within experiment 5 was analysed using a non-parametric Friedman Test.

6.3.1 Histology

17 animals completed behavioural testing and were perfused (n = 8 eticlopride; n = 9 eticlopride and amphetamine). Animals with one injector tip located minimally dorsal to the NAcb core border and one injector tip hitting the NAcb core were included in analysis. Animals with one or both injector tips located ventral to the NAcb core boarder, and therefore hitting the NAcb shell, were removed from analysis. After visual inspection of cannula placements, one animal was removed from the analysis of experiment 4 due to both the left and right hemisphere injector tips hitting the NAcb shell, and one animal was removed from experiment 5 due to the right hemisphere injector tip hitting the NAcb shell. Subsequently, data from 15 animals was used in statistical analysis (n = 7 experiment 4; n = 8 experiment 5). Figure 6.1 shows the location of injector tips amongst animals treated with eticlopride (experiment 4), and eticlopride in combination with systemic amphetamine (experiment 5). Photographs showing the location of injector tips from an animal in experiment 4 and an animal in experiment 5 are shown in Figures 6.2 - 6.5. For photographs showing the location of injector tips for each animal in experiments 4 and 5, see Appendices 3 and 4.

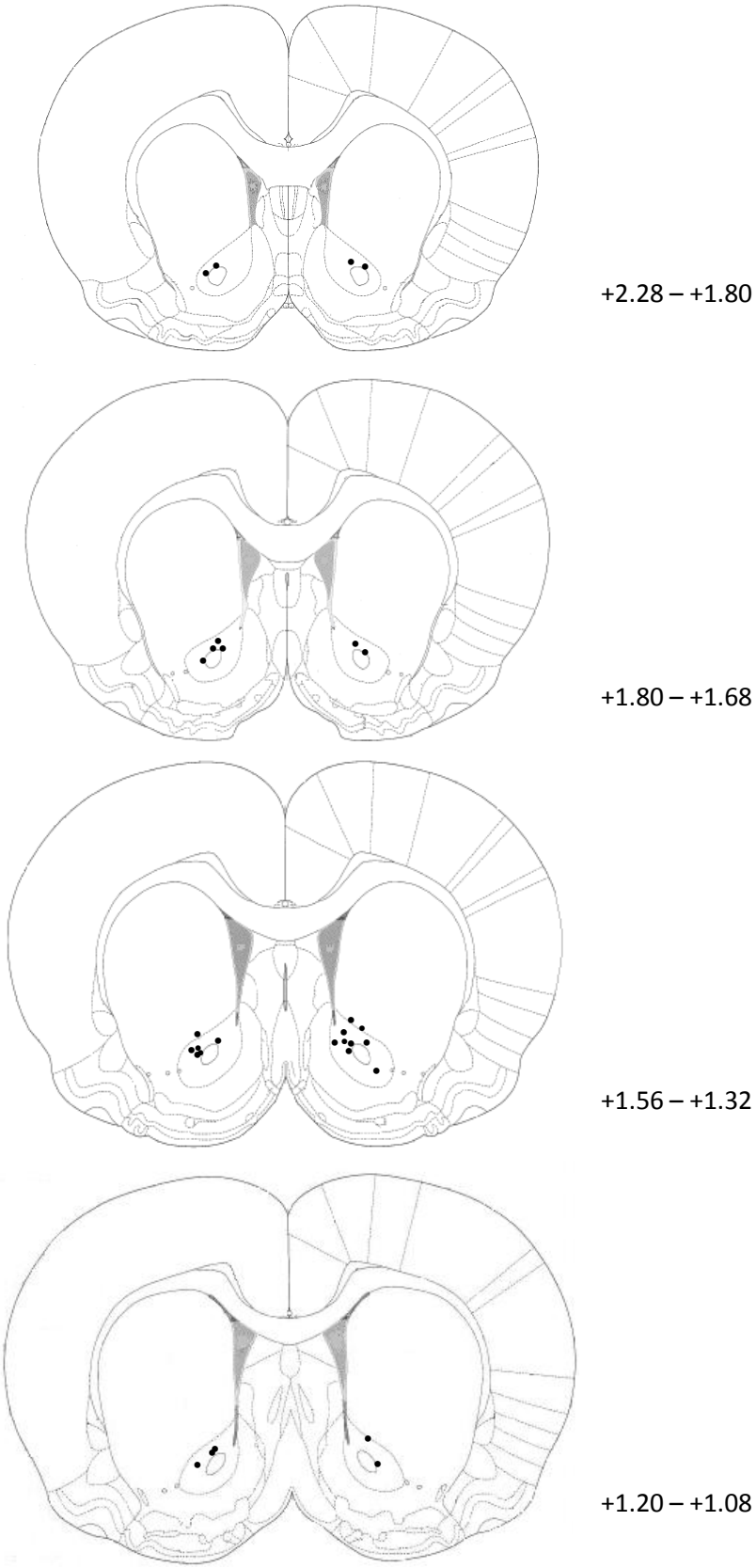


Fig. 6.1: Schematic illustrations of the rat brain taken from Paxinos and Watson Atlas (1998) demonstrating the location of injector tips relative to bregma in experiments 4 and 5.

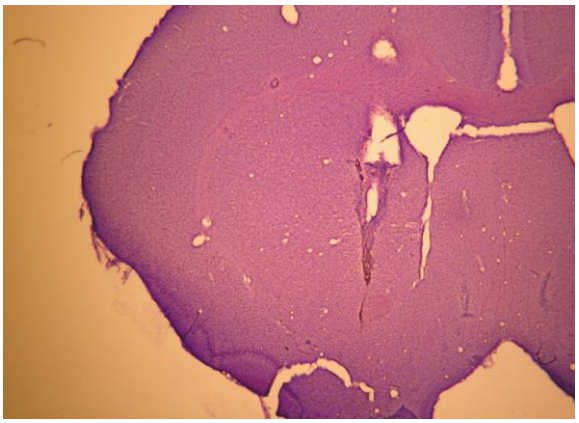


Fig. 6.2

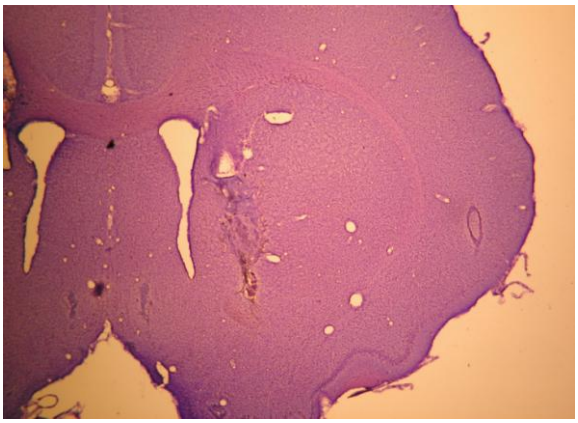


Fig. 6.3

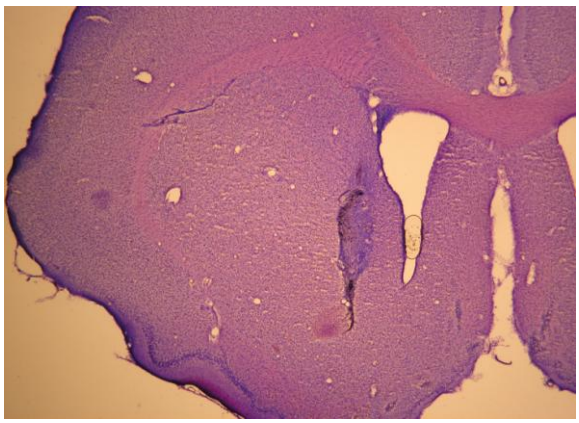


Fig. 6.4

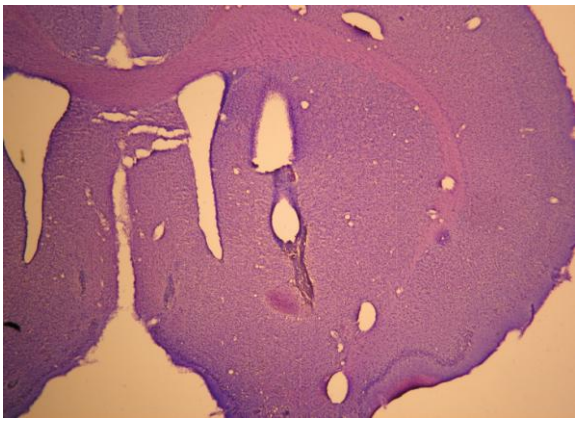


Fig. 6.5

Figures 6.2-6.5: Example pictures of brain slices taken during histology to confirm the location of injector tips in the Nucleus Accumbens core. Pictures are taken from an animal treated with eticlopride (**6.2**: left hemisphere; **6.3**: right hemisphere), and an animal treated with eticlopride and systemic amphetamine (**6.4**: left hemisphere; **6.5**: right hemisphere).

6.3.2 Experiment 4: The effects of eticlopride infusions into the NAcb core on behavioural inhibition

6.3.2.1 Performance Accuracy

Accuracy was not affected by eticlopride infusions. The total percentage of correctly completed trials ($F(3, 18) = 0.263$, NS), total percentage of correct Go trials ($F(3, 18) = 0.980$, NS) and the total percentage of correct No-go trials ($F(3, 18) = 0.423$, NS) did not significantly differ in comparison to the saline control following eticlopride infusions into the NAcb core (Figs. 6.6 – 6.8).

6.3.2.2 Speed of Responding

The speed of responding was also not affected by eticlopride infusions. Go trial latency ($F(3, 18) = 1.465$, NS), No-go trial incorrect response latency ($F(3, 18) = 0.302$, NS), Go trial magazine latency ($F(1.273, 7.738) = 0.902$, NS) and No-go trial magazine latency ($F(3, 18) = 2.675$, NS) did not significantly differ in comparison to the saline control following infusions of eticlopride into the NAcb core (Figs. 6.12 – 6.15).

6.3.2.3 Anticipatory Responding

Anticipatory responding was also not affected by eticlopride infusions. Go trial early responses ($F(3, 18) = 2.644$, NS), No-go trial early responses ($F(3, 18) = 0.292$, NS), Go trial inappropriate panel responses ($F(3, 18) = 1.961$, NS) and No-go trial inappropriate panel responses ($F(3, 18) =$ 1,961, NS) did not significantly differ in comparison to the saline control following eticlopride infusions into the NAcb core (Figs. 6.20 – 6.23).

6.3.3 Experiment 5: The effects of eticlopride pre-treatment into the NAcb core on systemic amphetamine induced behavioural disinhibition

6.3.3.1 Performance Accuracy

Eticlopride and systemic amphetamine produced to a main treatment effect in the total percentage correct of trials ($F(4, 28) = 10.779$, $p < 0.01$). Post hoc analysis of this main effect revealed a significant reduction in the total percentage of correctly completed trials following vehicle infusion + systemic amphetamine ($p = 0.002$) and 0.1 µg eticlopride + systemic amphetamine ($p = 0.038$) in comparison to vehicle infusion + systemic saline. A significant increase in the total percentage of correctly completed trials was found following 1.0 µg eticlopride + systemic amphetamine in comparison to vehicle infusion + systemic amphetamine ($p = 0.020$) (Fig. 6.9). A trend towards a significant increase in the total percent correct was detected following 0.3 µg eticlopride + systemic amphetamine in comparison to vehicle infusion + systemic amphetamine ($p = 0.075$). There was no significant difference between the percentage of correctly completed trials following administration of either 1.0 µg eticlopride + systemic amphetamine, or 0.3 µg eticlopride + systemic amphetamine ($p > 0.05$), in comparison to vehicle infusion + systemic saline (Fig. 6.9). Analysis of Go trials revealed no main effect of eticlopride and amphetamine treatment ($\chi(4) = 5.818$, NS) (Fig. 6.10). Analysis of No-go trials produced a main effect of eticlopride and amphetamine treatment ($F(4, 28) =$ 10.228, $p < 0.001$). Post hoc analysis of this main effect revealed a reduction in No-go trial

accuracy following vehicle infusion + systemic amphetamine in comparison to vehicle infusion + systemic saline ($p = 0.003$), and an increase in No-go trial accuracy following 1.0 µg eticlopride + systemic amphetamine in comparison to the vehicle infusion + systemic amphetamine ($p = 0.014$) (Fig. 6.11). A non-significant trend towards reduced No-go trial accuracy was found following 0.1 µg eticlopride + systemic amphetamine in comparison to the vehicle infusion + systemic saline ($p = 0.057$), and a non-significant trend towards increased No-go trial accuracy was found following 0.3 µg eticlopride + systemic amphetamine in comparison to the vehicle infusion + systemic amphetamine ($p = 0.076$) (Fig 6.11).

6.3.3.2 Speed of Responding

A main effect of eticlopride + systemic amphetamine treatment was found in Go trial response latencies ($F(4, 28) = 2.972$, $p < 0.05$), however, post hoc tests failed to detect any significant difference in response speed during Go trials between specific combination dosages (all $p > 0.05$) (Fig. 6.16). No-go trial response latencies did not differ following eticlopride + amphetamine combinations in comparison to the vehicle infusion + systemic saline combination ($F(4, 28) = 1.144$, NS) (Fig. 6.17). Go trial ($F(4, 28) = 0.683$, NS) and No-go trial magazine latencies ($F(4, 28) = 1.084$, NS) were also unaffected by eticlopride + systemic amphetamine treatment (Figs. 6.18 and 6.19).

6.3.3.3 Anticipatory Responding

Analysis of Go trial early responses produced a main effect of eticlopride + systemic amphetamine treatment ($F(4, 28) = 3.131$, $p < 0.05$), however, post hoc tests only detected a trend towards a significant increase in Go trial early responses following the vehicle infusion + systemic amphetamine in comparison to the vehicle infusion + systemic saline ($p = 0.09$) (Fig 6.24). Analysis of No-go trial early responses also produced a main effect of drug treatment ($F(4, 28) = 9.313$, $p < 0.001$). Post hoc analysis revealed that animals made significantly more early responses within No-go trials following either the vehicle infusion + systemic amphetamine ($p = 0.003$) or the 0.1µg eticlopride + systemic amphetamine ($p = 0.012$) treatment in comparison to the vehicle infusion + systemic saline. A trend towards a significant reduction in No-go trial early responses was detected following 1.0 µg eticlopride + systemic amphetamine in comparison to vehicle infusion + systemic amphetamine ($p = 0.085$). No-go trial early responses did not significantly differ between 1.0 µg eticlopride + systemic amphetamine and the vehicle infusion + systemic saline combination ($p > 0.05$) (Fig. 6.25). Go trial ($F(4, 28) = 2.223$, NS) and No-go trial inappropriate panel responses were unaffected by the eticlopride + systemic amphetamine treatments ($F(4, 28) = 1.338$, NS) (Figs. 6.26 and 6.27).

Eticlopride

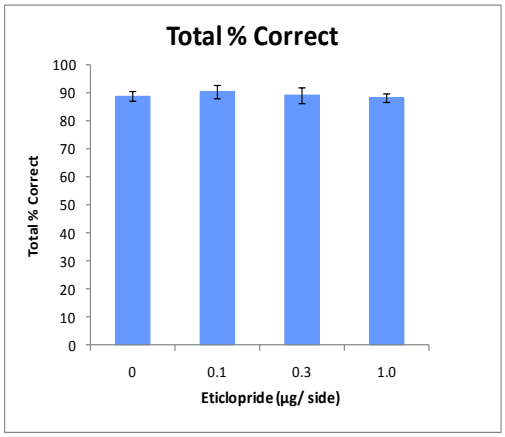


Fig. 6.6

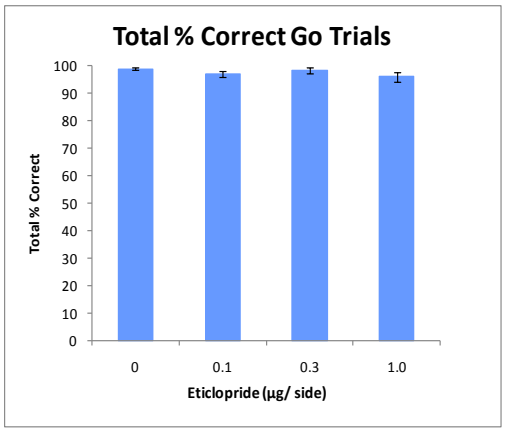


Fig. 6.7

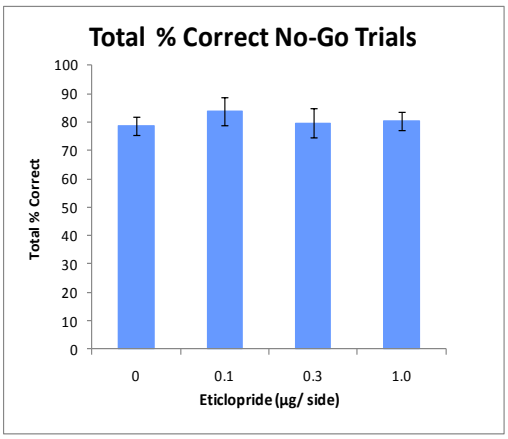


Fig. 6.8

Eticlopride + Amphetamine

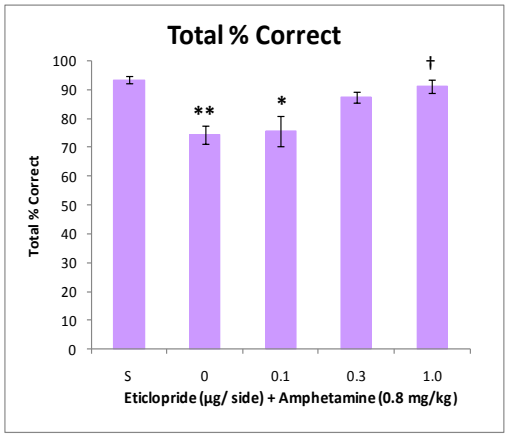


Fig. 6.9

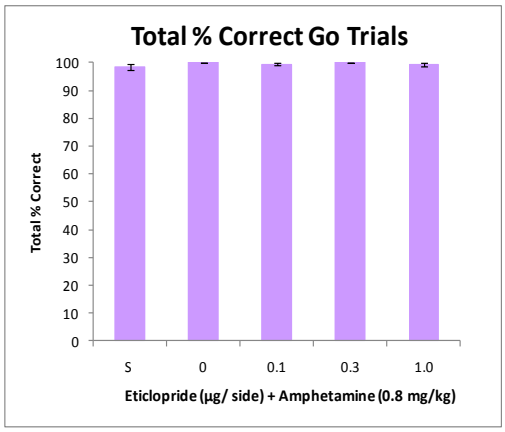


Fig. 6.10

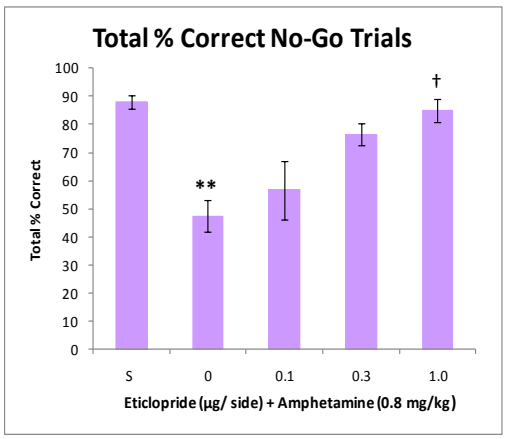


Fig. 6.11

Figures 6.6- 6.27: Performance following eticlopride infusions into the NAcb core on **(6.6)** the total percent correct of all trials, **(6.7)** the total percent correct Go trials, **(6.8)** the total percent correct No-go trials, **(6.12)** go trial latency, **(6.13)** No-go trial latency, **(6.14**) go trial magazine latency, **(6.15)** no-go trial magazine latency, **(6.20)** go trial early responses, **(6.21)** no-go trial early response, **(6.22)** go trial panel response and **(6.23)** no-go trial panel response. Performance following eticlopride infusions into the NAcb core and systemic amphetamine on **(6.9)** the total percent correct of all trials, **(6.10)** the total percent correct Go trials, **(6.11)** the total percent correct No-go trials, **(6.16)** go trial latency, **(6.17)** no-go trial latency, **(6.18)** go trial magazine latency, **(6.19)** no-go trial magazine latency, **(6.24)** go trial early responses, **(6.25)** no-go trial early response, **(6.26)** go trial panel response and **(6.27)** no-go trial panel response. *p<0.05, **p<0.01 and ***p<0.001 vs vehicle infusion + systemic saline combination. †p<0.05 vs vehicle infusion + systemic amphetamine combination. 'S' represents the vehicle infusion + systemic saline control combination. Values represent means and error bars represent the SEM.

Eticlopride

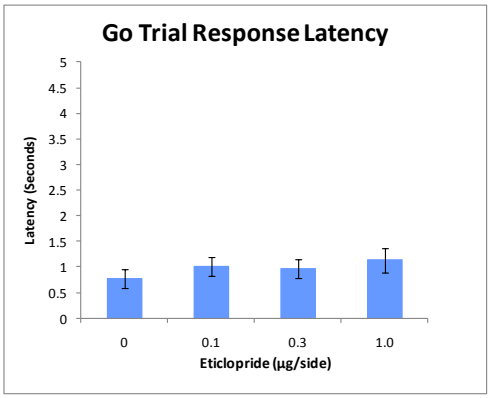


Fig. 6.12

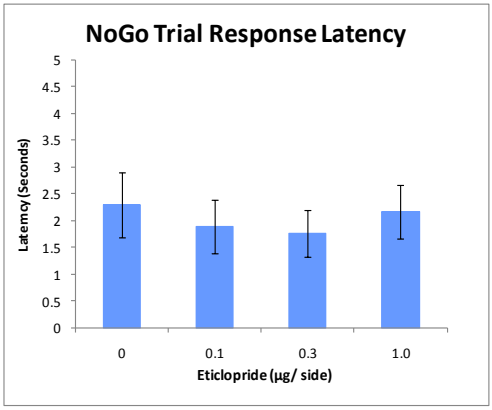


Fig. 6.13

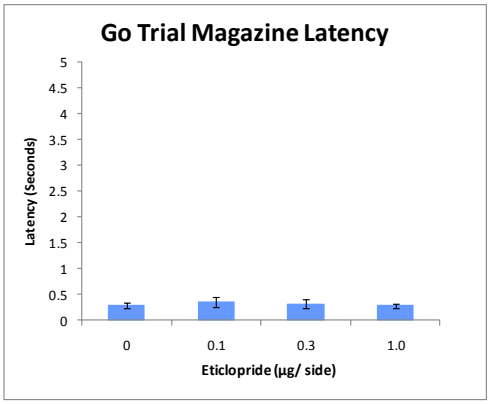


Fig. 6.14

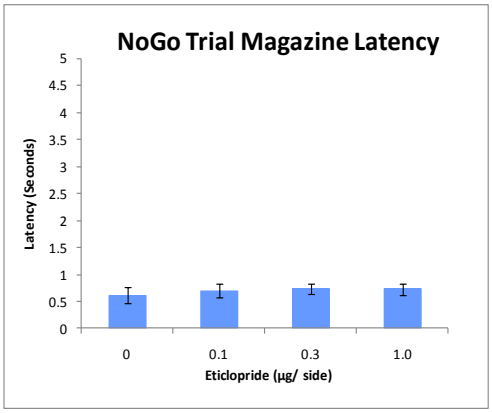


Fig.6.15

Eticlopride + Amphetamine

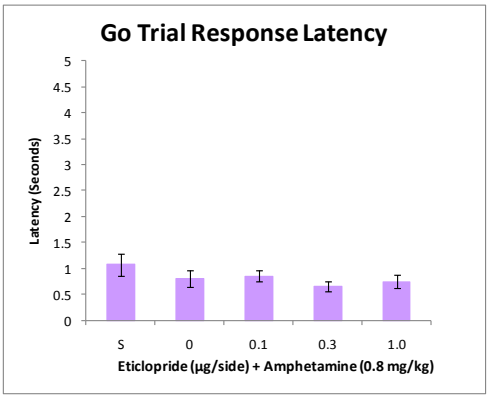


Fig. 6.16

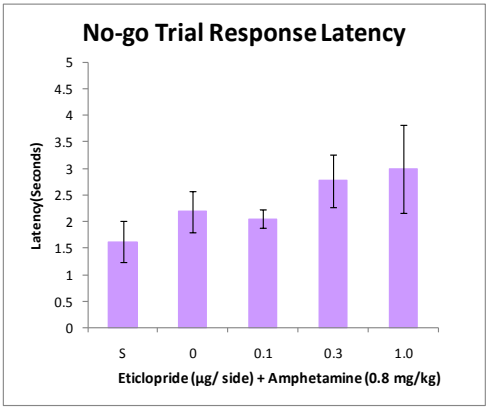


Fig. 6.17

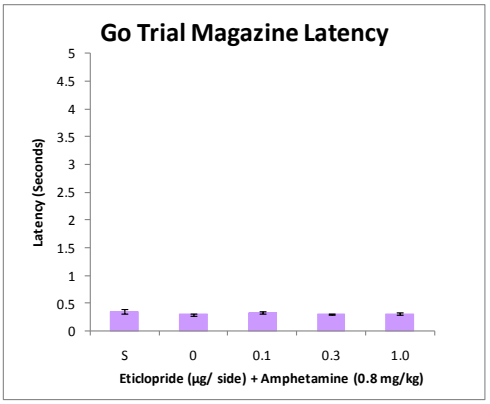


Fig. 6.18

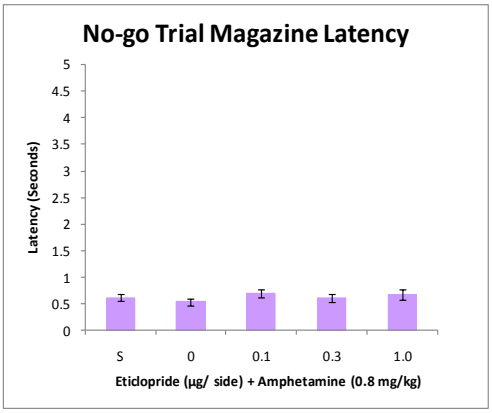


Fig. 6.19

Eticlopride | **Eticlopride + Amphetamine**

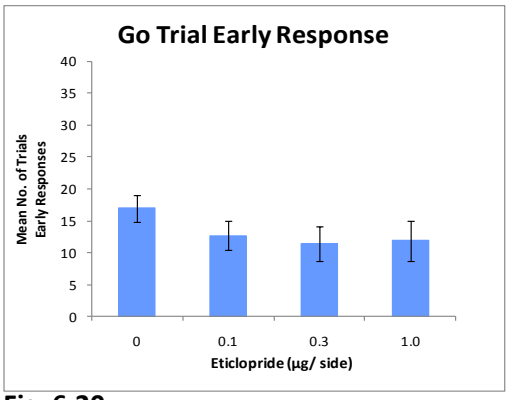


Fig. 6.20

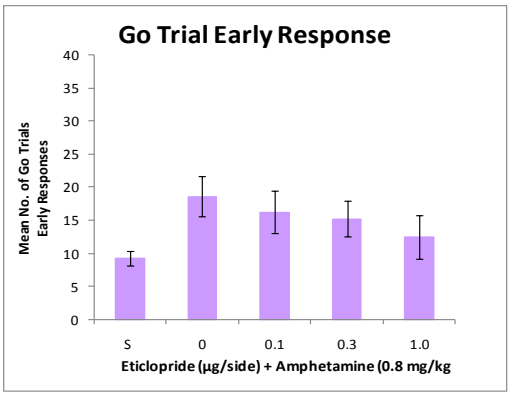


Fig. 6.24

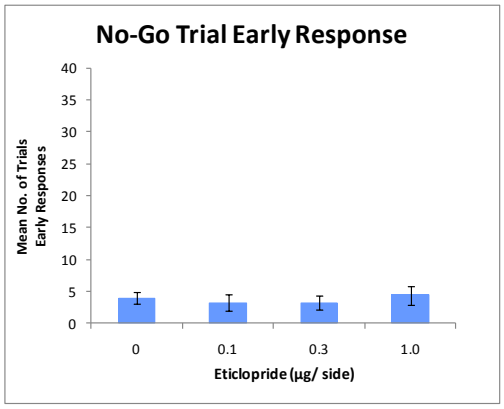


Fig. 6.21

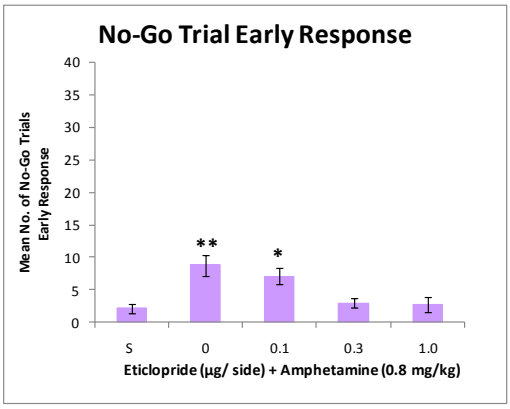


Fig. 6.25

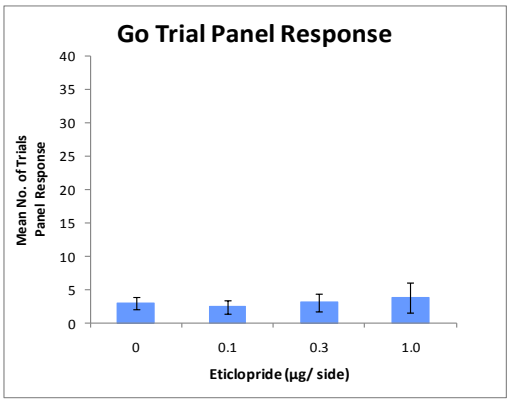


Fig. 6.22

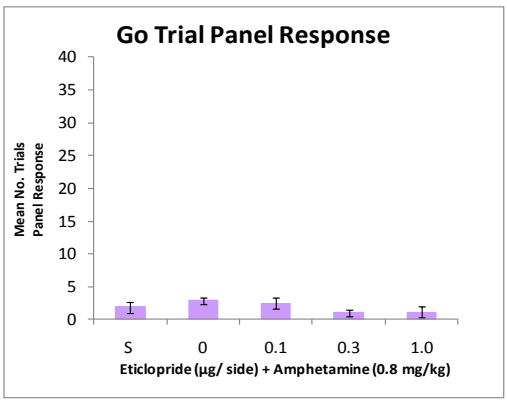


Fig. 6.26

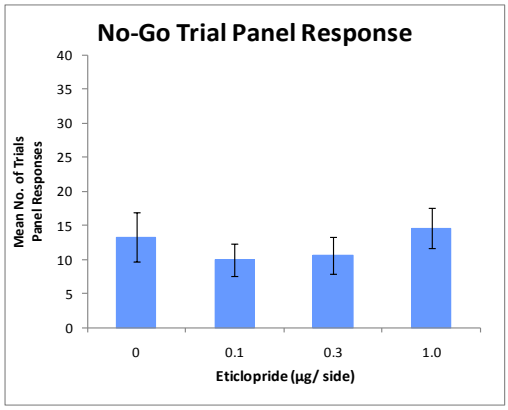


Fig. 6.23

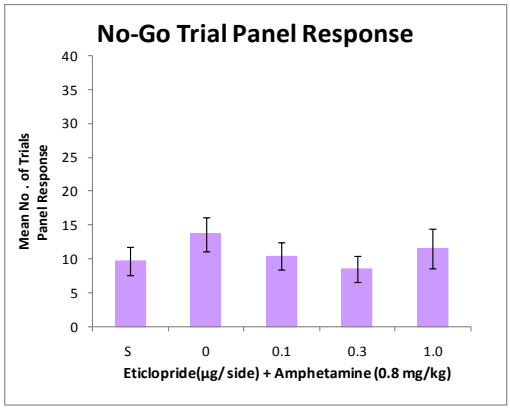


Fig. 6.27

6.4 Discussion

Eticlopride infused into the NAcb core alone did not disrupt any performance measures, indicating that D_2 receptors within the core do not mediate behavioural inhibition, speed of responding or anticipatory responding within a symmetrically reinforced Go/No-go task. Treatment with systemic amphetamine increased impulsivity in rats, demonstrated by reduced No-go trial accuracy and increased No-go trial early responses. Amphetamine induced reductions of No-go trial accuracy were reversed following 1.0 µg of eticlopride and increases in No-go trial early responses were partially reversed following 1.0 µg of eticlopride. Go trial accuracy, speed of responding and magazine latencies during Go and No-go trials were unaffected by eticlopride + systemic amphetamine treatment.

6.4.1 The effects of eticlopride infusions into the NAcb core on behavioural inhibition

Eticlopride infusions did not disrupt the speed of responding during either go or no-go trials, indicating that central infusions of the D_2 antagonist had no affect on the animals general motor performance on the task. D_2 receptor antagonism of the NAcb core via eticlopride and raclopride infusions has previously been shown to reduce locomotor activity in rats (Baldo et al., 2002; Boye, Grant, & Clarke, 2001) and increase response latencies on choice reaction time tasks (Pattij et al., 2007; Pezze, Dalley, & Robbins, 2006). However, dopamine depletion within the NAcb core via 6-hydroxdopamine infusions does not affect speed of responding within simple reaction time tasks where animals have limited response selection (Amalric & Koob, 1987) similar to that on the Go/No-go task. This suggests NAcb core D_2 mediated changes in locomotor activity might not be detected in tasks where behavioural responding is more controlled than locomotor (photobeam) measures and the 5CSRTT. Furthermore, the NAcb shell has been shown to have a preferential role in mediating locomotor activity in comparison to the core (Moreno et al., 2013; Parkinson et al., 1999) and $D_{2/3}$ agonists infused into the NAcb core have been shown to induce no effects on locomotion or rearing in rats (Ikemoto, 2002).

Dopaminergic innervation of the basal ganglia and systemic administration of D_2 antagonists have previously been associated with changes in timing perception in rats (Harrington, Haaland, & Hermanowitz, 1998; Jones et al., 2008; Meck, 1986). The lack of change in the speed of responding therefore additionally indicates that any D_2 mediated changes in time perception in rats did not significantly affect the speed of responding. Furthermore, 6-hydroxydopamine infusions into the NAcb core and shell do not change the temporal dynamics

of responding in rats performing a 'peak procedure' task designed to measure changes in the speed of the 'internal clock' in rats, whilst 6-hydroxydopamine infusions into the caudate-putamen complex does lead to a loss of temporal control (Meck, 2006), indicating that changes in timing perception might be located in more dorsal regions of the striatum than the NAcb core.

Systemic administration of eticlopride, however, has been shown to produce a reduction in the speed of responding in rats performing the Go/No-go task (Harrison et al., In prep) indicating that this task is sensitive to D_2 mediated changes in the speed of responding. The lack of change in response speed following eticlopride into the NAcb core, along with evidence for greater involvement of the NAcb shell in locomotor activity and the dorsal striatum in changes in time perception, therefore suggests that these systemic effects might have been produced though changes in D_2 mediated dopamine transmission in striatal sites outside the NAcb core.

Eticlopride infusions also did not disrupt the speed to collect food reward during either go or no-go trials, indicating that central infusions of the D_2 antagonist had no affect on the animals general motivation to perform the task. These findings are consistent with previous literature demonstrating that eticlopride induced changes in motivation occur following infusions of 2.0 µg eticlopride into the NAcb core, but not following 1.0 µg (Farrar et al., 2010), that was the highest dose in this study. However, these results contrast with the results of Pattij et al., (2007) that report increased omissions at all eticlopride doses infused into the core, the same doses to that employed in the current study, and increased response latencies following the highest (1.0 µg) dose. The differences in these results might relate to the fact that in the 5CSRTT there is much higher attentional demand than the Go/No-go task, meaning that D_2 mediated changes in motivation might be detected at earlier doses on the 5CSRTT than on the Go/No-go task.

Systemic administration of eticlopride does, however, cause an increase in No-go trial magazine latencies on the Go/No-go task (Harrison et al., In prep) indicating that this task is sensitive to D_2 mediated changes in motivation. The fact these changes were not observed in the NAcb core, alongside literature indicating that a dose above 2.0 µg eticlopride is required to significantly change motivation suggests that this dose range of eticlopride might not have been large enough to replicate the highest doses of systemic eticlopride that changes No-go magazine latencies. However, D_2 receptor antagonism within the NAcb shell has also been shown to induce motivational changes in rats (Farrar et al., 2010) also indicating that alternative neural targets of eticlopride might have caused this systemic effect.

The lack of effect of eticlopride infusions on performance accuracy demonstrates that the D_2 receptors within the core do not mediate behavioural inhibition measured by a symmetrically reinforced Go/No-go task. This is consistent with previous studies reporting that D_2 receptor antagonism within the core does not affect behavioural inhibition measured by premature responding on the 5CSRTT and action cancellation on the SST in rats (Eagle et al., 2011; Pattij et al., 2007). This is also in line with previous systemic and central literature reporting systemic D_1 but not D_2 receptor mediation of premature responding (Van Gaalen et al., 2006) and D_1 but not D_2 receptor antagonism of the core and shell reducing premature responding in rats performing the 5CSRTT (Pattij et al., 2007a). However, systemic eticlopride administration on the symmetrically reinforced Go/No-go task produces a reduction in Go trial accuracy indicative of disruptive performance effects following eticlopride on this task (Harrison et al., In prep). The lack of eticlopride effects on performance measures of the Go/No-go task observed following eticlopride infusions into the NAcb core therefore indicates that these systemic effects may be mediated through neural targets outside of the NAcb core. In consideration of the lack of NAcb core D_2 mediated changes on the 5CSRTT and SSRT (Eagle et al., 2011; Pattij et al., 2007) and further on the symmetrically reinforced Go/No-go task, this collectively suggests that D_2 receptors within the core are not involved in the expression of behavioural inhibition or action cancellation. Results from the Go/No-go task further add to this literature by demonstrating that the D_2 receptors might not be involved in behavioural inhibition even when this behaviour is directly reinforced.

6.4.2 The effects of eticlopride on amphetamine induced behavioural disinhibition

The combination of eticlopride and amphetamine treatment led to a main effect on Go trial response latencies. Although post hocs did not reveal any significant differences between specific doses, following amphetamine treatment Go trial response latencies were faster in comparison to the control treatment (Fig 6.16). This is generally consistent with previous literature reporting that acute amphetamine reduces response latencies on the 5CSRTT (Harrison et al., 1997; Pattij et al., 2007) and acutely increases locomotor behaviour in rat (Kuczenski & Segal, 2001). However, as post hoc tests were not significant in the analysis of Go trial response latencies, this suggests that changes in the speed of responding were not a substantial. The lack of change in Go trial response latencies between any eticlopride doses supports that eticlopride infusions did not cause any significant change in slowing response speed that might account for the reversal observed at the highest eticlopride infusion.

Amphetamine can acutely alter timing perception in rats through speeding of the 'internal clock' (Çevik, 2003; Maricq, Roberts, & Church, 1981; Meck, 1983; Taylor, Horvitz, & Balsam, 2007) that might be proposed to underlay reduced No-go trial accuracy as a consequence of miscalculation of the time period in which behavioural responding must occur. The lack of significant changes in the speed of responding and magazine latencies, however, indicates that any amphetamine induced speeding of time perception did not significantly affect the speed of response behaviour in rats. The lack of such changes might relate to the fact that there is low demand on the 'internal clock' on the Go/No-go task due to the continual presentation of Go and No-go cues. Continual visual cues guiding the correct response behaviour for food reward therefore reduced demand on the 'internal clock' to track the time period when responding must occur. Consequently, any amphetamine or eticlopride induced changes in timing perception do not appear to have disrupted response behaviour, and are therefore unlikely to account for No-go trial performance.

In addition no changes were observed in magazine latencies indicating that amphetamine did not produce any significant change in the animal's motivation to gain food reward. Systemic amphetamine treatment within a similar dose range to that employed in the current experiment (0.75 mg/kg) has been shown to acutely increase motivation for food reward indexed via greater engagement in high effort high reward behaviours in rats following systemic amphetamine (Bardgett et al., 2009). Changes in motivation following amphetamine may not have occurred on the Go/No-go task due to that fact behaviour is controlled to one of two trials and there is not a high element of choice over behaviour involved, meaning that subtle changes in motivation that might affect differences in choice behaviour are less likely to be detected on the Go/No-go task where behaviour is more controlled.

In addition, the lack of change in magazine latencies following eticlopride infusions in combination with systemic amphetamine indicates that motivational changes are unlikely to account for the eticlopride reversal of amphetamine induced No-go trial deficits. This is consistent with the lack of eticlopride induced change in magazine latencies when infused into the NAcb core alone, confirming that the dose range of eticlopride employed into the NAcb core does not affect motivation to perform the Go/No-go task, and previous literature indicating that a dose of 2.0 µg is required to affect motivation (Farrar et al., 2010).

Collectively, the combination of speed and anticipatory responding results confirm that changes in impulsivity and eticlopride mediated reversal of amphetamine induced impulsivity cannot be easily accounted for by any substantial locomotor, timing or motivational changes in behaviour caused by amphetamine and eticlopride combination treatments.

The reversal of amphetamine induced No-go trial accuracy deficits by intra-NAcb core eticlopride is consistent with NAcb core eticlopride mediated reversal of amphetamine induced premature responding in rats performing the 5CSRTT (Pattij et al., 2007). These findings therefore collectively support a role for the D_2 receptors within the NAcb core in the meditation of amphetamine induced behavioural disinhibition. The consistency in these results is interesting in light of the difference in effects observed following systemic D_1 and D_2 receptor antagonism on the 5CSRTT and the Go/No-go task (van Gaalen et al., 2006; Harrison et al., In prep). Evidence that amphetamine induced impulsivity on both tasks can be reversed by D_2 core antagonism therefore indicates that despite differences in overall system changes in D_1 and D_2 receptor activity on the 5CSRTT and Go/No-go tasks, changes at the D_2 receptors within the NAcb core is a shared neural substrate of amphetamine induced impulsivity on both tasks.

However, differences were observed in the results in the current experiment and (Pattij et al., 2007). In the current experiment only the highest dose of eticlopride (1.0 µg) produced full behavioural reversal of reduced No-go trial accuracy following amphetamine, whereas 0.3 µg of eticlopride produced full behavioural reversal of premature responding on the 5CSRTT (Pattij et al., 2007). Differences in the dose at which eticlopride reversed amphetamine induced impulsivity might relate to the fact that impulsive behaviour is more controlled by food reinforcement and visual cues within the symmetrically reinforced Go/No-go task than the 5CSRTT, meaning that higher doses of eticlopride might be required on the Go/No-go task to produce similar effects to that on the 5CSRTT.

Interestingly, amphetamine not only decreased No-go trial accuracy but also increased No-go trial early responses, which may be more comparable with premature responses during the 5CSRTT. Both measures indicate early inappropriate responding. Although pre-treatment with eticlopride into the NAcb core did not fully reverse this effect, there was evidence of a partial reversal at the highest doses of eticlopride where no significant difference was detected in No-go trial early responses following 1.0 and 0.3 µg eticlopride in comparison to control treatment, but both doses did not significantly differ from early responses performed following the amphetamine and vehicle combination and amphetamine and 0.1 µg eticlopride combination. These results therefore illustrate consistency in the role of the D_2 receptors within the core in mediating behavioural disinhibition in situations of attentional demand.

The lack of eticlopride induced effects on impulsivity on the Go/No-go task, in combination with eticlopride reversal of amphetamine induced impulsivity on this task, are also collectively interesting in relation to the role of the D_2 receptors within the NAcb core and impulsivity. The

lack of eticlopride induced effects on impulsivity when infused into the NAcb core alone, in contrast the reversal observed in combination with amphetamine, suggests that the D_2 receptors are only involved in the mediation of impulsive behaviour during Go/No-go task following increased activation. This is also consistent with recent evidence that highly impulsive animals on the 5CSRTT have supersensitive D_2 receptors within the NAcb core (Moreno et al., 2013). The D_2 receptors within the NAcb core might then only be detrimental in the expression of behavioural inhibition when they are over stimulated, either via drug induced hyperactivity or predisposing supersensitivity of the receptor.

However, it is also noteworthy that eticlopride also has an affinity for the D_3 receptors (IC_{50} (nM) = 113), although this is lower than eticlopride affinity for the D_2 sub-receptor (IC_{50} (nM) = 1.0), consequently it is possible that blockade of the D_3 receptors within the NAcb core might have contributed to the reversal of amphetamine induced impulsivity. Upregulation of the D_3 receptors has been observed in methamphetamine dependent subjects (Boileau et al., 2012), however, this has only been found in D_3 rich and not D_2 rich areas, as is the NAcb core (Le Moine & Bloch, 1996). In addition, systemic D_3 specific antagonists do not reverse amphetamine induced impulsivity on the 5CSRTT (van Gaalen et al., 2009) and increased impulsivity in rats performing the 5CSRTT following intra NAcb core infusions of the D_2 specific agonist quiniprole is not reversed by pretreatment with the preferential D_3 antagonist nafadotride (Moreno et al., 2013). These studies therefore support a more specific role for the D_2 receptors, and D_2 receptors within the NAcb core, in high levels of impulsivity in rats.

Within the NAcb core, the D_2 receptors exert inhibitory actions on cells via activation of G proteins that inhibit adenylate cyclase to hyperpolarise the cell membrane (Stoof & Kebabian, 1984). In contrast, the D1 receptors exert an excitatory action on cells via activating G proteins that activate adenylate cyclase to activate local depolarisation of the cell membrane (Stoof & Kebabian, 1984). The inhibitory and excitatory actions of the D_2 and D_1 receptors, respectively, can therefore exert an inhibitory or excitatory influence over afferent connections innervation the NAcb core and efferent connections leaving the NAcb core.

The D_2 receptors within the ventral striatum are located presynaptically on glutamate terminals innervating the NAcb core and postsynaptically on GABAergic medium spiny neurons that project from the NAcb core to descending structures (Albin, Young, & Penney, 1989; Goto & Grace, 2005; Kawaguchi, 1993; Surmeier et al., 2007). The D_2 receptors can therefore exert inhibitory control over excitatory prefrontal and limbic glutamatergic afferents innervating the NAcb core and GABAergic efferents leaving the NAcb core. This means that changes in D_2

receptor function within the NAcb core might consequently disrupt the integration of fronto-striatal activity, and or, disrupt the activity of descending GABAergic activity.

The NAcb core and shell connect to descending anatomical targets via GABA mediated *direct* and *indirect* ventral striato-pallidal pathways (See Chapter 1, Fig. 1.4). The direct ventral striato-pallidal pathway projects directly back to the VTA and substantia nigra (SN) and predominantly expresses D_1 receptors, whilst the indirect pathway projects indirectly to subthalamic and thalamic nuclei via the ventral pallidum, dorsomedial subthalamic nucleus, internal globus pallidum and substantia nigra pars reticular, and predominantly expresses D_2 receptors. GABAergic MSNs descending from the NAcb core and shell therefore modulate fronto-striatal activity through midbrain connections that ultimately influence the activation or deactivation of the thalamus (Feil et al., 2010; Carlezon & Thomas, 2009). Amphetamine induced activation of the inhibitory D_2 receptors within the NAcb core might therefore cause subsequent inhibition of the indirect pathway projecting from the NAcb core that, along with the indirect pathway, modulate the fronto-striatal activity through ventral striato-pallido-thalamic circuits (Bonelli & Cummings, 2007; Feil et al., 2010).

6.4.3 Key Findings

The main findings from this study are that infusions of the D_2 receptor antagonist eticlopride into the nucelus accumbens core do not affect response inhibition, but do reverse amphetamine induced response disinhibition in rats. The combination of these findings suggests that the D_2 receptors within the nucleus accumbens core are not involved in baseline expressions of response inhibition, but are involved in the expression of drug induced response disinhibition. The main aims of this study were to determine the role of the D_2 receptors within the nucelus accumbens core in mediating response inhibition and amphetamine induced response disinhibition on the Go/No-go task. These aims were established from broader objectives to elucidate how pathological changes in the D_2 receptors within the ventral striatum might contribute towards impulsive behaviour observed in addicts. Observations that the D_2 receptors within the nucleus accumbens core are specifically involved in mediating amphetamine induced response disinhibition, but not baseline levels of response inhibition, consequently addresses this broader objective by demonstrating that drug induced activation of the D_2 receptors within the nucleus accumbens core region of the ventral striatum can directly increase impulsive behaviour.

6.4.4 Limitations

One limitation of this study is that only the D_2 receptors within the nucleus accumbens core were investigated. It would have been useful to also investigate the role of the D_2 receptors in the nucleus accumbens shell to gain a broader neuroanatomical understanding of D_2 receptor function within the nucleus accumbens as a functional anatomical unit in relation to impulsivity and drug induced impulsivity. This study also did not investigate the role of the D_1 receptors within the nucleus accumbens core in impulsivity and amphetamine induced impulsivity on the Go/No-go symmetrically reinforced task. This consequently limits broader interpretations into roles of different dopamine sub-receptors within the ventral striatum in impulsivity and drug induced impulsivity.

An additional limitation of this study is that the role of the D_2 receptors within the nucleus accumbens was only investigated in relation to acute amphetamine induced impulsivity. Whilst these findings are useful for providing an initial indication of how the D_2 receptors might function in relation to drug induced impulsivity, they do not necessarily extend to explain how the D_2 receptors might be involved in impulsivity observed amongst addicts with a chronic history of drug use. The main findings of this study are therefore limited in terms of direct scalability to the role of the D_2 receptors within the ventral striatum in relation to impulsivity observed amongst drug addicts.

6.4.5 Future Research

In order to address the limitations of this study in regards to neuroanatomy, it would be useful for future research to investigate the role of the D_2 receptors within the nucleus accumbens shell in impulsivity and amphetamine induced impulsivity on the Go/No-go task. Previous research has suggested that the core and shell regions of the nucleus accumbens opponently modulate amphetamine induced impulsivity and that the $D_{2/3}$ receptors within the nucleus accumbens core and shell might have dissociable effects on impulsivity in the 5CSRTT (Besson et al., 2009; Murphy et al., 2008). Establishing the role of the D_2 receptors within the nucleus accumbens core and shell on accuracy, and drug induced changes in accuracy, within the Go/No-go symmetrically reinforced task will help to build a broader behavioural understanding of D_2 receptor function in regards to impulsivity.

In addition to investigating the D_2 receptors within the nucleus accumbens core, it would be useful for future research to explore the role of the D_1 receptors within the nucleus accumbens core and shell in impulsivity and drug induced impulsivity measured by the Go/No-go

symmetrically reinforced task. As discussed in the introduction of this chapter (pages 163 - 166) there are indications from systemic studies that the D_1 and D_2 receptors might have different effects on impulsivity measured by the 5CSRTT and Go/No-go symmetrically reinforced task (Harrison et al., In prep; van Gallen et al., 2006; 2006b). Considering that the results from this study found a similar role for the D_2 receptors within the nucleus accumbens core in amphetamine induced impulsivity, as has previously been reported by studies employing the 5CSRTT (Pattij et al., 2007), it would be useful to continue this line of research to fully establish the role of the D_1 and D_2 receptors in the core and shell on impulsivity measured by the Go/No-go symmetrically reinforced task. Further investigations into the role of the D_1 and D_2 receptors in response inhibition and drug induced response disinhibition measured by the Go/No-go task will help to build a more comprehensive picture of how the D_1 and D_2 receptors might be involved in the expression of impulsivity and drug induced impulsivity.

In order to address scalability limitations of this study to human addicts, it would be useful to explore the role of the D_2 receptors in drug induced impulsivity during, and following, more chronic self-administration drug regimes in rats. Future research employing a reliable animal model of human drug use alongside intra-nucleus accumbens D_2 receptor manipulation would help to elucidate how long-term drug exposure affects D_2 receptor function in relation to impulsive behaviour, and further, how such changes in receptor function might contribute towards elevated levels of impulsive behaviour observed amongst addicts. In addition, given that the D_2 receptors within the ventral striatum have been linked to trait impulsivity in drug addicts (Lee et al., 2009), it would be useful to examine whether animals screened for high and low levels of trait impulsivity at baseline, are differentially affected by D_2 antagonism within the nucleus accumbens core and shell on baseline levels of impulsivity, and drug induced impulsivity, measured by the Go/No-go symmetrically reinforced task. This would help to elucidate how the D_2 receptors within the ventral striatum function in relation to both trait and drug induced impulsivity.

In regards to future research directions concerned with the neurocircuitry of impulsivity and addiction, the next steps for elucidating how changes at the level of the D_2 receptors within the nucleus accumbens core might be involved in higher level circuit changes mediating impulse control, will be to explore how changes in D_2 receptor function affects the activity of neural targets in connection with the nucleus accumbens core, such as the ventral pallidum, prefrontal and orbitofrontal cortex. For example, it would be useful to measure GABA concentration in the ventral pallidum following D_2 receptor antagonism to establish how changes in accumbens core D_2 function affects the activity of descending neuroanatomical

sites. Alternatively, it would be useful to examine glutamate concentration in nucleus accumbens core following D_2 antagonism in order to indentify how amphetamine induced D_2 receptor activation affects glutamate afferents innervating the nucleus accumbens core. This would be useful for considering how changes in D_2 receptor activity might affect prefrontal glutamatergic afferents innervating the nucelus accumbens core, and thus help to further elucidate how changes in D_2 receptor activity more specifically disrupt fronto-striatal connections.

6.4.6 Conclusions

In summary, eticlopride infused into the NAcb core alone produced no effects on behaviour whilst eticlopride infused into the NAcb core in combination with systemic amphetamine led to reversal of amphetamine induced impulsivity. These finding further implicate the involvement the D_2 receptors within the NAcb core in amphetamine induced behavioural disinhibition and collectively suggest that stimulation of the D_2 receptors can lead to behavioural disinhibition. These findings expand on previous animal literature implicating the D_2 receptors within the NAcb core in amphetamine induced impulsivity (Pattij et al., 2007) by further identifying that the D_2 receptors within the NAcb core also mediate amphetamine induced impulsivity on a symmetrically reinforced Go/No-go task. Furthermore, these findings also contribute towards existing literature implicating a role for the D_2 receptors within the ventral striatum with impulsivity in stimulant addicts by further confirming that stimulant induced changes at the D_2 receptor within the NAcb core can increase impulsivity.

Chapter 7 The effects of NAcb $GABA_A$ agonism on behavioural inhibition and amphetamine induced behavioural disinhibition

7.1 Introduction

GABA is the brains major inhibitory neurotransmitter that is prevalent at approximately 40% of all synapses within the brain (Leonard, 2005). GABA transmission is modulated by three GABA sub-receptors: $GABA_A$, $GABA_B$ and $GABA_C$. The $GABA_A$ and $GABA_C$ receptors are ionotropic receptors meaning that activation of these receptors directly opens plasma membrane chloride (Cl^-) channels, causing an increase in internal Cl^- and hyperpolarizing the cell (Chebib & Johnston, 1999). $GABA_B$ receptors are alternatively metabotropic G-protein coupled receptors that activate potassium (K^+) and inhibit calcium (Ca^{2+}) channels leading to hyperpolarization of the cell (Chebib & Johnston, 1999). The $GABA_A$ and $GABA_C$ receptors both produce fast inhibition whilst the $GABA_B$ receptors produce show inhibition (Nicoll, Malenka, & Kauer, 1990). Out of the three GABA sub-receptors the $GABA_A$ receptor is the most widely distributed throughout the brain (Young & Snyder, 1974).

GABA transmission has been indirectly associated with a number of behavioural dimensions of impulsivity. Benzodiazepines that target sites of the $GABA_A$ receptor reduce impulsive aggression and behavioural dyscontrol in subjects with bipolar disorder (Cowdry & Gardner, 1988; Hollander et al., 2003) and benzodiazepines can also be prescribed as a second line treatment for ADHD (Nair & Mahadevan, 2009; Popper, 2000; Silva, Munoz, & Alpert, 1996). More recently, studies directly assessing the relationship between GABA and impulsivity have found a relationship between cerebrospinal fluid GABA concentration and trait impulsivity in humans (Lee, Petty, & Coccaro, 2009). GABA concentration within prefrontal regions of the brain can also predict rash impulsivity (Boy et al., 2011), distractibility and motor decision speed within healthy adults (Sumner et al., 2010). Additionally, children with ADHD have reduced GABA concentration within the cerebral cortex (Edden et al., 2012), indicating that hypoactive GABA circulation is prevalent in deficits of inhibitory control.

Investigation into the relationship between GABA and impulsivity using animal models has identified a role for GABA within the prefrontal cortex and behavioural inhibition in rats. Inhibition of GABA production within the PFC (infralimbic and prelimbic cortices) via infusion of the glutamate decarboxylase (GAD) inhibitor $_L$-allylglycine increases premature responding in rats without producing any changes in accuracy of attention, response latencies or omissions (Asinof & Paine, 2012). Infusion of the $GABA_A$ agonist muscimol into the infralimbic cortex, but

not prelimbic cortex, also increases premature responding within the 5CSRTT in rats (Murphy et al., 2012). Infusions of muscimol into the infralimbic cortex also produced some changes in accuracy, response latencies and omissions, however, only changes in premature responding were found to be site specific changes following muscimol (Murphy et al., 2012). In addition, highly impulsive rats on the 5CSRTT show reduced $GABA_A$ receptor availability within the ACC in comparison to less impulsive rats (Jupp et al., 2013) indicating that changes in GABA transmission via the $GABA_A$ sub-receptor within the ACC is associated with trait impulsivity in rats. Although only a small number of studies have investigated the effects of central GABA manipulation on impulsivity, these studies collectively confirm a relationship between GABA transmission and animal models of impulsivity.

The NAcb core has been identified as a neural mediator of impulsivity and amphetamine induced impulsivity (Cardinal et al., 2001; Murphy et a., 2008; Pattij et al., 2007). The principle neurons within the NAcb core are GABAergic medium spiny projection neurons and aspiny GABAergic interneurons (Groenewegen & Trimble, 2007; Meredith, 1999), meaning that the convergence of prefrontal, limbic and midbrain afferent signals within the NAcb core is modulated by GABAergic cells, and furthermore, that the output of cortical, limbic and midbrain integration within the NAcb core is transmitted to descending anatomical targets of the NAcb core via GABAergic signalling. In addition, the NAcb core also receives GABAergic afferents from the ventral pallidum and VTA (Churchill & Kalivas, 1994; Van Bockstaele & Pickel, 1995), consequently placing the NAcb core anatomically within an inhibitory GABAergic network.

Efferent GABA projections leaving the NAcb core constitute the direct and indirect ventral striato-pallidal pathways that through different anatomical midbrain connections ultimately influence the activation or deactivation of the thalamus (Carlezon & Thomas, 2009) (See Chapter 1, Fig 1.4). The direct ventral striato-pallidal pathway predominantly expresses D_1 receptors, whilst the indirect pathway predominantly expresses D_2 receptors, that exert excitatory and inhibitory actions, respectively (Albin, Young, & Penney, 1989; Surmeier et al., 2007). In consideration of evidence implicating changes at the D_2 receptors within the NAcb core in amphetamine induced impulsivity (Pattij et al., 2007; Chapter 6, section 6.3), this suggests that D_2 mediated changes in GABA release though the indirect pathway descending from the NAcb core might be an important neuropathology of drug induced impulsivity.

In summary, emerging evidence has indicated that changes in GABA transmission can affect impulsivity in humans and animal models of impulsivity. In addition, changes at the D_2 receptors within the NAcb core that can influence the activation or deactivation of GABAergic

cells within the NAcb core have been implicated in amphetamine induced impulsivity (Pattij et al., 2007; Chapter 6, Section 6.3), suggesting that changes in GABA transmission at the level of the NAcb core might be involved in impulsivity. The following set of experiments therefore sought to investigate the effects of direct GABA manipulation within the NAcb core on impulsivity and amphetamine induced impulsivity in rats using the symmetrically reinforced Go/No-go task. Given that the $GABA_A$ sub-receptor is the most predominant GABA sub-receptor within the rat brain (Young & Snyder, 1974), and that there are more $GABA_A$ type receptors within the NAcb than $GABA_B$ (Bowery, Hudson, & Price, 1987), the effects of $GABA_A$ inhibition within the NAcb core in impulsivity and amphetamine induced impulsivity on the symmetrically reinforced Go/No-go task was investigated.

7.1.1 Objectives

In order to investigate the involvement of GABA within the NAcb core on behavioural inhibition and amphetamine induced behavioural disinhibition, the following objectives of Chapter 7 were:

i) To determine the effects of $GABA_A$ agonism within the NAcb core on behavioural disinhibition measured by a symmetrically reinforced Go/No-go task (Experiment 6)

ii) To determine the effects of $GABA_A$ agonism within the NAcb core on amphetamine induced behavioural disinhibition measured by a symmetrically reinforced Go/No-go task (Experiment 7)

7.2 Methods

7.2.1 Subjects

18 male Lister Hooded rats (Charles River, UK) were housed in pairs upon arrival into Leeds Behavioural Neuroscience Laboratory and were maintained under a 12 hour light/dark cycle (lights on 0700 hours), in a temperature (21°C ± 2°C) and humidity (50% ± 5%) controlled environment. Throughout behavioural experimentation animals were placed on a food restriction schedule of 18.6g per day, maintaining animals above 85% of their adult free feeding body weight. Water was available ad libitum in home cages and feeding took place in the morning after behavioural testing. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996. All procedures were covered by Home Office Project Licence No. PIL 40/3606 and Home Office Personal Licence No. 40/1989.

7.2.2 Drugs

D-Amphetamine sulphate (Sigma Aldrich, UK) was dissolved in 0.9% physiological saline and was administered i.p. in a volume of 1 ml/kg body weight. D-amphetamine was weighed out and prepared in solution on the morning of test days. Muscimol (Tocris, UK) was dissolved in Dulbecco's phosphate buffered saline (Sigma Aldrich, UK) and was administered via intracerebral infusion into the NAcb core in a volume of 0.5 µl per hemisphere. Muscimol solutions were weighed out and prepared in solution upon the morning of test days. On occasion muscimol solutions were stored at ~-20°C until use (Tocris, UK storage guidelines).On test days, muscimol solutions were removed from freezing and left to sit at room temperature to defrost before use. Musicmol solutions stored overnight only underwent one freezing and de-frosting cycle; no solutions were re-frozen after experimental use. Muscimol dose were selected by literature review targeting studies administering solely muscimol into the brain, in combination with operant testing. Following assessment of the literature collected, a dose range of 30 ng (0.003µg) -1000ng (1.0µg) muscimol was documented (Cain, Denehy, & Bardo, 2007; Corbit & Balleine, 2011; Hodge, Chappelle, & Samson, 1995; Lê Dzung et al., 2008; Murphy et al., 2012; Yoon et al., 2009).

7.2.3 Apparatus

Behavioural testing took place in eight aluminium operant chambers (30.5 x 24.1 x 21 cm, Med Associates Inc., USA) placed inside sound attenuating and ventilated cubicles (63.5 x 49.1 x

39.4 cm, Med Associates Inc., USA). Chambers were controlled, and all data was recorded from chambers, using MED-PC IV software (Med Associates Inc., USA). Administration of intracerebral drug infusions was performed using an infusion pump (KD Scientific, model 200). For a more detailed description of test chambers refer to Chapter 2 section 2.1.3, page 60.

7.2.4 Behavioural Testing

Behavioural inhibition was assessed via the symmetrically reinforced go/no-go visual discrimination paradigm. Behavioural training took place for approximately 8 weeks until animals reached stable performance of 85% total percent correct (+/-5%). Upon stable baseline performance animals were entered into the experiment. All behavioural training and testing took place between 0700-1030 hours during the light phase of animals Light/Dark cycle. For a detailed outline of the task refer to Chapter 2 section 2.1.4, page 61.

7.2.5 Surgery

Once behavioural performance was stable at the minimum criteria of 85% total percent correct (+/-5%), animals were removed from daily test sessions and placed on a free-feeding schedule for a minimum of 3 days prior to receiving surgery. Rats were anaesthetised using a combination of 4% isoflurane gas and oxygen (flow rate 3 l/min) before being placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) continuing to deliver 4% isoflurane gas and oxygen. Rats were secured into flat skull position via ear bars and a nose clamp with the incisor bar set to -3.3 - -4.7, depending on flat skull measurements from each rat. 22 gauge bilateral guide cannulae (centre-to-centre distance between each cannula: 3.8 mm; pedestal height: 10.41 mm; stainless steel length below pedestal: 7 mm; pedestal diameter: 6.12 mm; Plastics One Inc., USA) were positioned 2 mm above the NAcb core via the following stereotaxic coordinates: +1.5 mm anterior to bregma (AP), +/- 1.9 mm lateral to midline (ML), -5.2 mm ventral to skull surface (DV) calculated from Paxinos and Watson (1998). Co-ordinates were selected via a literature review identifying studies targeting the NAcb core in the administration of intracerebral drug infusions. Cannulas were fixed to the skull via dental cement (Kemdent Dental Cement, Associated Dental Products Ltd, UK) and three stainless steel screws (Plastics One Inc., USA) were fixed to the skull posterior to the cannula. 29 gauge stylets extending 1 mm below the guide cannula and sitting 1 mm above the NAcb core were inserted following secure placement of the guide cannula to the skull. Prior to removing animals from the stereotaxic frame, rats received a 0.2 ml intramuscular injection of buprenorphine (Vetergesic, Animal Health Ltd, Essex) to alleviate post-operative pain. Buprenorphine dosages were selected based upon previous literature reviewing the effects of

buprenorphine upon food and water consumption when administered post-operatively (Liles & Flecknell, 1992). Animals subsequently remained in their home cages on a free-feeding schedule for a minimum of 7 days post surgery. Following the demonstration of surgical recovery via weight gain and healing of the scalp wound surrounding the guide cannulae, animals were reintroduced to their original cage mate and were re-housed in pairs. On occasion animals continued to be singly housed following surgery due to failure to re-habituate to their cage mates. Following surgical recovery, animals were placed back on a food restriction schedule of 18.6 g per day and behavioural training recommenced.

7.2.6 Microinfusion Procedure

Muscimol dosages were infused in a volume of 0.5 µl/ side over a period of 1 minute (rate 0.5 µl/min) via a 25µl Hamilton syringe driven by an infusion pump (KD Scientific, model 200). To infuse, stylets were removed from guide cannulae and a 28 gauge internal injector (centre-to-centre distance, 3.8 mm; injector length from top of pedestal, 11 mm; Plastics One Inc., Roanoke, VA, USA) extending 2mm ventral to the tip of the guide cannulae was inserted delivering muscimol into the NAcb core. Injectors were inserted 1 minute pre-drug infusion and were left in place for a further 1 minute post-drug infusion to allow for the diffusion of liquid. The infusion rate, infusion volume per hemisphere, pre- and post-injection times were based upon previous literature administering intracerebral muscimol infusions (Murphy et al., 2012; Yoon et al., 2009). After drug infusions, stylets were replaced and animals in experiment 7 were immediately administered with either systemic amphetamine or saline. Animals were subsequently tested 10 minutes later. Pre-treatment time was based on previous findings that intracerebral infusions of muscimol take approximately 5 minutes to manifest behaviourally (Cain, Denehy, & Bardo, 2007; Spanis et al., 1999).

7.2.7 Design and Procedure

Following recovery from surgery and the return of stable baseline task performance, animals were assigned to either experiment 6 or 7. These groups were counterbalanced according to their baseline performance accuracy, programmed levers, and stimulus-response contingencies. A within subject design was employed in both experiment 6 and 7.

7.2.8 Experiment 6: The effects of muscimol infusions into the NAcb core on behavioural inhibition

Animals (N=8) received i.c. infusions of (0ng, 120ng, 240ng, 480ng and 960ng/ 0.5µl/ side) muscimol into the NAcb core 10 minutes before behavioural testing according to a Latin square design, with a minimum of 72 hours between each drug infusion to encourage site preservation between intracerebral injections. Prior to all drug treatments animal's task performance had returned to post-surgery baseline levels.

7.2.9 Experiment 7: The effects of pre-treatment of muscimol infusions into the NAcb core upon amphetamine induced behavioural disinhibition

Animals (N=10) received the following dosages of muscimol (i.c. 10 minutes prior to testing) immediately followed by amphetamine (i.p.); 0 ng + 0 mg/kg, 0ng + 0.8 mg/kg, 120ng + 0.8 mg/kg, 240 ng + 0.8 mg/kg, 480 ng + 0.8 mg/kg and 960 ng + 0.8 mg/kg (ng/0.5 µl). A minimum of 72 hours was employed between each drug infusion to encourage site preservation between intracerebral injections and to allow for a 3 day wash out period of amphetamine between subsequent amphetamine administrations. Animals were also required to re-stabilise behavioural performance between intracerebral infusions to a criteria of +/-10% of their average baseline performance following surgery. All drug treatments were administered in a latin-square design.

7.2.10 Assessment of Cannulae Placement

Following completion of experimental procedures animals were administered i.p. with a lethal dose of pentobaribital sodium solution (Animal Health Ltd, Essex, UK). Conscious responsiveness was checked via assessment of the pedal withdrawal and corneal reflex. Upon termination of respiration, the heart was subsequently perfused via injection of 100ml of 0.9% NaCl into the left ventricle of the heart, followed by 400ml of 4% paraformaldehyde (Sigma Aldrich, UK) in 0.2M phosphate buffer. Brains were immediately removed and stored in 4% paraformaldehyde solution at 4°C. Brains were transferred into 25% sucrose solution 48 hours prior to slicing. Rat brains were sliced in 40 µm coronal sections using a cryostat (Bright OTF 5000). Slices were mounted onto microscope slides and stained using working cresyl violet solution to determine the location of cannulae placements. Cresyl violet solution was mixed into a working solution within Leeds Behavioural Neuroscience Laboratory using cresyl violet acetate salt (Sigma Aldrich, UK).

7.2.11 Statistical Analysis

All data was initially checked for normality through Shaprio-Wilk tests and was appropriately transformed via arcsine, log10, square root and reciprocal transformations following any violations to normality. Homogeneity of variance was checked via Mauchley's test of Sphericity and following any significant violation of equal variances, the GLM degrees of freedom were adjusted using the Greenhouse- Geisser correction. All data was analysed via one-way repeated measures ANOVAs with 'drug treatment' entered as the within-subjects factor. The effects of muscimol infusions into the NAcb core upon behavioural disinhibition was assessed via 1 X 5 repeated measures ANOVAs across all behavioural parameters. The effects of muscimol pre-treatment within the NAcb core upon amphetamine induced behavioural disinhibition were assessed via 1 X 6 repeated measures ANOVAs across all behavioural parameters. Significant main effects of drug dose were followed up with Sidak post-hoc analyses. $\alpha < 0.05$ was employed across all statistical analysis.

7.2.12 Histology

18 animals completed behavioural testing and were perfused (n = 8 muscimol; n = 10 muscimol and amphetamine). Animals with both injector tips located dorsal to the NAcb core border were removed from analysis. Animals with one injector tip located minimally dorsal to the NAcb core border and one injector tip hitting the NAcb core were included in analysis. After visual inspection of cannula placements, one animal was removed from the analysis of experiment 6 due to a loss of histological data following an unsuccessful perfusion, and one animal was removed from experiment 7 due to both the left and right hemisphere injector tips falling dorsal to the NAcb core border. No injector tips were found to cross the ventral border of the NAcb core into the NAcb shell. Subsequently, the data from 16 animals was used in statistical analysis (n = 7 experiment 6; n = 9 experiment 7). Figure 7.1 shows the location of injector tips amongst animals treated with muscimol (experiment 6), and muscimol in combination with systemic amphetamine (experiment 7). Photographs showing the location of injector tips from an animal in experiment 6 and an animal in experiment 7 are shown in Figures 7.2 – 7.5. For photographs showing the location of injector tips for each animal in experiments 6 and 7, see Appendices 5 and 6.

7.3 Results

After checks of normality, the following transformations were performed*:* Experiment 6: Log_{10} (Go trial latency, No-go trial incorrect latency, Go trial panel responses); Experiment 7: Log_{10} (Go trial latency, No-go trial incorrect latency, Go trial magazine latency, No-go trial magazine latency, Go trial panel responses, No-go trial panel responses). In order to transform Go trials to a normal distribution, the total percentage correct of Go trials was analysed as a percentage change of the previous test day (baseline).

7.3.1 Experiment 6: The effects of muscimol infusions into the NAcb core on behavioural inhibition

7.3.1.1 Performance Accuracy

Accuracy was not affected by muscimol infusions. The total percentage correct ($F(1.800, 10.798) = 0.738$, NS), total percentage correct of Go trials ($F(4, 24) = 1.518$, NS) and the total percentage correct of No-go trials ($F(4, 24) = 0.718$, NS) did not significantly differ following muscimol infusions into the NAcb core (Figs. 7.6 – 7.8).

7.3.1.2 Speed of Responding

The speed of responding was not affected by muscimol infusions. Go trial response latency ($F(4, 24) = 2.404$, NS), No-go trial incorrect response latency ($F(2, 24) = 0.958$, NS), Go trial magazine latency ($F(2, 24) = 1.787$, NS) and No-go trial magazine latency ($F(2,24) = 0.710$, NS) did not significantly differ following infusions of muscimol into the NAcb core (Figs. 7.12 – 7.15).

7.3.1.3 Anticipatory Responding

Anticipatory responding was also not affected by muscimol infusions. Go trial early responses ($F(4, 24) = 0.473$, NS), No-go trial early responses ($F(1.414, 8.484) = 1.175$, NS), Go trial inappropriate panel responses ($F(2, 24) = 1.402$, NS), and No-go trial inappropriate panel responses ($F(1.553, 9.319) = 0.264$, NS) did not significantly differ following infusions of muscimol into the NAcb core (Figs. 7.20 – 7.23).

7.3.2 Experiment 7: The effects of muscimol pre-treatment into the NAcb core on systemic amphetamine induced behavioural disinhibition

7.3.2.1 Performance Accuracy

Muscimol and systemic amphetamine treatment produced a main effect of drug treatment on the total percent of correctly completed trials ($F(5, 40) = 7.213$, $p = 0.001$). Post hoc analysis revealed a reduction in the total percent of correctly completed trials following vehicle infusion + systemic amphetamine in comparison to the control treatment (0 ng + 0 mg/kg amphetamine) ($p < 0.05$) and a non-significant trend towards an increase in accuracy was found following 960 ng muscimol + systemic amphetamine in comparison to vehicle infusion + systemic amphetamine ($p = 0.07$) and in comparison to 120 ng muscimol + systemic amphetamine ($p = 0.08$). No significant differences in the total percent of correctly completed trials were detected between remaining muscimol + systemic amphetamine treatment combinations (all $p > 0.05$) (Fig. 7.9). Independent analysis of Go trials revealed no main effect of drug treatment ($F(5, 40) = 1.269$, NS) (Fig. 7.10), however, independent analysis of No-go trials did reveal a significant main effect of drug treatment ($F(5, 40) = 7.182$, $p = 0.001$). Post hoc analysis subsequently revealed a reduction in No-go trial accuracy following vehicle infusion + systemic amphetamine in comparison to control treatment ($p < 0.01$), and a non-significant trend towards a significant increase in No-go trial accuracy following 960 ng muscimol + systemic amphetamine in comparison to 120 ng muscimol + systemic amphetamine treatment was detected ($p = 0.07$) (Fig. 7.11). No significant differences in No-go trial accuracy were detected between remaining muscimol + systemic amphetamine treatment combinations (all $p > 0.05$).

7.3.2.2 Speed of Responding

Muscimol and systemic amphetamine treatment produced a main effect of drug treatment on Go trial response latencies ($F(5, 40) = 3.016$, $p < 0.05$) (Fig. 7.16), however, post hoc tests failed to reveal any significant differences in the speed of responding during Go trials between specific muscimol + systemic amphetamine treatment combinations (all $p > 0.05$). Analysis of No-go trial response latencies ($F(5, 40) = 1.771$, NS), Go trial magazine latencies ($F(1.765, 14.118) = 1.653$, NS) and No-go trial magazine latencies ($F(1.700, 13.598) = 0.483$, NS) all revealed no main effect of drug treatment (Figs. 7.17 – 7.19).

7.3.2.3 Anticipatory Responding

Analysis of Go trial early responses revealed no main effect of drug treatment ($F(5, 40) = 1.435$, NS) (Fig. 7.24). Analysis of No-go trial early responses, however, produced a main effect of muscimol and systemic amphetamine treatment ($F(5, 40) = 5.229$, $p = 0.001$), however, post hoc analysis of this effect only detected a trend towards a significance increase in No-go trial early responding following 0 ng muscimol + systemic amphetamine in comparison to control treatment ($p = 0.08$) and a trend towards a reduction in No-go trial early responses following 960 ng muscimol + systemic amphetamine in comparison to vehicle infusion + systemic amphetamine treatment ($p = 0.07$) (Fig. 7.25). No-go trial early responses did not significantly differ between any remaining muscimol + systemic amphetamine treatment combinations (all $p > 0.05$). Analysis of Go trial inappropriate panel responses ($F(1.560, 12.478) = 0.987$, NS) and No-go trial inappropriate panel responses revealed no main effect of drug treatment ($F(5, 40) = 0.883$, NS) (Figs. 7.26 – 7.27).

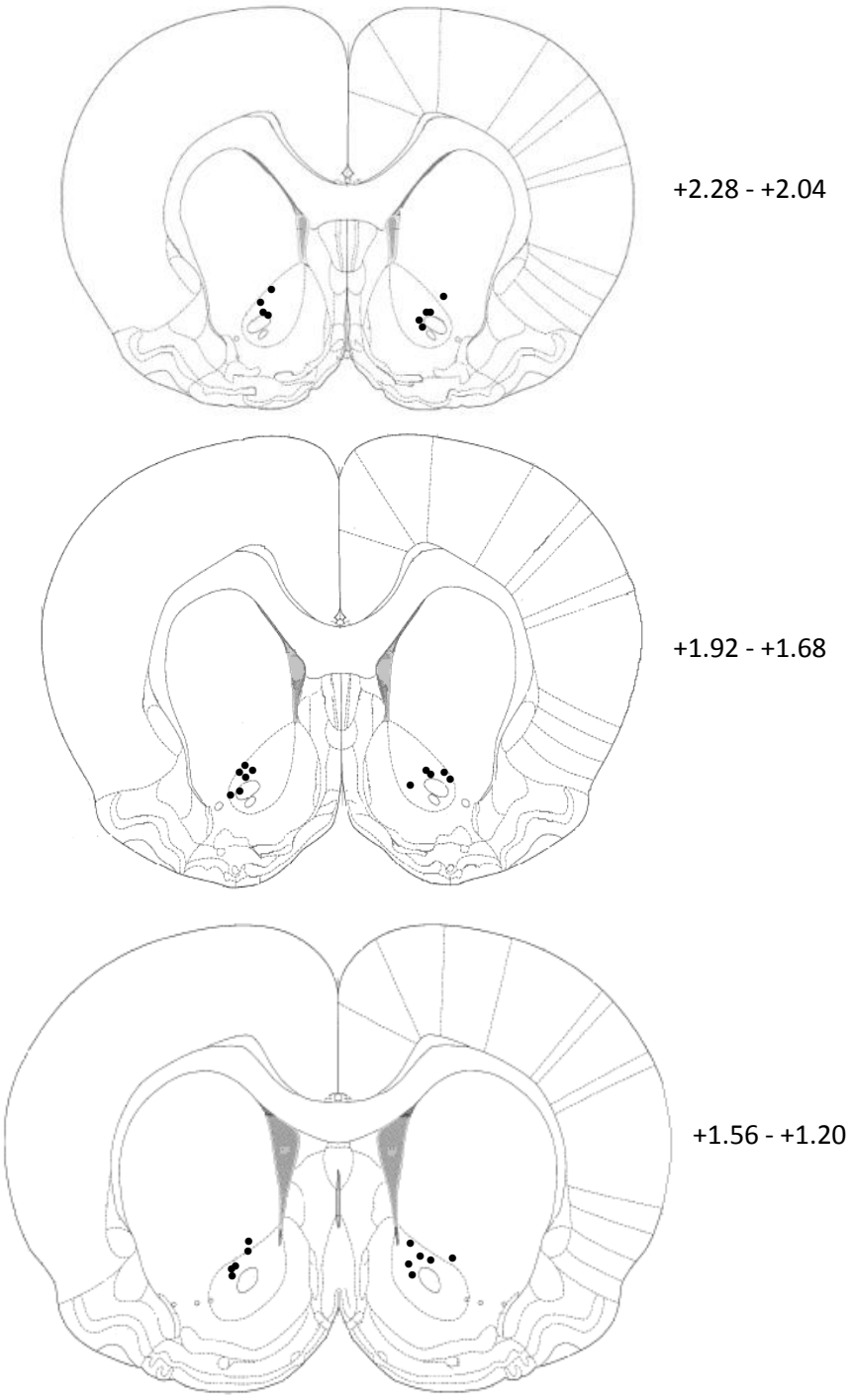


Figure 7.1: Schematic illustrations of the rat brain taken from Paxinos and Watson Atlas (1998) demonstrating the location of injector tips relative to bregma in experiments 6 and 7.

Fig. 7.2

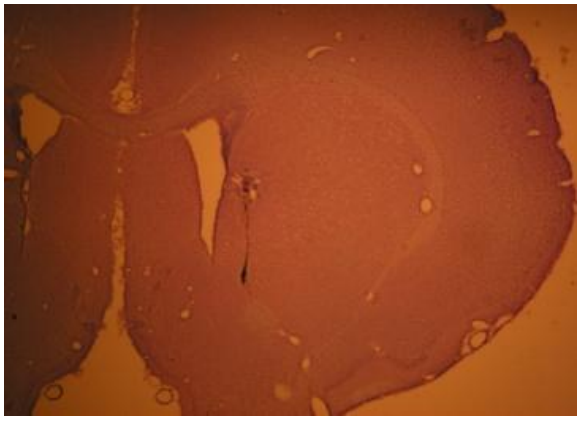


Fig. 7.3

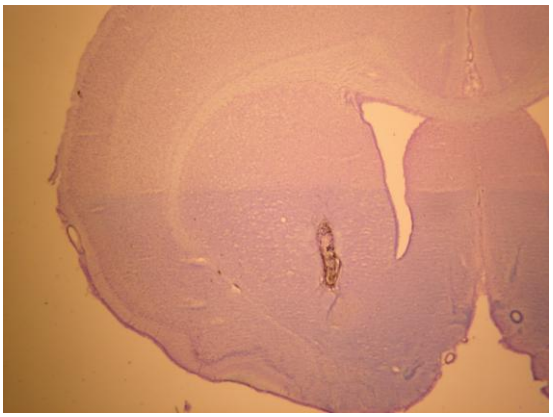


Fig. 7.4

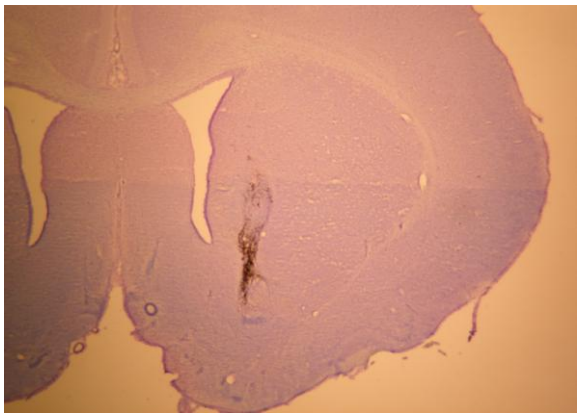


Fig. 7.5

Figures 7.2 – 7.5: Example pictures of brain slices taken during histology to confirm the location of injector tips in the Nucleus Accumbens core. Pictures are taken from an animal treated with muscimol **(7.2: left hemisphere; 7.3: right hemisphere)**, and an animal treated with muscimol and systemic amphetamine **(7.4 left hemisphere; 7.5: right hemisphere).**

Muscimol

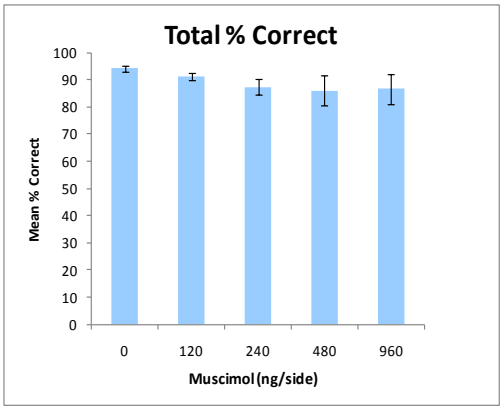


Fig. 7.6

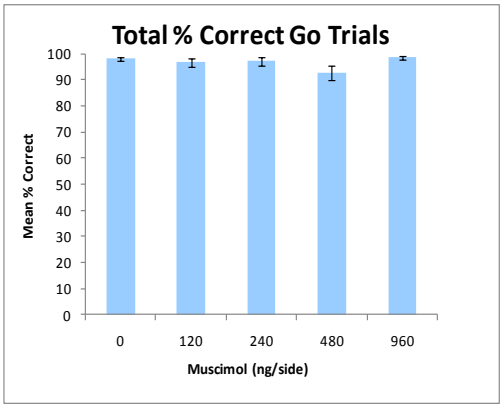


Fig. 7.7

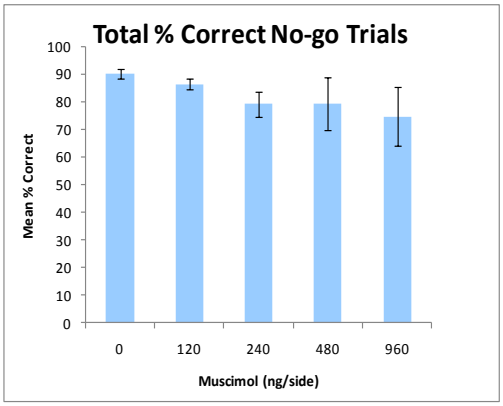


Fig. 7.8

Muscimol + Amphetamine

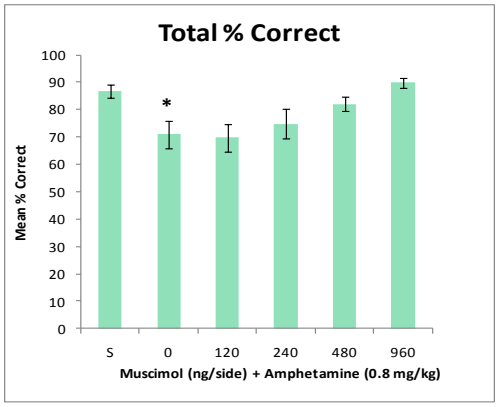


Fig. 7.9

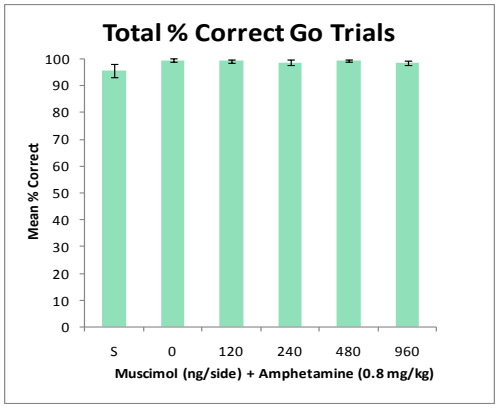


Fig. 7.10

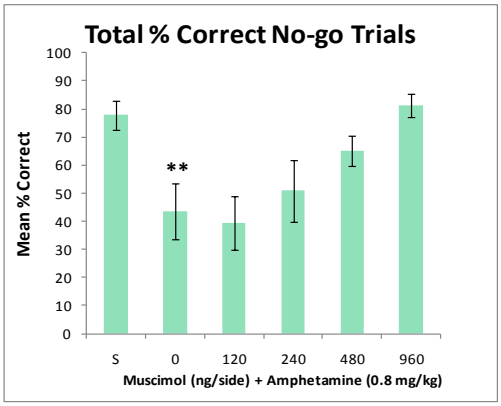


Fig. 7.11

Figures 7.6 – 7.11: Performance following muscimol infusions into the NAcb core on **(7.6)** the total percent correct of trials, **(7.7)** the total percent correct Go trials, **(7.8)** the total percent correct No-go trials, **(7.12)** Go trial latency, **(7.13)** No-go trial latency, **(7.14)** Go trial magazine latency, **(7.15)** No-go trial magazine latency, **(7.20)** Go trial early responses, **(7.21)** No-go trial early response, **(7.22)** Go trial panel response and **(7.23)** No-go trial panel response. Performance following muscimol infusions into the NAcb core and systemic amphetamine on **(7.9)** the total percent correct of all trials, **(7.10)** the total percent correct Go trials, **(7.11)** the total percent correct No-go trials, **(7.16)** go trial latency, **(7.17)** no-go trial latency, **(7.18)** go trial magazine latency, **(7.19)** no-go trial magazine latency, **(7.24)** go trial early responses, **(7.25)**no-go trial early response, **(7.26)** go trial panel response and **(7.27)** no-go trial panel response. *p<0.05, **p<0.01 and ***p<0.001 vs vehicle infusion + systemic saline combination. 'S' represents the vehicle infusion + systemic saline control combination. Values represent means and error bars represent SEM.

Muscimol

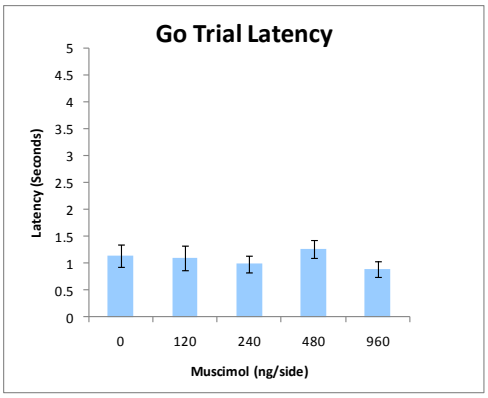


Fig. 7.12

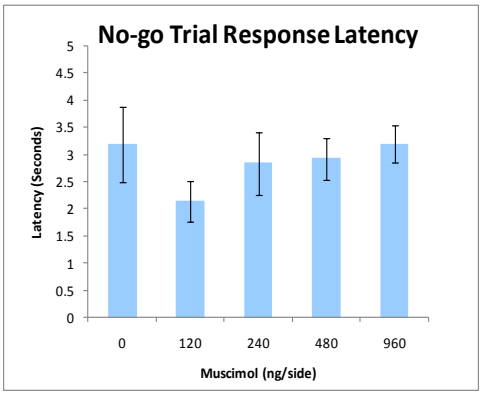


Fig. 7.13

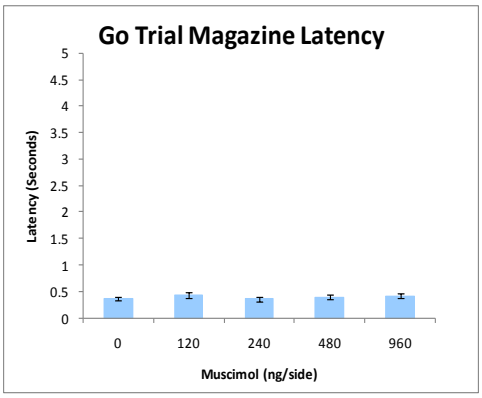


Fig 7.14

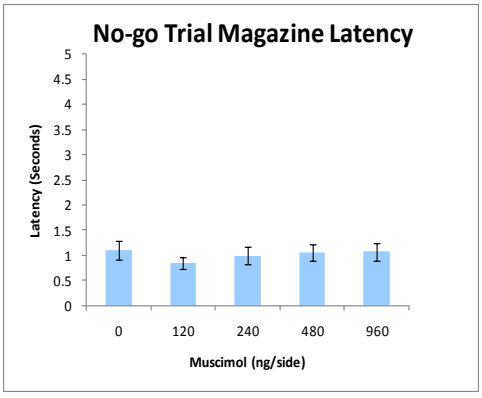


Fig. 7.15

Muscimol + Amphetamine

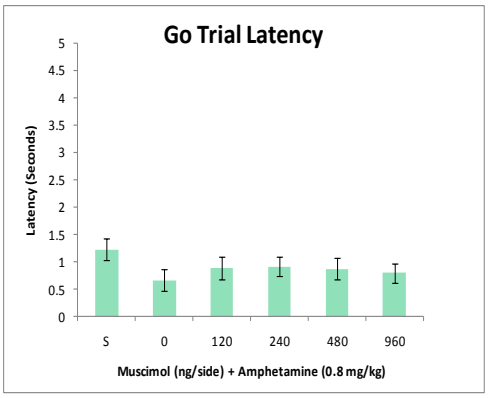


Fig. 7.16

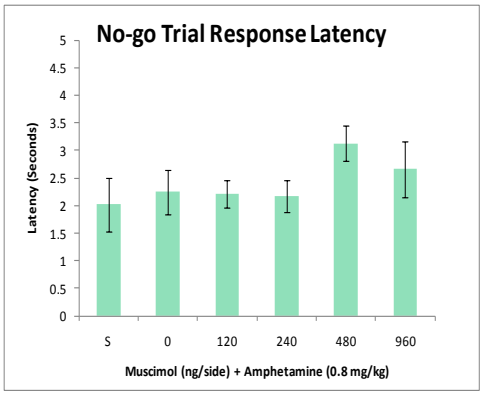


Fig. 7.17

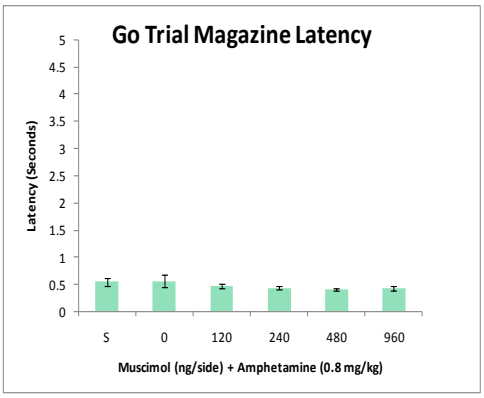


Fig. 7.18

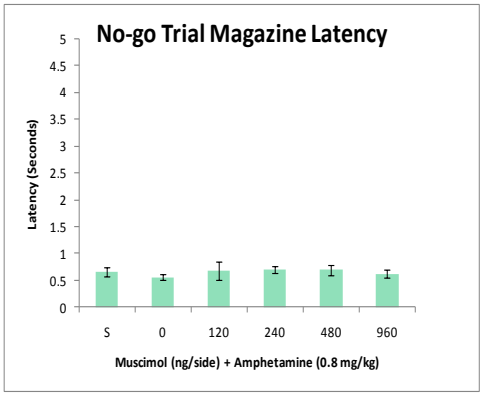
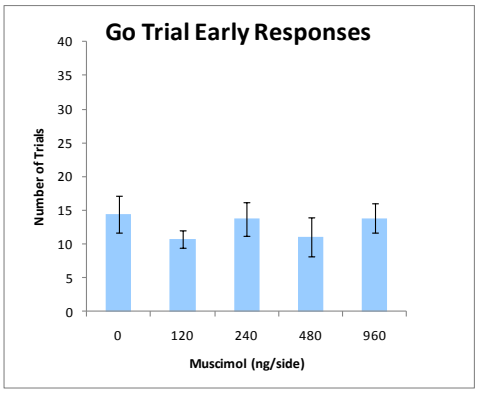


Fig. 7.19

Muscimol



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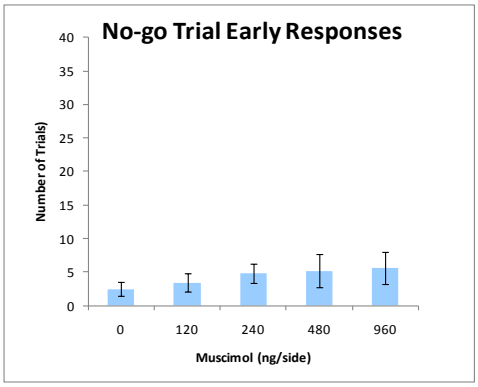

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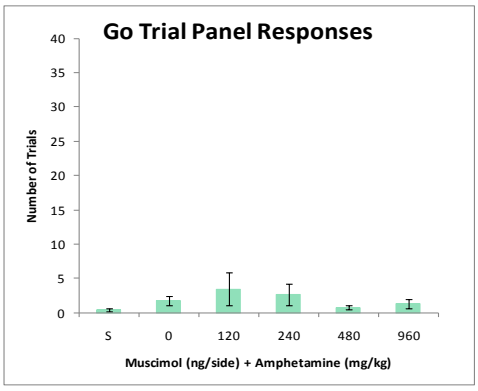

Fig. 7.22

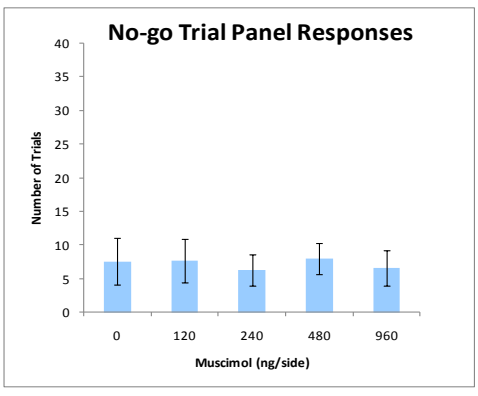

Fig 7.23

Muscimol + Amphetamine

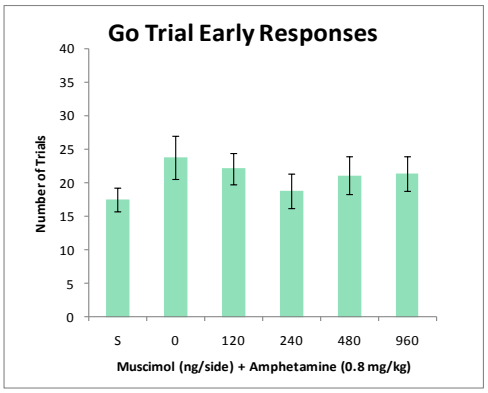

Fig. 7.24

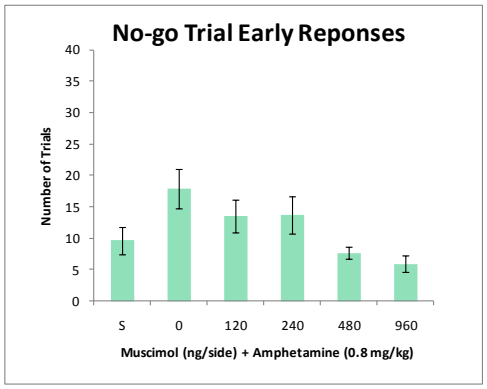

Fig. 7.25

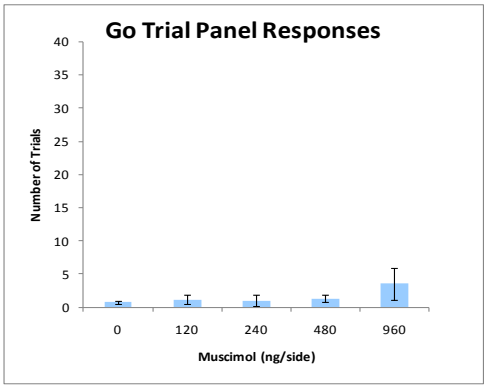

Fig. 7.26

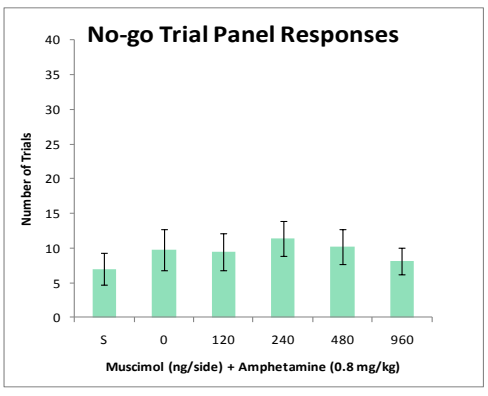

Fig 7.27

7.4 Discussion

Intra-NAcb core muscimol infusions did not disrupt accuracy, speed or anticipatory responding, indicating that under basal conditions, stimulation of the $GABA_A$ receptors within the NAcb core does not mediate behavioural inhibition measured by the Go/No-go symmetrically reinforced task. Treatment with vehicle infusion and systemic amphetamine led to a reduction in No-go trial accuracy in comparison to control treatment, indicating that animals became acutely more impulsive following systemic amphetamine. No-go trial accuracy did not differ from control treatment following intra-NAcb core muscimol infusions in combination with systemic amphetamine, indicating that activation of the $GABA_A$ receptors within the NAcb core led to partial reversal of amphetamine induced impulsivity on the symmetrically reinforced Go/No-go task.

7.4.1 The effects of muscimol infusions into the NAcb core on behavioural inhibition

Although systemic administration of muscimol can produce sedative (sleep inducing) effects in rats (DeFeudis, 1980; Lancel, Crönlein, & Faulhaber, 1996; Scheel-Krüger, Christensen, & Arnt, 1978) no changes were observed in response latencies following intra-NAcb core infusions of muscimol. This suggests that activation of the $GABA_A$ receptors within the NAcb core does not lead to sedation in rats performing a symmetrically reinforced Go/No-go task. Considering that the sedative effects of GABAergic drugs has been located to activation of the $GABA_A$ receptors (Reynolds et al., 2003; Rowlett, Platt, Lelas, Atack, & Dawson, 2005; Rudolph et al., 1999; Vinkers et al., 2009) the lack of change in response latencies following $GABA_A$ receptor activation might appear surprising. However, despite the role of the $GABA_A$ receptors in sedation, there is little direct evidence linking the $GABA_A$ receptors within the NAcb core specifically to the sedative effects of muscimol. Sedation via $GABA_A$ receptor activation has rather been neurally located to anatomical regions constituting sleep pathways, such as the tuberomammillary nucelus in the hypothalamus (Nelson et al., 2002). In following, the lack of sedation observed in rats following intra-NAcb core infusions of muscimol might relate to the fact the NAcb core is not anatomically connected with neural sleep pathways.

Infusions of muscimol (100 ng) and GABA (33 ng) into the NAcb has, however, previously been shown to reduce locomotor activity in rats, measured by photobeam counts (Andén, Grabowska-Andén, & Wachtel, 1979; Jones, Mogenson, & Wu, 1981), and these effects have been assumed to reflect GABA induced inhibition of dopamine mediated locomotor behaviour (Arnt, Christensen, & Scheel-Krüger, 1978; Jones et al., 1981; Scheel-Krüger et al., 1978). In

addition, intra-NAcb core muscimol-baclofen ($GABA_B$ agonist) infusions (combined dose 75 ng) can lead to slower response speed in rats performing operant behavioural tasks (Floresco et al., 2006; Ghods-Sharifi & Floresco, 2010). These studies therefore contrarily suggest that muscimol infusions into the NAcb core might be expected to produce some reduction of motor activity as a consequence of GABA-dopamine interactions within the NAcb that mediate locomotor activity in rats (Arnt et al., 1978; Jones et al., 1981). In addition, considering that the dose range employed in the current study was much higher than the doses employed by these studies, this further suggests that the lack of changes in response latencies following muscimol infusions into the NAcb core appear surprising.

However, intra-NAcb infusions of a high muscimol dose (500 ng), comparable to the 480 ng dose in this study, does not change active or inactive lever responding in rats performing a FR1 schedule of food reinforcement (Yoon et al., 2009) indicating that high doses of muscimol infused into the NAcb does not necessarily lead to a changes in motor behaviour that might affect operant performance. Furthermore, infusions of the $GABA_B$ agonist baclofen into the NAcb have been shown to significantly suppress locomotor behaviour, whist infusion of $GABA_A$ agonists into the NAcb do not significantly change locomotor activity (Wong, Eshel, Dreher, Ong, & Jackson, 1991). Such differences in $GABA_A$ vs $GABA_B$ receptor mediation of locomotor activity within the NAcb core might then explain why muscimol-baclofen infusions into the NAcb core can produce changes in response latencies on behavioural tasks (Floresco et al., 2006; Ghods-Sharifi & Floresco, 2010) but muscimol infusions alone into the NAcb core did not affect response latencies in the current experiment. In addition, Floresco et al (2006) detected slower response latencies in rats during a maze based task whereby changes in locomotor activity would be more easily detected than on the Go/No-go task as trial completion on this task requires greater locomotor activity than trial completion on the Go/No-go task. In addition, slower response latencies reported by Ghods-Sharifi & Floresco (2010) were identified in an effort discounting task where animals could make choices in the magnitude of effort engaged in to obtain reward. This type of task enables a greater level of individual difference in response behaviour between rats than the Go/No-go task, and therefore changes in locomotor activity might be more easily detected on this task than on the Go/No-go task where animals do not have a choice in the behaviour they must employ to on each trial.

In following, whilst there is literature to suggest that increasing, or mimicking, GABA activity within the NAcb core might lead to changes in locomotor activity (Jones et al., 1981), changes in the speed of responding following GABA agonists into the NAcb core has only been observed alongside $GABA_B$ agonism and on tasks where there is greater motor activity required and greater sensitivity to individual differences in animal behaviour than on the

Go/No-go task employed in the current set of experiments. Consequently, changes in $GABA_A$ activation within the NAcb core on the Go/No-go symmetrically reinforced task, where behavioural responding is relatively controlled, would not necessarily be expected to produce the same behavioural effects as these studies.

The lack of drug induced changes of magazine latencies observed following muscimol infusions into the NAcb core is also indicative that muscimol did not produce any changes in motivation for food reward. These effects are generally consistent with previous literature that has implicated GABA activity within the NAcb shell, but not the core, in the motivation to earn food reward on progression ratio tasks (Basso & Kelley, 1999; Stratford & Kelley, 1997; Zhang, Balmadrid, & Kelley, 2003).

Increased variance at higher muscimol doses was observed in No-go trial accuracy (Fig. 7.8). The decrease in No-go trial accuracy and increase in variance observed as muscimol doses increase is representative of one animal in the group displaying decreased No-go trial accuracy as muscimol doses were increased. This change in No-go trial accuracy was, however, only observed in one animal. When this animal's placement was checked it was found to fall within the boundaries of the NAcb core (injector tips: +1.20 anterior to bregma left and right hemispheres) therefore the animal remained in the inclusion of data analysis. This visual change in No-go trial accuracy and increase in variance is therefore a consequence of the variance contributed by one animal that displayed decreased No-go trial accuracy following muscimol infusions.

In summary, the lack of change in any performance measures following muscimol infusions into the NAcb core on the symmetrically reinforced Go/No-go task indicates that $GABA_A$ receptor agonism within the NAcb core does not affect impulsivity, response speed or motivation to perform the task. Whilst the lack of effects on this task might initially appear surprising, considering that previous studies have found behavioural effects at lower doses to that employed in the current study, on closer analysis of these studies the specific role for the NAcb core vs. shell and $GABA_A$ vs $GABA_B$ in the mediation of sedative, locomotor and motivational effects can account for some of the differences in results between studies. Consequently, the current findings expand on previous literature by demonstrating that a wide range of muscimol doses infused into the NAcb core does not affect impulsivity, response speed or motivation for food reward in rats.

7.4.2 The effects of muscimol infusions into the NAcb core on amphetamine induced behavioural disinhibition

The combination of intra-NAcb core muscimol infusions and systemic amphetamine treatment was found to significantly affect Go trial response latencies. Although no significant post hoc effects were detected following this main effect, Go trial response latencies were faster following vehicle infusion and systemic amphetamine than the control treatment (Fig. 7.16). This is consistent with faster response latencies reported following systemic amphetamine on the 5CSRTT in rats (Harrison, Everitt, & Robbins, 1997; Pattij et al., 2007). However, the lack of significant difference between individual treatment combinations indicates that any changes in the speed of responding were not substantial. The lack of significant change in the speed of responding following drug treatment also indicate that any amphetamine induced changes in timing perception in rats (Meck, 1996) appear not to have affected the timing of response behaviour on the Go/No-go task. Consequently, amphetamine induced changes in locomotor activity or timing perception do not appear to have substantially affected response behaviour in rats performing the Go/No-go task. As such, changes in motor activity and timing perception are unlikely to account for amphetamine induced impulsivity observed, or for the apparent muscimol induced partial reversal of amphetamine induced impulsivity.

Considering that GABA-dopamine interactions within the NAcb have been found to mediate locomotor activity in rats (Jones et al., 1981) the lack of change in response latencies might appear surprising. Muscimol infusions into the NAcb core have previously been shown to reduce stimulant (cocaine, methylphenidate) induced increases in locomotor activity (Scheel-Krüger et al., 1978). However, indications of amphetamine induced changes in the speed of responding were subtle in the current experiment as no post hoc differences in the speed of responding were found on Go trial response latencies (Fig. 7.16). This might suggest that any GABA attenuating effects on amphetamine induced effects on motor behaviour were subsequently less likely to be detected due to the fact amphetamine only subtly changed the speed of responding in rats. In following, although previous studies have implicated GABA-dopamine interactions within the NAcb on stimulant induced motor activity (Arnt et al., 1978; Jones et al., 1981; Scheel-Krüger et al., 1978) such effects might not have been detected in the current experiment due to the fact that any amphetamine induced changes in locomotor activity did not significantly change the speed of responding between doses in rats on the Go/No-go task.

The lack of drug effects on the speed to collect the food reward also indicates that animals remained motivated to perform the Go/No-go task and receive food reward following intra-NAcb core muscimol infusions and systemic amphetamine treatment. Although amphetamine can increase motivation to earn food reward in rats (Bardgett et al., 2009) suggesting that magazine latencies might have been expected to increase following systemic amphetamine, the lack of change between intra-NAcb core muscimol and systemic amphetamine combination treatments is consistent with GABAergic activity NAcb shell, and not core, in mediating motivation to earn food reward (Basso & Kelley, 1999; Stratford & Kelley, 1997; M. Zhang et al., 2003). Unaffected magazine latencies therefore additionally indicate that intra-NAcb core muscimol infusions in combination with systemic amphetamine did not caused any changes in motivation that can account for the muscimol induced changes in No-go trial accuracy.

Intra-NAcb core muscimol infusions and systemic amphetamine treatment was found to significantly affect No-go trial early responses (Fig. 7.25). Although post hoc tests failed to produce any significant findings, a non-significant trend towards a significant increase in No-go trial early responses following vehicle infusion and systemic amphetamine in comparison to control treatment ($p = 0.08$) and non-significant trend towards a reduction following 960 ng muscimol and amphetamine in comparison to the vehicle infusion and amphetamine ($p = 0.07$) were detected. These p-values indicate trends towards amphetamine induced impulsivity and muscimol (960 ng) induced reversal of impulsivity, when measured by anticipatory responding. These trends are therefore consistent with amphetamine induced impulsivity observed on No-go trials and muscimol induced partial reversal of amphetamine induced impulsivity measured by No-go trial accuracy.

Partial reversal of amphetamine induced impulsivity via activation of the $GABA_A$ receptors within the NAcb core presents novel findings in relation to the neural substrates mediating amphetamine induced impulsivity. These findings are the first to indicate that changes in GABA transmission within the NAcb core is involved in drug induced impulsivity, and specifically amphetamine induced impulsivity. A relationship between GABA and impulsivity is consistent with recent research identifying that changes in GABA in the rat prefrontal cortex is involved in impulsivity on the 5CSRTT, and that the expression of the $GABA_A$ receptor within regions of the frontal cortex (ACC) is associated with highly impulsive rats on the 5CSRTT (Asinof & Paine, 2012; Jupp et al., 2013; Murphy et al., 2012). Evidence that GABA activity within the NAcb core plays a role in mediating drug induced impulsivity consequently builds on this previous literature by indicating that GABA transmission within ventral regions of the striatum is involved in mediating drug induced impulsivity.

Full behavioural reversal of amphetamine induced impulsivity, however, was not detected in the analysis of No-go trial accuracy, and interestingly, no approaching significance was detected between any muscimol dose in combination with systemic amphetamine in comparison to vehicle infusion and systemic amphetamine. Despite the overall mean for the highest muscimol (960 ng) and systemic amphetamine dose visually appearing to fully reverse (Fig. 7.11), from closer inspection of the data presented in experiment 7, four out of the nine rats that received muscimol and systemic amphetamine treatment did not fully reverse following the highest muscimol dose and systemic amphetamine (range: 7.5 – 35 % lower than No-go trial accuracy following control combination), and out of the five animals that did display full behavioural reversal, performance following 960 ng muscimol and systemic amphetamine tended to surpass No-go trial accuracy following control treatment (range: + 7.5 – 32.5 % higher than No-go trial accuracy following control combination) (See Appendix 7). This means that although the average of No-go trial accuracy following 960 ng muscimol and systemic amphetamine visually appears to have produced full behavioural reversal, only five out of the nine rats actually displayed this 'full' profile of reversal, whilst a convincing number of the animals tested did not fully reverse. This level of variance at the 960 ng muscimol dose consequently explains why, despite this effect visually appearing to have produced full behavioural reversal, this is not statistically close to significance.

However, despite the high variation in No-go trial accuracy at the highest muscimol doses, all rats did demonstrate higher No-go trial accuracy at higher muscimol doses in combination with systemic amphetamine (480, 960 ng) than vehicle infusion and lower muscimol doses in combination with systemic amphetamine (120, 240 ng) (Fig. 7.11), supporting that higher doses of muscimol treatment did induce some reversal of amphetamine induced impulsivity. In addition, the lack of significant difference between all muscimol doses and systemic amphetamine in comparison control treatment indicates that muscimol did cause some reversal from vehicle infusion and systemic amphetamine treatment.

The $GABA_A$ receptors are located both presynaptically on dopamine terminals innervating the NAcb and postsynaptically on medium spiny projection neurons and aspiny interneurons within the NAcb (Ferraro et al., 1996; Krebs, Kemel, Gauchy, Desban, & Glowinski, 1993; Ronken, Mulder, & Schoffelmeer, 1993; Schwarzer et al., 2001). It is most likely that the muscimol induced changes in No-go trial accuracy represent a presynaptic action of muscimol at the $GABA_A$ receptors located on dopaminergic nerve terminals innervating the NAcb core. This location of muscimol action would consequently cause an increase in presynaptic $GABA_A$ mediated inhibition over dopamine release into the NAcb core. Given that dopamine activity within the NAcb core has been found to mediate amphetamine induced impulsivity (Pattij et

al., 2007; Chapter 6, Section 6.3) this location of muscimol action within the NAcb core fits with the existing neurochemical framework within the NAcb core that is assumed to mediate amphetamine induced impulsivity. Muscimol infusions into the NAcb of anesthetised rats has previously been found to reduce dopamine concentration, whilst the $GABA_A$ antagonist bicuculline increases dopamine concentration in the NAcb in both anesthetized and freely moving rats (Ferraro et al., 1996; Yan, 1999). These findings therefore indicate that the $GABA_A$ receptors within the NAcb exert an inhibitory role over dopamine innervation into the NAcb, consistent with an inhibitory influence of muscimol over NAcb core dopamine induced impulsivity.

However, opposing effects of muscimol on dopamine concentration within the NAcb have been reported when muscimol is infused into the NAcb of freely moving rats. Aono et al., (2008) found that muscimol produces an increase in basal dopamine flux within the NAcb in freely moving rats, contrarily suggesting the possibility that muscimol infused into the NAcb might bind to postsynaptic $GABA_A$ receptors located on inhibitory interneurons, consequently inhibiting the release of GABA at dopamine terminal innervating the core, leading to an increase in dopamine release. However, it is also noteworthy that Aono et al., (2008) report that the $GABA_A$ antagonist bicuculline also increase basal dopamine flux within the NAcb. Although animals in the Aono et al., (2008) study are comparable to the animals in the current study because they were freely moving rats, a postsynaptic action of muscimol within the NAcb core is unlikely to explain muscimol induced changes in No-go trial accuracy as a muscimol induced increase in dopamine within the NAcb core would mimic the actions of amphetamine in the core, and would therefore be expected to potentiate amphetamine induced impulsivity. A postsynaptic action of muscimol within the NAcb core is therefore at odds with muscimol induced increases in No-go trial accuracy. In addition, the infusion of muscimol by Aono et al., (2008) is much lower (25- 200 pmol) than that employed in the current study (120 – 960 ng), indicating that the action of muscimol within the NAcb reported by Aono et al., (2008) and the current study might not be comparable.

In following, given that an action of muscimol within the NAcb core at the $GABA_A$ receptors located on GABAergic interneurons is at odds with muscimol induced increases in No-go trial accuracy, alongside the fact that much higher doses were employed in the current study to Aono et al., (2008), this suggest that changes in amphetamine induced impulsivity in the current study is more likely to reflect a presynaptic action of muscimol at the $GABA_A$ receptors located on the terminal of dopamine neurons innervating the NAcb core. If muscimol did produce the apparent partial reversal of amphetamine induced impulsivity through presynaptic activation of the $GABA_A$ receptors located on dopamine terminals innervating the

NAcb core, this might subsequently suggests muscimol indirectly reduced the effects of amphetamine induced dopamine activity at the D_2 receptors within the NAcb core that are known to mediate amphetamine induced impulsivity in rats (Pattij et al., 2007; Chapter 6, Section 6.3).

Interestingly, however, the data set did produce some indications of muscimol induced potentiation of amphetamine induced impulsivity at the lowest (120 ng) doses in a subset of animals (See Appendix 7). Although these effects were non-significant, this suggests that lower doses of muscimol might have acted at different $GABA_A$ receptor locations (presynaptic vs postsynaptic) within the NAcb core than higher muscimol doses in some animals. These non-significant changes in the data set are generally consistent with previous observations that intra-NAcb muscimol infusions can produce biphasic effects on behaviour in rats (Scheel-Krüger et al., 1980) possibly in relation to different pre-synaptic and post-synaptic $GABA_A$ targets within the NAcb.

7.4.3 Key Findings

The main findings from this study are that infusion of the $GABA_A$ agonist muscimol into the nucleus accumbens core does not affect baseline levels of response inhibition but can partially reverse amphetamine induced response disinhibition in rats. These findings suggest that changes to GABA function within the nucelus accumbens core might be involved in drug induced impulsivity. The main aim of the current study was to assess the involvement of GABA in response inhibition and amphetamine induced response disinhibition on the Go/No-go symmetrically reinforced task. These aims attempted to address broader objectives into the neurochemical mediators of impulsivity and drug induced impulsivity within the ventral striatum. The observation that muscimol partially revered amphetamine induced impulsivity, but did not affect baseline levels of response inhibition, consequently addresses these objectives by confirming that GABA activity might be specifically involved in drug induced impulsivity.

7.4.4 Limitations

The main limitation in the design of this study is that through administering muscimol to test GABAergic involvement in response inhibition and amphetamine induced response disinhibition, the partial reversal of amphetamine induced impulsivity observed may reflect either a pharmacological effect involving the $GABA_A$ receptors, or a GABA induced reversible lesion of the nucelus accumbens core. It is therefore difficult to interpret whether the $GABA_A$

receptors are specifically involved in mediating amphetamine induced impulsivity or whether through inhibiting the nucelus accumbens as an entire site was responsible for partial reversal of impulsivity. It should therefore be noted that the current study does not confirm GABAergic involvement in drug induced impulsivity exclusively but might instead represent more general nucleus accumbens core site involvement in amphetamine induced impulsivity.

7.4.5 Future Research

Having established that mimicking GABAergic activity within the nucleus accumbens core can partially reverse amphetamine induced response disinhibition on the Go/No-go task, it would be useful for future research to more directly assess the role of the $GABA_A$ and $GABA_B$ receptors within the nucleus accumbens core and shell in response disinhibition. Such research efforts would help to elucidate whether specific changes in GABA sub-receptors are involved in the ventral striatal neuropathology linked to impulsivity and drug addiction. In addition, it would be useful for future research to investigate involvement of GABAergic activity within regions of the prefrontal cortex in response inhibition measured by the Go/No-go task. Changes in GABA concentration in regions of the prefrontal cortex have been linked to trait impulsivity in human subjects and direct manipulation of GABA in the infralimbic cortex of rats has been found to affect premature responding in rats (Boy et al., 2011; Murphy et al., 2012). It would therefore be useful to further investigate the role of GABA in response inhibition in on the Go/No-go task which can more directly ascertain response inhibition than the 5CSRTT. Further research aiming to further elucidate the relationship between GABAergic changes and impulsivity will be useful for considering how different neurochemical pathways may contribute towards impulsivity and addiction. Subsequently, understanding this relationship better will help give insight into potential novel pharmacological approaches for the treatment of drug addiction.

7.4.6 Conclusions

In conclusion, the lack of effects observed following $GABA_A$ activation within the NAcb core on impulsivity, alongside NAcb core $GABA_A$ mediated partial reversal of amphetamine induced impulsivity, implicates a role for GABA transmission within the NAcb core in amphetamine induced impulsivity. These findings consequently expand on previous animal and human literature implicating a role for GABA transmission in impulsive behaviour (Asinof & Paine, 2012; Boy et al., 2011; Edden et al., 2012; Jupp et al., 2013; Murphy et al., 2012) by identifying a novel anatomical location whereby changes in GABA activity can reduce drug induced impulsivity in rats. These findings also expand on previous literature that has implicated

dopamine activity within the NAcb core in amphetamine induced impulsivity in rats (Pattij et al., 2007; Chapter 6, Section 6.3) by indicating that changes in both dopamine and GABA activity within the NAcb core might be involved in the neurochemical pathology of amphetamine induced impulsivity in rats. These findings therefore provide novel findings into the neural substrates mediating amphetamine induced impulsivity in rats.

Chapter 8 General Discussion

The current sets of experiments provide evidence that sub-chronic and chronic amphetamine treatment can cause transient reductions in behavioural inhibition in rats measured by a symmetrically reinforced Go/No-go task. In addition, the D_2 receptors, and to a lesser extent $GABA_A$ receptors, within the NAcb core were identified as neural substrates mediating amphetamine induced impulsivity on the Go/No-go task. These results therefore present novel findings on the relationship between subchronic and chronic amphetamine use and impulsivity and on the neural substrates of amphetamine induced impulsivity. These findings will be discussed below.

8.1 Subchronic and chronic amphetamine and impulsivity

8.1.1 Feeding behaviour and impulsivity observed during 4-day amphetamine treatment and amphetamine withdrawal

Reduced food intake observed during 4-day amphetamine treatment and for up to 60 hours of amphetamine withdrawal suggests that this amphetamine regime might cause some alterations in the motivation for food in rats. Interestingly, however, no significant changes in magazine latencies were detected amongst rats treated with the 4-day amphetamine regime in comparison to controls, either during amphetamine treatment or during withdrawal from amphetamine. This suggests that animals treated with 4-day amphetamine conversely remained active and motivated to gain food reward during drug treatment and withdrawal. The combination of these findings therefore suggests that whilst 4-day amphetamine treatment might produce some motivational effects on food intake in rats, any such changes did not appear to affect motivation to receive food reward on the Go/No-go task. These differences might relate to differences in the baseline motivational state of animals between studies (eg. free-feeding vs. food-restricted). The lack of change in magazine latencies amongst animals that were food restricted might suggest that the effects of 4-day amphetamine on motivation for food were less disruptive to these animals than animals on a free-feeding schedule that had access to food at any time. The combination of these results suggests that the food restriction schedule employed in Chapter 4 might prevent significant treatment induced changes in feeding motivation that might disrupt performance on the Go/No-go task.

8.1.2 Patterns of amphetamine use and impulsivity

Treatment with a 4-day amphetamine binge and 11-day chronic amphetamine regime both reduced behavioural inhibition in rats during drug treatment. The combination of these results demonstrates that amphetamine can increase impulsivity during different patterns of use. Amphetamine can be abused in a variety of different settings and for different purposes (Davey, Richards, & Freeman, 2007; Degenhardt et al., 2007; Hall, Darke, Ross, & Wodak, 1993; McCabe, Teter, & Boyd, 2006; Riley, James et al., 2001; Teter et al., 2006) meaning that these findings are particularly useful for elucidating how different patterns of amphetamine use per se might affect impulsivity amongst amphetamine users.

Different patterns of drug use can occur at different stages of addiction (Perry, 2008). In following, the 4-day and 11-day amphetamine regimes can be broadly mapped onto different stages of addiction. For example, the binge regime most closely maps onto a stage of escalating drug use, whilst the longer-term 11-day treatment more closely maps onto a stage of maintained drug use. Amphetamine induced impulsivity during both of these regimes therefore supports that behavioural inhibition might change as a consequence of the pattern of drug use employed during distinct stages of addiction. Increased impulsivity during escalation and maintenance of amphetamine use might then increase the chance of subsequent drug use as a consequence of reduced inhibitory control. In addition elevated impulsivity might reduce inhibitory control over other risk factors for addiction during phases of escalation and maintenance. For example, stress can facilitate escalation and maintenance of stimulant use in rats (Miczek et al., 2004; Piazza & Le Moal, 1996) and is a prominent risk factor for escalating drug use in humans (Sinha, 2008). Drug induced reductions in behavioural inhibition might then reduce inhibitory control over concurrent risk factors for addiction, such as stress, rendering drug users more vulnerable to developing, and or maintaining, drug addiction.

Interestingly, previous investigations into the relationship between impulsivity and phases of escalation and maintenance have found that greater levels of trait impulsivity can increase escalation but not the maintenance of stimulant use in rats (Anker et al., 2009; Belin et al., 2008; Dalley et al., 2007; Perry et al., 2005; Perry, Nelson, & Carroll, 2008). When considered with the present findings, this might suggest that greater levels of trait impulsivity can differentially affect the vulnerability to distinct stages of addiction, whilst the direct effects of stimulant use may produce common effects in elevating impulsivity during distinct stages of addiction. However, as the effects of amphetamine on impulsivity interact with baseline levels of inhibitory control in animals and humans, and stimulants can in fact improve inhibitory

control in highly impulsive subjects (Dalley, Fryer, et al., 2007; de Wit et al., 2002), amphetamine induced deficits in behavioural inhibition observed amongst animals in the current set of experiments might more accurately represent the causal effects of amphetamine on impulsivity in a population that display an existing level of inhibitory control at baseline.

Whilst the 4-day and 11-day amphetamine regimes both produced a common outcome of increased impulsivity during drug use, the time course of which these effects developed was different. During treatment with the 4-day amphetamine binge a significant reduction in behavioural inhibition was observed following a cumulative dose of 6 mg/kg (day 1: 1, 2, 3 mg/kg) (See Figs. 4.1 - 4.2). In contrast, during treatment with the 11-day amphetamine regime, reductions in behavioural inhibition occurred following a cumulative dose of 30 mg/kg (days 1 – 4: 1, 2; 3, 4; 5, 5; 5, 5 mg/kg) (See Figs. 5.1 – 5.2). The combination of these findings presents an interesting difference in amphetamine dose vs. pattern effects on impulsivity and suggests that a small cumulative amount of amphetamine administered in a short period of time can cause faster changes in behavioural inhibition than a larger cumulative amount of amphetamine administered over a longer period of time. The 4-day amphetamine regime therefore appears to be more detrimental to behavioural inhibition than the 11-day regime, based on the observation that this regime induced earlier reductions in behavioural inhibition and at a lower cumulative dose of amphetamine than the 11-day regime. Intense periods of binge intoxication are a hallmark feature of addiction and represent a phase of uncontrolled drug use (Koob & Le Moal, 2008; Perry et al., 2008), whilst more maintained patterns of drug use do not necessarily indicate phases of drug use that is out of control (Perry et al., 2008). The present findings therefore support that impulsivity might have a preferential relationship with, or be more detrimental to, periods of fast and frequent drug use (binge) relative to more maintained patterns of drug use (Perry et al., 2008).

To date, previous investigations into the relationship between binge drug use and impulsivity have found that trait impulsivity can predict binge patterns of drug use in humans (Carlson et al., 2010; Semple, Zians, Grant, & Patterson, 2005) therefore suggesting that the direction of this relationship exists with impulsivity acting as a predictive cause of bingeing and not necessarily a consequence of drug use itself. The present findings therefore expand on this literature by demonstrating that not only might reduced inhibitory control increase the chance of engaging in an escalating and uncontrolled pattern of drug use, but this pattern of use might in itself also produce greater deficits in inhibitory control relative less intense patterns of use.

8.1.3 Patterns of amphetamine on short-term withdrawal induced impulsivity

Both the 4-day and 11-day amphetamine regimes increased impulsivity during short-term amphetamine withdrawal (withdrawal week one) indicating an overall similarity in the duration of elevated impulsivity following the termination of two different patterns of amphetamine administration. However, further analysis of the specific duration of these effects demonstrated that treatment with the 4-day amphetamine regime caused a longer duration of increased impulsivity (12 -36 hrs, and 156 hrs of withdrawal) than that observed by 11-day amphetamine treatment (See Figs. 4.2 and 5.2). These differences in withdrawal induced impulsivity are therefore useful for considering how different patterns of amphetamine use, and possibly stages of addiction, might affect impulsivity during early phases of drug withdrawal where vulnerability to relapse is high.

Greater withdrawal induced impulsivity following 4-day amphetamine in comparison to 11-day amphetamine suggests that treatment with the 4-day amphetamine binge was more detrimental to behavioural inhibition than the 11-day amphetamine regime during short-term amphetamine withdrawal. Reduced behavioural inhibition during the first two days of withdrawal (up to 36 hrs withdrawal) following 4-day amphetamine treatment only, is particularly interesting for considering how inhibitory control between these two patterns of amphetamine use might affect impulsive urges to seek and take drugs during acute drug withdrawal. Acute drug withdrawal marks a period of heightened vulnerability to relapse (Koob & Le Moal, 2008) during which sensitivity to drug related cues and cue-elicited craving are thought to play a significant role in drug seeking and relapse (Childress et al., 1993; Myers & Carlezon Jr, 2010; Robinson & Berridge, 1993; Rohsenow et al., 1991; Wise, 1988). The lack of significant difference between amphetamine treated animals and controls during the first two days (up to 36 hrs withdrawal) of 11-day amphetamine withdrawal suggests that animals moving into this phase of acute withdrawal following treatment with a relatively maintained pattern of amphetamine use might be able to exert stronger inhibitory control over risk factors for relapse during this phase, such as sensitivity to drug cues. Reduced behavioural inhibition observed by animals treated with the 4-day amphetamine binge alternatively indicates that this pattern of amphetamine use might lead to a state of compromised inhibitory control over acute withdrawal symptoms, consequently increasing the vulnerability of relapse. This suggests that a binge pattern of amphetamine use might lead to a greater chance of subsequence drug use than a more maintained pattern of amphetamine use as a consequence of greater deficits in inhibitory control over risk factors for drug seeking and relapse, such as

increased sensitivity to drug cues and craving (Robinson & Berridge, 1993), during short-term amphetamine withdrawal.

Collectively, observations that 4-day amphetamine treatment is more detrimental to behavioural inhibition than 11-day amphetamine during drug treatment, alongside greater inhibitory deficits during withdrawal from 4-day amphetamine to that observed by 11-day amphetamine, suggests that binge use of amphetamine causes quicker and longer lasting deficits in behavioural inhibition in comparison to longer-term and more maintained use of amphetamine. Consequently, this pattern of use might render amphetamine users more vulnerable to repeated drug use both throughout escalating patterns of use, consequently fuelling prolonged binge intoxication, or through increasing the chance of relapse during short-term drug withdrawal, fuelling the transition into dependence and/or maintaining dependence.

The differences observed in the onset and duration of impulsivity as a consequence of 4-day or 11-day amphetamine treatment might relate to differences in the neurochemical changes induced by these different patterns of amphetamine treatment. The main differences in 4-day and 11-day amphetamine treatment was the frequency of administration per day and the duration of amphetamine treatment, both of which factors are known to influence neurochemical changes in dopamine during amphetamine withdrawal (Paulson & Robinson, 1995; Robinson & Becker, 1986; Robinson & Berridge, 1993). Amphetamine withdrawal is associated with reduced dopamine concentration within the striatum and ventral striatum (Paulson & Robinson, 1996; Rossetti, Hmaidan, & Gessa, 1992) and partial dopamine agonists can reverse withdrawal induced reductions in motivational deficits (Orsini et al., 2001). These findings indicate that changes in dopamine functioning within striatal regions are concomitant with amphetamine withdrawal. Changes in dopamine functioning within the ventral striatum are thought to be associated with amphetamine induced behavioural inhibition in rats (Pattij et al., 2007) and in Chapter 6 changes in dopamine activity within the ventral striatum is shown to be associated with amphetamine induced behavioural inhibition measured by the symmetrically reinforced Go/No-go task. When considered together these findings may suggest that the differential changes in dopamine function in withdrawal relate to the differences observed in the significance of withdrawal induced deficits in behavioural inhibition between amphetamine treatment regimes.

8.1.4 Patterns of amphetamine on long-term withdrawal and impulsivity

Neither 4-day amphetamine nor 11-day amphetamine caused any long-term changes in behavioural inhibition in rats. The combination of these results indicates that increased impulsivity following subchronic and chronic amphetamine is a transient effect that is recoverable long-term. Different patterns of amphetamine use might then only pose differential effects on impulsivity, and subsequently differential effects on the vulnerability to addiction/ maintenance of addiction, during periods of drug use and short-term withdrawal.

In addition, neither regime caused any changes in long-term sensitivity to the acute effects of amphetamine on impulsivity. Given that more intermittent patterns of amphetamine administration are usually required to induced sensitisation to the acute effects of amphetamine (Robinson & Becker, 1986; Robinson & Berridge, 1993) these findings are generally consistent with previous literature. Furthermore, amphetamine regimes that produce sensitisation to the stereotypic effects of amphetamine in rats have also been shown not to affect sensitisation to the impulsive effects of amphetamine (Stanis et al., 2008) indicating that regimes that typically induce sensitisation to locomotor behaviour may not produce similar effects on impulsive behaviour.

However, contrasting effects were observed during Go trial performance following 4-day and 11-day amphetamine treatment (See Figs. 4.4 - 5.4). Animals treated with 4-day amphetamine showed tolerance to disruptive performance effects following 1.2 mg/kg amphetamine in comparison to animals treated with saline, whilst animals treated for 11-days with amphetamine showed sensitivity to these effects, performing Go trials less accurately than controls following 1.2 mg/kg. These differences might relate to the fact that during the 4-day regime there was less time (6 hrs/ 12 hrs) between each injection than the 11-day (12 hrs). Given that tolerance is usually observed following continuous drug regimes whilst sensitisation is usually observed following intermittent drug regimes (Post, 1980), the smaller duration between amphetamine injections during 4-day amphetamine treatment might then explain why these animals displayed some evidence of tolerance in response to acute amphetamine, whilst animals with a longer duration between injections displayed some evidence of sensitisation.

8.1.5 Conclusions and Future Research

In conclusion, the results presented demonstrate that both subchronic and chronic amphetamine treatment can reduce behavioural inhibition in rats both during drug treatment

and short-term drug withdrawal when measured by a symmetrically reinforced Go/No-go task. In addition, more specific analysis of these effects indicates that the pattern of amphetamine administration can affect both the onset and duration of drug induced impulsivity, with indications that subchronic high frequency regimes (binge) leading to earlier and more long-term effects on impulsivity than chronic low frequency regimes. These findings therefore present novel findings in the relationship between to subchronic and chronic amphetamine administration and impulsivity in rats. The prevalence of both subchronic and chronic amphetamine induced deficits on behavioural inhibition during drug treatment and withdrawal therefore warrants future research into how such withdrawal induced deficits in behavioural inhibition might relate to relapse in animal models. Furthermore, given that much research has implicated changes in the D_2 receptors in stimulant addicts during phases of abstinence (Lee et al., 2009) and impulsivity (Van Gaalen et al., 2009; Van Gaalen et al., 2006; Van Gaalen et al., 2006b) future research investigating the role of the D_2 sub-receptors in withdrawal induced impulsivity would be useful for elucidating possible neurochemical changes driving impulsivity that occur as a consequence of stimulant use.

8.2 Central Dopamine and GABA manipulations and Impulsivity

Investigation into the neural substrates of impulsivity and amphetamine induced impulsivity revealed that the D_2 and $GABA_A$ receptors within the NAcb core are involved in the expression of amphetamine induced disinhibition, but not behavioural inhibition, in rats performing a symmetrically reinforced Go/No-go task. Whilst the D_2 receptors with the NAcb core have previously been found to mediate amphetamine induced impulsivity on the 5CSRTT (Pattij et al., 2007), this is the first study to identify that the D_2 receptors within the NAcb core mediate drug induced impulsivity that is associated with a direct loss of reward, consequently implicating this receptor in the mediation of a motivationally significant loss of inhibitory of control. Similarly, no previous literature to date has investigated the involvement of the $GABA_A$ receptors within the NAcb core in impulsivity or amphetamine induced impulsivity in rats. The combination of these studies therefore presents novel findings into the behavioural profile of behavioural disinhibition that is mediated by the D_2 and $GABA_A$ receptors within the NAcb core, and more broadly, into the neurochemical systems within the NAcb core that mediate drug induced disinhibited behaviour in rats.

8.2.1 Neural substrates of impulsivity

Infusions of eticlopride and muscimol into the NAcb core both produced no effects on any measures of the Go/No-go task, indicating that dopamine neurotransmission at the D_2 receptors and exogenous activation of the $GABA_A$ receptors within the NAcb core does not disrupt baseline performance on the Go/No-go task. D_2 receptors are located presynaptically on dopamine nerve terminals forming inhibitory autoreceptors (Benoit-Marand, Borrelli, & Gonon, 2001; Zhang & Sulzer, 2012) and within the ventral striatum the D_2 receptors are also located postsynaptically on medium spiny projection neurons and cholinergic interneurons, and there are also evidence that the D_2 receptors are located presynaptically on glutamate terminals innervating the NAcb (Albin, Young, & Penney, 1989; Goto & Grace, 2005; Kawaguchi, 1993; Surmeier et al., 2007). The lack of effect eticlopride produced on baseline performance of behaviour inhibition therefore indicates that the blockade of the D_2 receptors at any of these potential sites of action might not change the expression of baseline behavioural inhibition on the Go/No-go task. Infusion of eticlopride into the NAcb core has previously been shown to facilitate prefrontal glutamate input into the NAcb core (Goto & Grace, 2005) suggesting that these results might reflect a lack of involvement in of presynaptic D_2 receptors located on glutamate terminals innervating the NAcb core. However, Goto & Grace, (2005) infused much higher eticlopride doses (20 µM) to that in the current these (0.1 - 1.0 µg), therefore, the site of action of eticlopride might not necessarily be comparable between these two studies.

This lack of eticlopride induced effects on baseline behavioural inhibition is consistent with previous literature that has demonstrated a role for the D_1 but not D_2 receptors within the NAcb core in the mediation of baseline behavioural inhibition on the 5CSRTT (Pattij et al., 2007b). In addition, the D_1 receptors within the NAcb core have been shown to produce greater deficits in the processing of pavlovian cues that influence instrumental responding (PIT) than the D_2 receptors within the NAcb core (Lex & Hauber, 2008), suggesting that the D_1 receptors within the NAcb core might be more preferentially involved in the mediation of cue elicited behavioural activation than the D_2 receptors. The Go/No-go task requires intact associations between the introceptive value of Go and No-go cues with active and passive response behaviour. When considered together, this might suggest that intact D_1 receptor functioning following eticlopride infusions enabled normal processing of the introceptive values of Go and No-go visual cues in relation to reward.

The $GABA_A$ receptors are located postsynaptically on medium spiny projection neurons and aspiny interneurons, and presynaptically on dopamine terminals innervating the NAcb core

(Ferraro et al., 1996; Krebs et al., 1993; Ronken et al., 1993; Schwarzer et al., 2001). The lack of muscimol induced effects on baseline behavioural inhibition on the Go/No-go task therefore suggests that activation of the $GABA_A$ receptors at any of these potential sites of action might not affect baseline behavioural inhibition on the Go/No-go task. Muscimol infused into the NAcb has previously been shown to exert effects that are reflective of both a presynaptic action on dopamine terminals and postsynaptic action on medium spiny and aspiny neurons (Aono et al., 2008; Ferraro et al., 1996; R. Mitchell, 1980; Yan, 1999). Consequently, the lack of effects of muscimol might reflect a lack of $GABA_A$ receptor involvement at any or all of these sites.

Aside from dopamine and GABA systems within NAcb core, there are multiple neurochemical systems that innervate the NAcb core that might potentially be involved in the processing of behavioural inhibition under baseline conditions. The NAcb core is heavily innervated by prefrontal, limbic and thalamic glutamate afferents (DeFrance et al., 1985; Fuller, Russchen, & Price, 1987; McDonald, 1991), and as such, these afferent projections might affect processing of behavioural inhibition within the NAcb core on the Go/No-go task. AMPA and NMDA receptor antagonism within the NAcb core and shell has recently been shown to increase behavioural disinhibition, measured by the ratio of responding to non-rewarded auditory stimuli in rats (Ambroggi et al., 2011). Interestingly, AMPA and NMDA antagonism within the NAcb core, but not shell, additionally led to a significant reduction in the correct discrimination of an auditory tone signalling reward (Ambroggi et al., 2011), consistent with a specific role for the NAcb core in processing the incentive value of cues in rats (Parkinson et al., 1999). These findings therefore suggest that glutamate activity within NAcb core might mediate behavioural inhibition through processing the incentive value of stimuli that guides appropriate behavioural activation and inhibition for reward. Consequently, if glutamate signalling remained intact following infusions of eticlopride and muscimol into the NAcb core, then the incentive value of Go and No-go trial visual cues would remain intact, guiding the correct response behaviour to receive reward.

Another neurochemical system that might have been unaffected by eticlopride and muscimol infusions and that might plausibly mediate behavioural inhibition within the NAcb core on the Go/No-go task is serotonin. Antagonism of the $5\text{-}HT_{2a}$ and $5\text{-}HT_{2c}$ sub-receptors have both been found to mediate changes in premature responding on the 5CSRTT (Robinson et al., 2007). Although antagonism of NAcb $5\text{-}HT_{2a}$ and $5\text{-}HT_{2c}$ receptors on the 5CSRTT might not be directly comparable to the role of serotonin within the NAcb on the Go/No-go task, especially since differences in omissions were observed between these effects (Robinson et al., 2007), these findings are useful in considering how changes in alternative systems within the NAcb core

might mediate behavioural inhibition. Furthermore, central serotonin depletion in rats has previously been shown to impair the acquisition of, and performance on, the symmetrically reinforced Go/No-go task employed in the current thesis (Harrison et al., 1999) indicating that changes in 5-HT function within the NAcb core might a potential neurochemical substrate of behavioural inhibition on the Go/No-go task.

Noradrenalin terminals also innervate the NAcb, suggesting the possibility that this neurotransmitter might also play a role in behavioural inhibition. However, it is probably unlikely that noradrenalin represents a potential neurochemical system outside of dopamine and GABA within the NAcb core that mediates behavioural inhibition on the Go/No-go task, as firstly, immunoreactivity for the noradrenalin enzyme dopamine β-hydroxylase is much greater in the NAcb shell than NAcb core (Berridge et al., 1997), and secondly, infusions of the selective noradrenalin reuptake inhibitor atomoxetine has recently been shown to only affect premature responding in rats when infused into the NAcb shell, and not the core (Economidou et al., 2012). However, without investigation into the direct effects of noradrenalin manipulation within the NAcb core on the Go/No-go task, it remains possible this noradrenalin might additionally play a role in the neurochemical mediation of behavioural inhibition on the Go/No-go task that might not have been affected following eticlopride and muscimol infusions into the NAcb core.

8.2.2 Neural substrates of amphetamine induced impulsivity

Eticlopride mediated reversal of amphetamine induced impulsivity on the Go/No-go task demonstrates that amphetamine induced activation of the D_2 receptors within the NAcb core is involved in the expression of amphetamine induced disinhibition on this task. It is well known that acute amphetamine causes an increase in dopamine flux within the NAcb (Carboni et al., 1989) and that acute amphetamine reduces behavioural inhibition in rats (Cole & Robbins, 1989; Harrison et al., 1997; Harrison et al., In prep; Murphy et al., 2008; Pattij et al., 2007). These findings therefore help to elucidate how amphetamine induced elevations in dopamine release within the core sub-region of the NAcb might contribute towards the expression of acute disinhibition observed following systemic amphetamine in rats. These findings are consistent with a role for the D_2 receptors within the NAcb core in the mediation of amphetamine induced premature responding on the 5CSRTT (Pattij et al., 2007) and additionally contribute towards elucidating the behavioural profile of disinhibited behaviour mediated by the D_2 receptors within the NAcb core by implicating amphetamine induced activation of the D_2 receptors in a the loss of behavioural inhibition that costs the loss of reward. Taken together these findings provide consistent evidence for changes at the D_2

receptors within the NAcb core mediating the expression of amphetamine induced disinhibition, and indicate that changes at the D_2 receptors can lead to disinhibited behaviour despite associated negative outcomes, consistent with the behavioural profile of impulsivity observed in drug users.

Muscimol induced partial reversal of amphetamine induced impulsivity additionally indicates that activation of the $GABA_A$ receptors within the NAcb core can reduce the expression of amphetamine induced behavioural inhibition on Go/No-go task. These findings elucidate the involvement of potential neurochemical systems other than dopamine within the NAcb core that acute amphetamine might alter, either directly or indirectly, and that contribute towards acute changes in behavioural inhibition following amphetamine. When considered with the results observed with eticlopride, collectively these findings indicate that both dopamine and GABA systems within the NAcb core are involved in amphetamine induced behavioural disinhibition on the Go/No-go task.

Interestingly, both eticlopride and muscimol infusions into the NAcb core produced a similar pattern of behavioural effects on the Go/No-go task. The only difference between the effects of eticlopride and muscimol infusions on Go/No-go performance was in the significance of full behavioural reversal at the highest dose of each drug. The similarities in these results might reflect the fact that eticlopride and muscimol both ultimately targeted dopamine activity within the NAcb core, meaning that both drugs shared a common pharmacological outcome of reducing dopaminergic action within the NAcb core that is responsible for disinhibited behaviour (Pattij et al., 2007). However, the differences in full behavioural reversal might reflect the fact that this common action of eticlopride and muscimol on dopamine was produced via different direct and indirect actions, respectively. Eticlopride directly blocks dopamine at the D_2 receptors where as muscimol indirectly reduces dopamine by activation of inhibitory $GABA_A$ receptors (Ferraro et al., 1996; Köhler, Hall, & Gawell, 1986; Yan, 1999). Differences in the direct and indirect actions of eticlopride and muscimol might then explain why muscimol produced a similar pattern and direction of reversal to that observed with eticlopride, and consequently why this was not as significant as the effects of eticlopride. In addition, the similar pattern of results between eticlopride and muscimol infusions might also suggest that muscimol indirectly inhibited dopamine action at the D_2 receptors. However, without knowing whether the D_1 receptors within the NAcb core are involved in amphetamine induced disinhibition on the Go/No-go task, muscimol induced reductions on dopamine might have affected the action of dopamine at both the D_1 and D_2 receptors within the NAcb core, along with the subsequent effects both receptors might play in behavioural disinhibition.

As previously mentioned, the D_2 receptors are located both pre and postsynaptically on cells within the NAcb (Albin, Young, & Penney, 1989; Goto & Grace, 2005; Kawaguchi, 1993; Surmeier et al., 2007). Hypothetically, eticlopride induced antagonism of the D_2 receptors within the NAcb core could reduce amphetamine induced disinhibition either through a presynaptic action on PFC NAcb-projecting glutamatergic neurons innervating the core, or through a postsynaptic action on GABAergic medium spiny neurons projecting to the ventral pallidum from the NAcb core (See Fig 1.4). Both of these locations would theoretically block the effects of amphetamine induced elevations of dopamine release on inhibitory D_2 receptors that can attenuate PFC input (Goto & Grace, 2005) and attenuate the activation of medium spiny projection neurons through the indirect ventral striato-pallidal pathway (Albin et al., 1989; Surmeier et al., 2007; West & Grace, 2002).

As previously mentioned, the $GABA_A$ receptors are located presynaptically on dopamine terminals innervating the NAcb core and postsynaptically on medium spiny projection neurons and aspiny interneurons (Ferraro et al., 1996; Krebs et al., 1993; Ronken et al., 1993; Schwarzer et al., 2001). Theoretically, a presynaptic effect of muscimol would be expected to reduce amphetamine induced disinhibition though presynaptic dopamine inhibition, whilst a postsynaptic effect would be expected to potentiate amphetamine induced disinhibition through mimicking the actions of dopamine at inhibitory D_2 receptors.

The fact that muscimol did not potentiate the effects of amphetamine induced disinhibition on the Go/No-go task is therefore useful for considering whether changes on the post-synaptic membrane of MSNs, and subsequently changes in ventral striato-pallidal activity, are in involved in amphetamine induced disinhibition. The lack of potentiation in amphetamine induced disinhibition following muscimol might then suggest that changes in the activation of the indirect ventral striato-pallidal pathway are not involved in mediating amphetamine induced disinhibition. When considered with the eticlopride study, this might suggest that eticlopride induced reversal is more likely to reflect blockade of amphetamine induced increases in the activation of inhibitory D_2 receptors located on PFC glutamate afferents as opposed to a postsynaptic D_2 receptors on MSNs, consistent with previous literature indicating that activation/ inactivation of the D_2 receptors can attenuate/ facilitate PFC glutamate release into the NAcb core (Goto & Grace, 2005) and that glutamate release in the NAcb core is required for behavioural inhibition in rats (Ambroggi et al., 2011). However, it is also possible that muscimol did not bind to the $GABA_A$ receptors located on medium spiny projection neurons but rather preferentially bound to presynaptic $GABA_A$ receptors on dopamine terminals (Ferraro et al., 1996; Yan, 1999) leaving open the possibility that indirect changes in the activation of the ventral striato-pallidal pathway, through a presynaptic inhibition of

dopamine by muscimol and direct blockade of the D_2 receptors on MSNs by eticlopride, might be involved in reductions/ reversal the amphetamine induced disinhibition observed.

8.2.3 Summary and Future Research

In summary, in terms of the neural circuits that might mediate amphetamine induced disinhibition on the symmetrically reinforced Go/No-go task, the effects of eticlopride and muscimol on amphetamine induced impulsivity collectively support the involvement of circuits innervating and projecting from the NAcb core in the mediation of amphetamine induced disinhibition, and might specifically support VTA dopamine release causing a shift from top-down frontal processing to bottom up dopamine modulated processing via disconnection of PFC glutamate input into the NAcb core. However, due to the fact that a presynaptic action of muscimol on dopamine receptors cannot be ruled out, it remains possible that changes in ventral striato-pallidal signalling from the NAcb core might also contribute toward amphetamine induced behavioural disinhibition.

In order to better elucidate the specific involvement of prefrontal and ventral striato-pallidal circuits in amphetamine induced impulsivity, future research into the functional involvement of prefrontal and subcortical targets of the NAcb core, such as the ventral pallidum, are required with the Go/No-go task. Given that the NAcb core receives glutamatergic afferents from dorsal prelimbic and lateral orbitofrontal areas, it would be particularly useful to investigate the role of glutamate antagonism within these areas to establish whether inhibiting descending prefrontal networks to the NAcb core affects behavioural inhibition on the Go/No-go task. Alternatively, given that the D_2 receptors within the NAcb core exert an inhibitory role over medium spiny projection neurons to the ventral pallidum, it would be particularly interesting to investigate the involvement of GABA function within the ventral pallidum during amphetamine induced impulsivity, in order to establish whether changes in the descending processing from the NAcb core through ventral striato-pallidal circuits might also contribute towards amphetamine induced behavioural disinhibition. Furthermore, given that emerging evidence has implicated a specific role for the D_2 mediated indirect pathway leaving the NAcb core in the development of disinhibited drug seeking in rats (Bock et al., 2013), it would be interesting to establish the role of this pathway, either through glutamatergic activation within the NAcb core or GABAergic manipulation within the ventral pallidum in animal models of impulsivity.

8.2.4 Conclusions

In conclusion, the effects of eticlopride and muscimol infusions into the NAcb core on impulsivity measured by the symmetrically reinforced Go/No-go task have suggested that baseline expression of behavioural inhibition on this task is not mediated activity at the D_2 or $GABA_A$ receptors. In contrast, amphetamine induced impulsivity on this task appears to be mediated by stimulation of the D_2 receptors within the NAcb core, suggesting that amphetamine induced elevations in dopamine activity at the D_2 receptors within the NAcb core is involved in the manifestation of acute disinhibition of response behaviour following amphetamine. In addition, evidence that stimulation of the $GABA_A$ receptors within the NAcb core can reduce amphetamine induced impulsivity is indicative of both dopamine and GABA systems within the NAcb core contributing towards the expression of amphetamine induced impulsivity on the symmetrically reinforced Go/No-go task. The combination of these effects lends support to a break down in fronto-striatal connectivity in the manifestation of drug induced impulsivity (Dalley, Everitt, & Robbins, 2011; Jentsch & Taylor, 1999) but also implicate possible changes in the activation of descending ventral striato-pallidal pathways projecting from the NAcb core in amphetamine induced disinhibition. These findings consequently present novel findings in the neural substrates of amphetamine induced impulsivity and warrant future research into the role of different dopamine and GABA sub-receptors within the NAcb core in drug induced impulsivity, and research into fronto-striatal and ventral striato-pallidal circuits in the involvement of drug induced impulsivity.

8.3 Thesis Limitations

One of the major limitations of this thesis, which has been discussed in experimental chapters, is that no animal models of addiction were employed. Non-contingent drug administration was employed within all experimental procedures, and as such, there are scalability limitations of the results in this thesis to human populations. In order to overcome this limitation in future research, it would be useful to employ non-contingent self-administration regimes alongside measuring performance the Go/No-go task. This would, firstly, enable a more transferable method of examining impulsive behaviour and addiction in rats to human populations, and secondly, allow a more direct assessment of specific phases of addiction, such as, escalation, maintenance and relapse. Whilst this thesis employed non-contingent regimes that broadly map onto patterns of drug use within specific phases of addiction, eg. 4-day binge (escalation) and 11-day chronic (maintenance), these regimes do not directly model these phases of addiction. It would therefore be useful for future research to employ self-administration

regimes that model escalation and maintenance phases of addiction whilst measuring impulsivity on the Go/No-go task during these treatment phases and subsequent short and long-term withdrawal. These alternations would give a greater level of scalability in the data collected and would therefore be more useful for considering how changes in impulse control might facilitate drug addiction in humans.

The research presented in this thesis has only investigated the effects of amphetamine on one dimension of impulsivity in rats, limiting broad interpretations from results in relation to impulsivity as a multi-dimensional construct. Sub-dimensions of impulsivity have been suggested to be both distinct (Broos et al., 2012b; Reynolds, 2006) and related (Robinson et al., 2009). It would have been useful, therefore, to examine whether the drug regimes employed in this thesis produced similar or differential effects on different behavioural measures of impulsivity. In order to address this limitation in future research, it would be useful to employ models of both impulsive action and choice in combination with chronic drug treatment. This would help to identify whether different dimensions of impulsivity are similarly or differentially affected by a specific set of dosing parameters employed within one study.

Rats were not split into groups of high and low levels of trait impulsivity in this thesis. There is a well established relationship exists between trait impulsivity and the effects of amphetamine (de Wit, 2000; Eagle et al., 2007; Hand, Fox & Reilly, 2009) and between trait impulsivity and drug addiction (Broos et al., 2012; Dalley et al., 2007; Diergaarde et al., 2009; Kollins, 2003; Perry, 2005; 2008). Consequently, a limitation of this thesis is that it does not account for how individual differences might contribute the effects of amphetamine on impulsivity observed. It would have been useful to consider a method of screening trait impulsivity in rats on the Go/No-go task, and subsequently to include groups expressing high and low trait impulsivity in the studies conducted. In order to avoid this limitation in future research it will be important to develop a screening method to detect high and low levels of trait impulsivity on the Go/No-go symmetrically reinforced task. Previous research investigating the relationship between trait response disinhibition and addiction has largely been conducted with the 5CSRTT. In consideration of main differences discussed between the 5CSRTT and the Go/No-go task (see pages 57-58), once a screening method for detecting trait impulsivity on the Go/No-go symmetrically reinforced task has been established, it will be useful to compare how highly impulsive rats on this task respond to self-administration models of acquisition, escalation, maintenance and relapse. This will help to consider how trait impulsivity on a more direct animal model of response inhibition in comparison to the 5CSRTT affects vulnerability to distinct stages of addiction.

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Appendix 1: Chapter 4: 4-Day Amphetamine: Corrected p-values using the Benjamini-Hochberg correction

Experiment 2a: 4 – Day Amphetamine

Performance Accuracy

Table 1a: Total percent correct

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 2	6.206	0.024	0.050 *
2	Day 1	7.838	0.013	0.033 *
3	Day 3	14.965	0.001	0.017 **

Table 1b: Total percent correct No-go trials

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 2	4.479	0.050	0.050
2	Day 3	10.848	0.005	0.033 *
3	Day 1	11.253	0.004	0.017 *

Speed of responding

Table 1c: No-go trial incorrect response latency

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 1	2.846	0.098	0.050
2	Day 3	0.289	0.593	0.033
3	Day 2	2.287	0.137	0.017

Anticipatory Responding

Table 1d: Go trial panel responses

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 1	0.269	0.611	0.050
2	Day 2	3.059	0.098	0.033
3	Day 3	12.140	0.003	0.017 **

Experiment 2b: Withdrawal week one

Performance Accuracy

Table 1e: Total percent correct. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction	
1	60 hrs	0.671	0.425	0.050	
2	108 hrs	0.858	0.368	0.044	
3	84 hrs	4.925	0.041	0.038	
4	132 hrs	5.671	0.030	0.031	*
5	156 hrs	6.519	0.021	0.025	*
6	18 hrs	7.694	0.014	0.019	*
7	36 hrs	12.920	0.002	0.013	**
8	12 hrs	40.014	0.000	0.006	***

Table 1f: Total percent correct No-go trials. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction	
1	60 hrs	0.952	0.344	0.050	
2	108 hrs	1.043	0.322	0.044	
3	84 hrs	4.231	0.056	0.038	
4	132 hrs	5.228	0.036	0.031	
5	18 hrs	6.148	0.025	0.025	*
6	156 hrs	8.762	0.009	0.019	**
7	36 hrs	15.368	0.001	0.013	**
8	12 hrs	17.893	0.001	0.006	***

Speed of Responding

Table 1g: Go trial correct response latency. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction	
1	108 hrs	0.493	0.493	0.050	
2	84 hrs	1.432	0.249	0.044	
3	132 hrs	1.637	0.219	0.038	
4	156 hrs	2.120	0.165	0.031	
5	36 hrs	4.407	0.052	0.025	
6	12 hrs	5.175	0.037	0.019	
7	18 hrs	5.211	0.036	0.013	
8	60 hrs	10.298	0.005	0.006	**

Table 1h: No-go trial magazine latency

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction
1	12 hrs	2.735	0.101	0.050
2	84 hrs	0.081	0.777	0.044
3	36 hrs	0.945	0.333	0.038
4	108 hrs	1.059	0.305	0.031
5	132 hrs	1.543	0.216	0.025
6	60 hrs	2.877	0.092	0.019
7	156 hrs	2.950	0.088	0.013
8	18 hrs	6.953	0.009	0.006

Anticipatory Responding

Table 1i: No-go trial early responses

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction
1	132 hrs	0.000	0.990	0.050
2	156 hrs	0.011	0.917	0.044
3	84 hrs	0.535	0.466	0.038
4	18 hrs	1.273	0.261	0.031
5	108 hrs	1.781	0.184	0.025
6	60 hrs	2.343	0.128	0.019
7	12 hrs	3.081	0.082	0.013
8	36 hrs	3.496	0.064	0.006

Experiment 2c: Withdrawal week two

Performance Accuracy

Table 1j: Total percent correct. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Day	F-Value	P-Value	Benjamini-Hochberg Correction	
1	Day 10	0.260	0.617	0.050	
2	Day 14	0.469	0.503	0.043	
3	Day 11	1.449	0.246	0.036	
4	Day 9	1.894	0.188	0.029	
5	Day 12	4.070	0.061	0.021	
6	Day 13	4.514	0.050	0.014	
7	Day 8	16.958	0.001	0.007	***

Anticipatory Responding

Table 1k: No-go trial panel responses

Index	Withdrawal Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 11	0.086	0.769	0.050
2	Day 12	0.166	0.685	0.043
3	Day 14	0.242	0.624	0.036
4	Day 10	0.718	0.399	0.029
5	Day 13	1.975	0.163	0.021
6	Day 8	2.212	0.140	0.014
7	Day 9	2.265	0.135	0.007

Appendix 2: Chapter 5: 11-Day Amphetamine: Corrected p-values using the Benjamini-Hochberg correction

Experiment 3a: **11 – Day Amphetamine**

Performance Accuracy

Table 2a: Total percent correct. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction	
1	Day 1	0.007	0.932	0.050	
2	Day 2	0.154	0.696	0.045	
3	Day 3	4.343	0.039	0.040	*
4	Day 5	5.140	0.025	0.035	*
5	Day 6	6.420	0.012	0.030	*
6	Day 4	8.054	0.005	0.025	**
7	Day 10	8.873	0.003	0.020	**
8	Day 8	11.733	0.001	0.015	***
9	Day 9	11.847	0.001	0.010	***
10	Day 7	15.605	0.000	0.005	***

Table 2b: Total percent correct No-go trials. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction	
1	Day 2	0.679	0.422	0.050	
2	Day 1	1.322	0.267	0.045	
3	Day 5	3.362	0.085	0.040	
4	Day 6	3.645	0.074	0.035	
5	Day 3	4.473	0.050	0.030	
6	Day 7	6.127	0.024	0.025	*
7	Day 9	6.845	0.018	0.020	*
8	Day 10	8.214	0.011	0.015	*
9	Day 8	8.297	0.011	0.010	*
10	Day 4	8.398	0.010	0.005	**

Speed of Responding

Table 2c: Go trial correct response latency.

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 1	0.077	0.786	0.050
2	Day 6	0.647	0.433	0.045
3	Day 2	0.826	0.377	0.040
4	Day 4	2.072	0.169	0.035
5	Day 5	2.561	0.129	0.030
6	Day 3	2.654	0.123	0.025
7	Day 8	5.048	0.039	0.020
8	Day 9	7.031	0.017	0.015
9	Day 10	7.078	0.017	0.010
10	Day 7	7.838	0.013	0.005

Table 2d: No-go trial magazine latency. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction	
1	Day 4	0.005	0.944	0.050	
2	Day 6	0.193	0.667	0.045	
3	Day 2	0.957	0.343	0.040	
4	Day 3	1.794	0.199	0.035	
5	Day 1	1.958	0.181	0.030	
6	Day 5	2.074	0.169	0.025	
7	Day 10	2.956	0.105	0.020	
8	Day 8	3.316	0.087	0.015	
9	Day 7	13.982	0.002	0.010	**
10	Day 9	15.649	0.001	0.005	***

Anticipatory Responding

Table 2e: Go trial early responses. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction	
1	Day 4	0.098	0.758	0.050	
2	Day 1	0.516	0.483	0.045	
3	Day 6	0.751	0.399	0.040	
4	Day 2	0.922	0.351	0.035	
5	Day 8	1.353	0.262	0.030	
6	Day 5	1.572	0.228	0.025	
7	Day 10	2.162	0.161	0.020	
8	Day 3	5.116	0.038	0.015	
9	Day 7	11.341	0.004	0.010	**
10	Day 9	13.973	0.002	0.005	***

Table 2f: Go trial panel responses.

Index	Drug Day	F-Value	P-Value	Benjamini-Hochberg Correction
1	Day 6	0.120	0.733	0.050
2	Day 10	0.200	0.660	0.045
3	Day 8	0.219	0.646	0.040
4	Day 1	0.232	0.636	0.035
5	Day 3	0.701	0.414	0.030
6	Day 2	1.652	0.216	0.025
7	Day 4	1.788	0.199	0.020
8	Day 5	1.840	0.193	0.015
9	Day 7	2.419	0.138	0.010
10	Day 9	4.685	0.044	0.005

Experiment 3b: Withdrawal week one

Performance Accuracy

Table 2g: Total percent correct. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction	
1	132 hrs	0.039	0.845	0.050	
2	156 hrs	1.285	0.274	0.044	
3	84 hrs	5.123	0.038	0.039	*
4	108 hrs	6.880	0.018	0.033	*
5	36 hrs	8.716	0.009	0.028	**
6	18 hrs	8.868	0.007	0.022	**
7	60 hrs	9.793	0.006	0.017	**
8	42 hrs	12.217	0.003	0.011	**
9	12 hrs	14.694	0.001	0.006	***

Table 2h: Total percent correct No-go trials. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction
1	132 hrs	0.021	0.886	0.050
2	156 hrs	1.656	0.217	0.044
3	108 hrs	3.603	0.076	0.039
4	84 hrs	3.729	0.071	0.033
5	60 hrs	4.641	0.047	0.028
6	42 hrs	4.667	0.046	0.022
7	18 hrs	6.340	0.023	0.017
8	36 hrs	7.856	0.013	0.011
9	12 hrs	9.103	0.008	0.006

Speed of Responding

Table 2i: Go trial correct response latency.

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction
1	156 hrs	0.127	0.727	0.050
2	42 hrs	0.767	0.934	0.044
3	108 hrs	1.214	0.287	0.039
4	84 hrs	2.063	0.170	0.033
5	132 hrs	2.526	0.132	0.028
6	36 hrs	3.946	0.064	0.022
7	60 hrs	4.302	0.055	0.017
8	18 hrs	5.903	0.027	0.011
9	12 hrs	6.207	0.024	0.006

Table 2j: No-go trial incorrect response latency. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction	
1	156 hrs	0.397	0.539	0.050	
2	132 hrs	0.538	0.474	0.044	
3	36 hrs	0.694	0.417	0.039	
4	12 hrs	2.293	0.149	0.033	
5	60 hrs	2.516	0.132	0.028	
6	42 hrs	2.668	0.122	0.022	
7	84 hrs	2.743	0.117	0.017	
8	108 hrs	7.042	0.017	0.011	
9	18 hrs	13.718	0.002	0.006	**

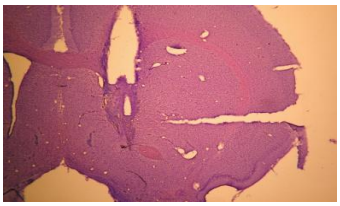
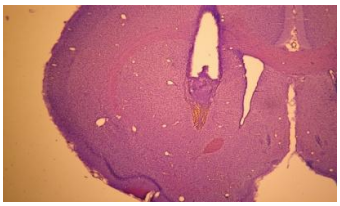
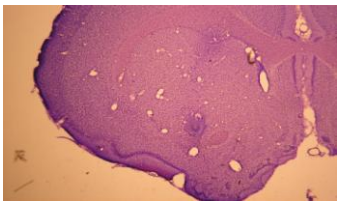
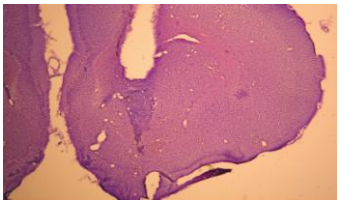
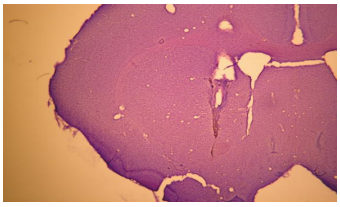
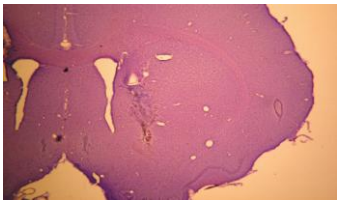
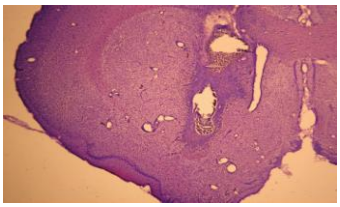
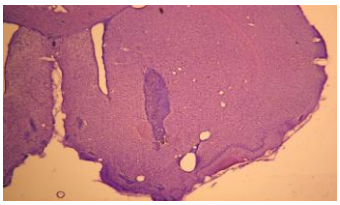
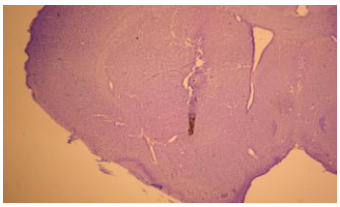
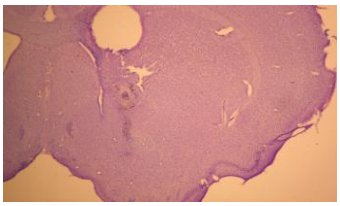
Anticipatory Responding

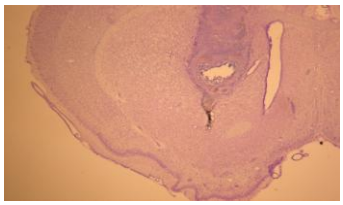
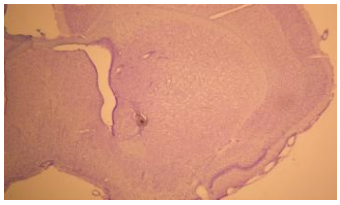
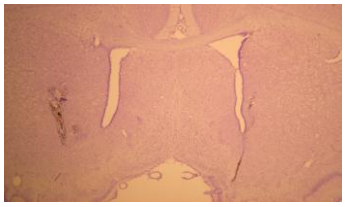
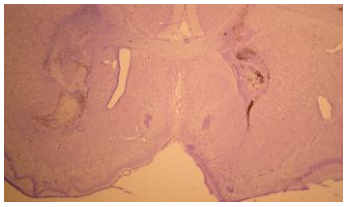
Table 2k: Go trial early responses.

Index	Withdrawal Hour	F-Value	P-Value	Benjamini-Hochberg Correction
1	108 hrs	0.151	0.703	0.050
2	156 hrs	1.067	0.317	0.044
3	18 hrs	1.159	0.298	0.039
4	42 hrs	2.268	0.152	0.033
5	132 hrs	2.230	0.147	0.028
6	84 hrs	3.453	0.082	0.022
7	60 hrs	4.675	0.046	0.017
8	36 hrs	5.268	0.036	0.011
9	12 hrs	6.821	0.019	0.006

Appendix 3: Experiment 4: Photographs of cannlue placements

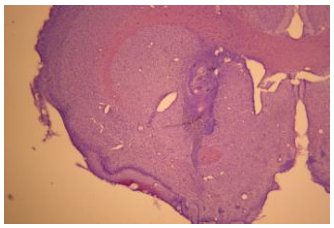
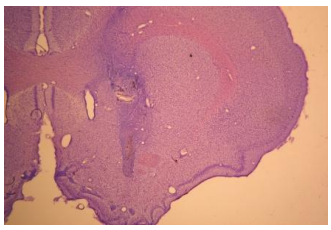
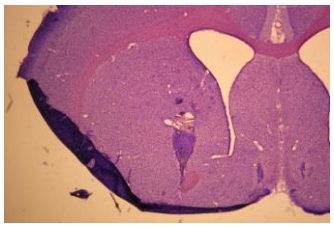
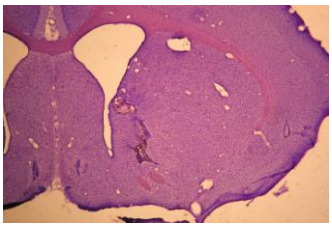
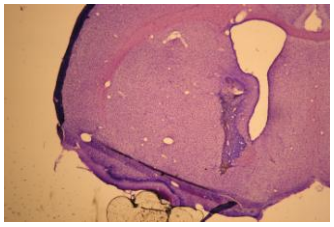
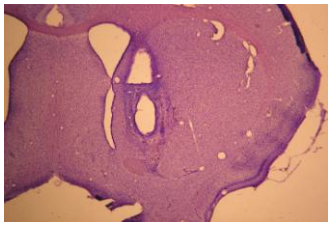
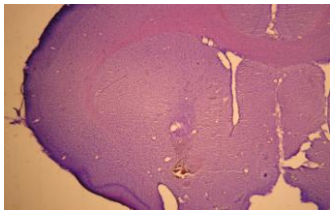
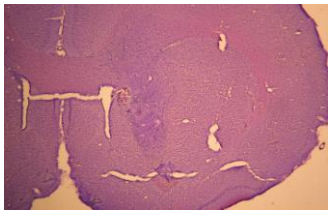
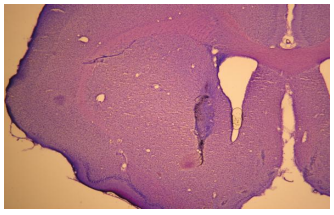
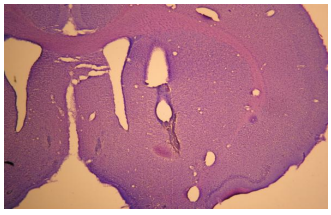
Table 3: Photographs showing the location of injector tips (mm from bregma) for each animal treated with eticlopride infusions into the NAcb core.

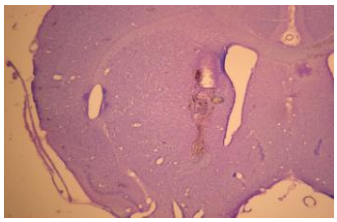
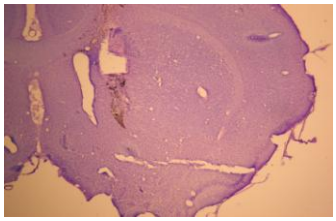
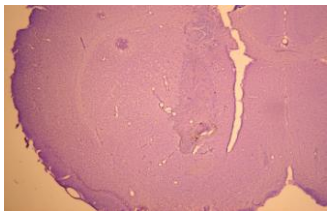
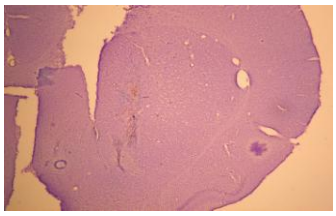
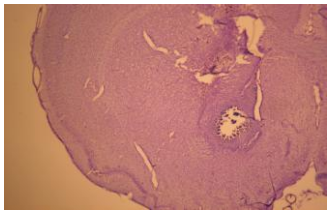
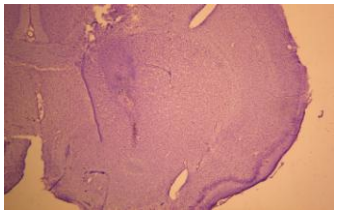
Animal	Left Hemisphere	LH Location (mm)	Right Hemisphere	RH Location (mm)
1		+ 1.56		1.44
2		+ 2.28		2.52
3		+ 1.32		1.56
4		+ 1.68		1.80
5		+ 1.56		1.56

6		+ 1.20		1.08
7		+ 1.44		1.56

Appendix 4: Experiment 5: Photographs of cannlue placements

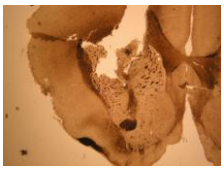
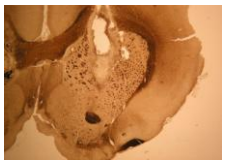
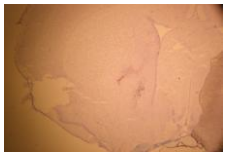
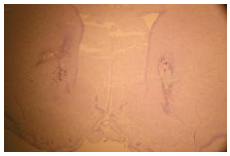
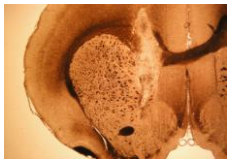
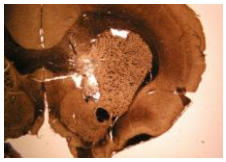
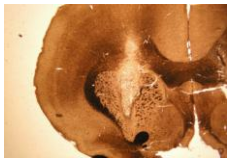
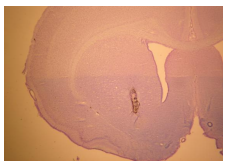
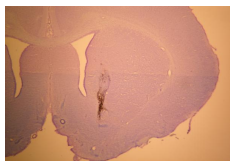
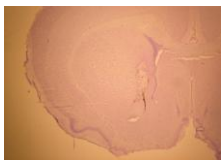
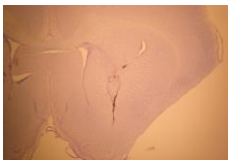
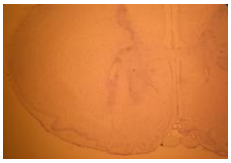
Table 4: Photographs showing the location of injector tips (mm from bregma) for each animal treated with eticlopride infusions into the NAcb core with systemic amphetamine.

Animal	Left Hemisphere	LH Location (mm)	Right Hemisphere	RH Location (mm)
1		+ 1.92		+ 2.04
2		+ 1.68h		+ 1.68 - 1.56
3		+ 1.08 - 0.96		+ 1.08
4		+ 1.68		+ 1.80
5		+ 1.68		+ 1.56

6		+ 1.56		+ 1.56
7		+ 1.08		+ 1.32
8		+ 1.44		+ 1.44

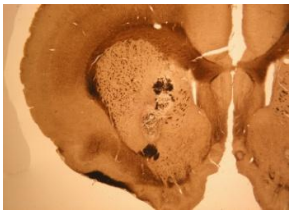
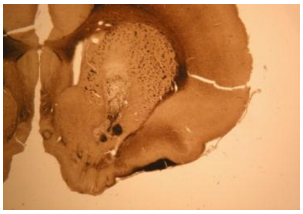
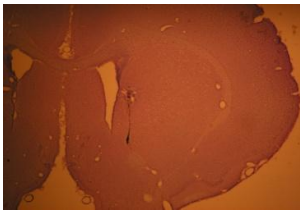
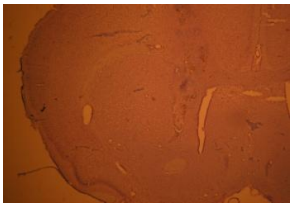
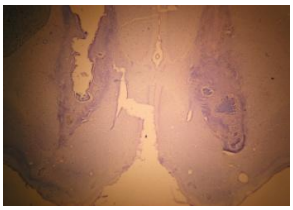
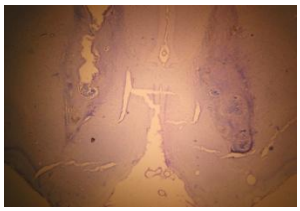
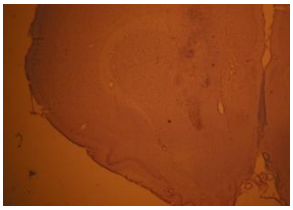
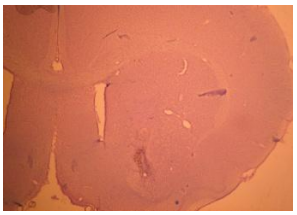
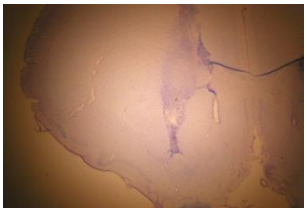
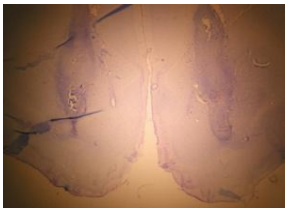
Appendix 5: Experiment 6: Photographs of cannlue placements

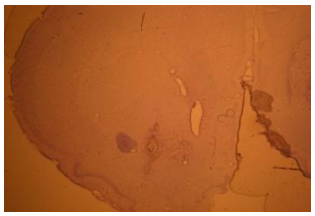
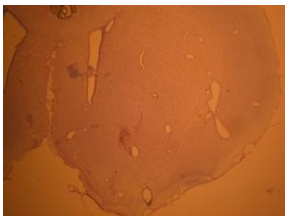
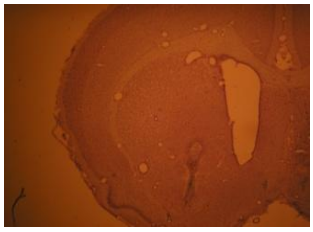
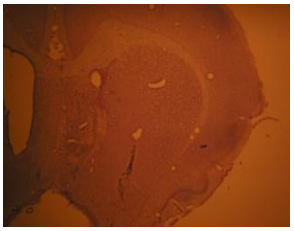
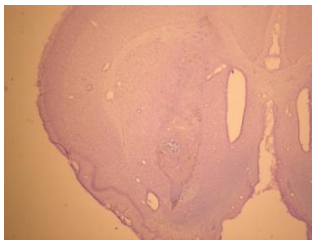
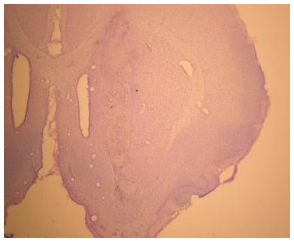
Table 5: Photographs showing the location of injector tips (mm from bregma) for each animal treated with muscimol infusions into the NAcb core.

Animal	Left Hemisphere	LH Location	Right Hemisphere	RH Location
1		+2.28		+2.04
2		+1.20		+1.20
3		+1.44		+1.56
4		+2.04		+2.24
5		+1.44-1.32		+1.56-1.44
6		+1.68		+1.68
7		+1.92		+2.16

Appendix 6: Experiment 7: Photographs of cannlue placements

Table 6: Photographs showing the location of injector tips (mm from bregma) for each animal treated with muscimol infusions into the NAcb core with systemic amphetamine.

Animal	Left Hemisphere	LH Location	Right Hemisphere	RH Location
1		+2.04		+2.04
2		+1.68		+1.56
3		+1.20		+1.44
4		+1.80		+1.92
5		+2.28		+1.92
6		+2.16		+2.28

7		+1.92		+1.80
8		+1.56-1.44		+1.56
9		+1.68		+1.68

Appendix 7: Experiment 7: No-go trial accuracy following muscimol and systemic amphetamine treatment

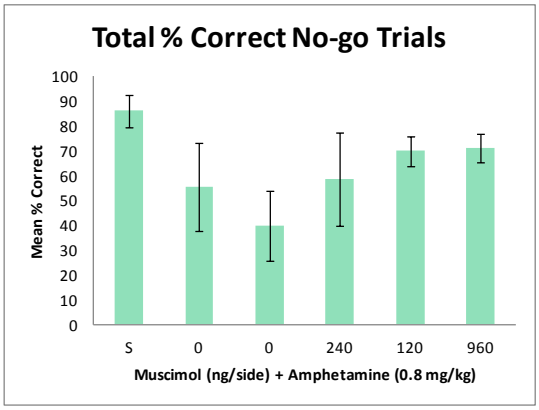


Fig. 7a

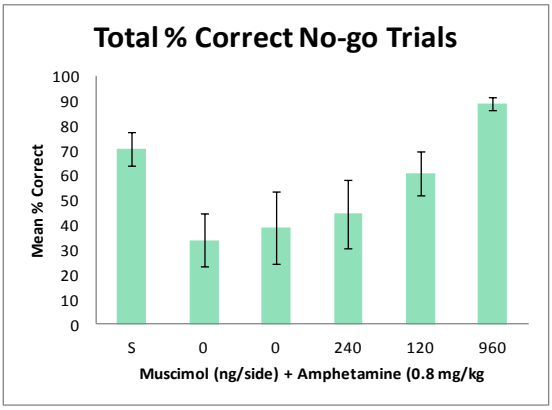


Fig. 7b

Figures 7a: No-go trial accuracy in (n = 4) rat that did not display full behavioural reversal of amphetamine induced impulsivity. **Figure 7b:** No-go trial accuracy in (n = 5) rats that did display full behavioural reversal of amphetamine induced impulsivity.