The effect of methamphetamine on habits, response conflict and neural morphology.

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The effect of methamphetamine on habits, response conflict and neural morphology.

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The first experiments in this thesis examined the influence of chronic methamphetamine and methamphetamine-paired contexts on S-R habits in undertrained animals. We found that chronic methamphetamine exposure prior to instrumental learning caused a rapid transition to the dominance of S-R habits over goal-directed actions. This was not due to chronic methamphetamine entirely abolishing the capability of goal-directed behaviour. When instrumental learning occurred prior to training but after test goal-directed behaviour was observed. We also found evidence of a return to goal-directed behaviour following a period of abstinence prior to training. Lastly, we found distinct differences in behavioural control when animals were tested in a methamphetamine- or saline-paired context. A second series of experiments examined the influence of acute and chronic methamphetamine on animals' ability to use contextual information to resolve conflict using a contemporary animal model of human executive function. Here we found a difference between the effects of acute and chronic methamphetamine administration on executive function. Acutely, methamphetamine had no impact on animals' ability to use contextual information to resolve conflict. However, chronic methamphetamine led to significantly impaired performance in this task. A third set of experiments investigated whether an identical chronic methamphetamine regimen caused drug-dependent neural plasticity in brain regions known to be involved in S-R habits and executive function, and to ascertain whether different periods of abstinence had any effect on methamphetamine-dependent neural plasticity. We found evidence of persistent changes in spine density of pyramidal cells in the prelimbic and infralimbic cortices. This implies that perhaps neurochemical adaptations occur over time in order to restore balance to the system, despite enduring changes to structure of the systems. Such restoration of balance may allow for a return to normal function.

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Abstract

The first series of experiments in the present thesis examined the influence of chronic methamphetamine and methamphetamine-paired contexts on S-R habits in animals that had undergone a limited amount of instrumental training. We found that chronic methamphetamine exposure prior to instrumental learning caused a rapid transition to the dominance of S-R habits over goal-directed actions. However, this was not due to chronic methamphetamine entirely abolishing the capability of goal-directed behaviour. When instrumental learning occurred prior to training but after test, goal-directed behaviour was observed. We also found evidence of a return to goal-directed behaviour following a long period of abstinence prior to instrumental training. Lastly, we found distinct differences in behavioural control when animals were tested in a methamphetamine- or saline-paired context. In a second series of experiments we examined the influence of acute and chronic methamphetamine on animals' ability to use contextual information to resolve conflict and behave appropriately using a contemporary animal model of human executive function. Here we found a clear difference between the effects of acute and chronic methamphetamine administration on executive function. Acutely, methamphetamine had no impact on animals' ability to use contextual information to resolve conflict. However, chronic methamphetamine led to significantly impaired performance in this task. In a third set of experiments we investigated whether the identical chronic methamphetamine regimen used in our previous experiments caused drug-dependent neural plasticity in brain regions known to be involved in S-R habits and executive function, and to ascertain whether different periods of abstinence have any effect on methamphetamine-dependent neural plasticity. Here we found evidence of persistent changes in spine density of pyramidal cells in the prelimbic and infralimbic cortices. This implies that perhaps neurochemical

adaptations occur over time in order to restore balance to the system, despite enduring changes to structure of the systems. Such restoration of balance may allow for a return to normal function.

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List of Publications

Oral Presentations

Pacitti, H.D., Balleine, B.W. & Killcross, A.S. (2011). Methamphetamine induced habits: the role of context. *UNSW Psychology Postgraduate Seminar,* Sydney, NSW, Australia.

Pacitti, H.D. & Killcross, A.S. (2012). Habits govern instrumental performance in methamphetamine-paired contexts. *UNSW Science Postgraduate Research Competition,* Sydney, NSW, Australia.

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Pacitti, H.D., Balleine, B.W. & Killcross, A.S. (2015). The effects of chronic methamphetamine on habits and synaptic plasticity. *UNSW Psychology Postgraduate Seminar,* Sydney, NSW, Australia.

Poster Presentations

Pacitti, H.D., Balleine, B.W. & Killcross, A. S. (2012). The role of context in methamphetamine-induced habits. 32nd Australian Neuroscience Society (ANS). Gold Coast, QLD, Australia.

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Pacitti, H.D., Balleine, B.W. & Killcross, A. S. (2015). Effects of chronic methamphetamine on habits and cognitive control. 42nd Australasian Experimental Psychology Conference (EPC), Sydney, NSW, Australia.

Pacitti, H.D., Balleine, B.W. & Killcross, A. S. (2015). Effect of methamphetamine on habits, cognitive control and structural plasticity. 4th APSAAR/5th IDARS Conference: Frontiers in Addiction, Sydney, NSW, Australia.

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Awards

Australian Postgraduate Award (2011-2014).

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Care and Use of Animals

The experiments reported in the present thesis conformed to the guidelines of the ethical use of animals maintained by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th and 8th Editions) and all procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales (ACEC: 12/69B). All efforts were made to reduce both suffering and the number of animals used.

List of Abbreviations

A	Action
A1	Auditory cue 1
A2	Auditory cue 2
A_{2A}	Adenosine 2A receptor
ACC	Anterior Cingulate Cortex
AMPA	α-amino-3-hydroxy-5-methylisoxalzole-4-proprionic acid
C1	Context 1
C2	Context 2
CNS	Central nervous system
CPP	Conditioned Place Preference
CPu	Caudate-putamen
CS	Conditioned Stimulus
D1	Dopamine 1 receptor
D2	Dopamine 2 receptor
DAT	dopamine transporter
DLS	dorsolateral striatum
DMS	dorsomedial striatum
fMRI	Functional magnetic resonance imaging
GFP	Green Fluorescent Protein

IL	Infralimbic cortex
ITI	Inter-trial interval
LiCl	Lithium chloride
LP1	Lever press 1
LP2	Lever press 2
LTD	Long Term Depression
LTP	Long Term Potentiation
mPFC	medial Prefrontal cortex
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance Spectroscopy
MSN	Medium spiny neurons
Mm	Micrometre
NA	Numerical aperture
NAcc	Nucleus accumbens
NAC	N-acetylcysteine
O	Outcome
O1	Outcome 1
O2	Outcome 2
PET	Positron emission tomography
PFC	Prefrontal cortex
PL	Prelimbic cortex
R	Response

RI	Random interval
RT	Random time
S	Stimuli
UNODC	United Nations Office of Drugs and Crime
US	Unconditioned stimuli
WCST	Wisconsin Card Sort Test
V1	Visual cue 1
V2	Visual cue 2
VTA	ventral tegmental area

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Chapter 1

The "Ice" Age: Methamphetamine use a growing public health concern.

The illegal use of methamphetamine poses significant risk to both its users and to the community. In April 2015, the then Prime Minister of Australia, Tony Abbott, announced the implementation of the "National Ice Action Strategy" (C.O.A.G., 2015). The aim of this initiative was to combat the growing problem of illicit methamphetamine (AKA "ice") use in Australia. According to the Australian Institute of Health and Wellbeing's Household Drug Survey (A.I.H.W., 2014), 50% of the stimulant-using population in Australia named methamphetamine as their drug of choice. This percentage had doubled from the previous survey in 2010 (A.I.H.W., 2011), in which only 22% of the stimulant using population reported methamphetamine as their drug of choice. Further highlighting this surge in use, the reports indicated that daily or weekly use of methamphetamine increased from 9.3% in 2010 to 15.5% in 2013. While these prevalence rates are alarming, Australia is not alone in this "ice epidemic". According to the United Nations Office of Drugs and Crime's (U.N.O.D.C.) World Drug Report (U.N.O.D.C., 2012), 80% of global stimulant seizures were of methamphetamine, specifically 114 tons, a quantity that had almost quadrupled since 2008; and worldwide 96% of laboratories that were illegally producing stimulants were exclusively manufacturing methamphetamine. There is no doubting that it was survey data such as these that contributed to the UNODC declaring methamphetamine a global public health concern.

Although there are issues with studies that rely on self-reported illicit substance use, this apparent rise in Australian's use of methamphetamine is supported by studies of waste water. A recent study by Tscharke and colleagues (Tscharke, Chen, Gerber, &

White, 2015) analysed samples of waste water for traces of drugs of abuse, thus providing an unbiased measure of population drug use. The results from this work indicate that, if anything, methamphetamine use was under-reported in the Household Drug Survey (A.I.H.W., 2014). Tscharke and colleagues found methamphetamine to be the most prevalent illicit substance detected in the waste water that they analysed. This increased use of methamphetamine in the Australian population (A.C.C., 2015; A.I.H.W., 2011, 2014; Tscharke et al., 2015), coupled with the significant burden methamphetamine use and related harms places on the nation's health and judicial systems (C.O.A.G., 2015), highlights the importance of research that aims to uncover the biological and psychological effects of both acute recreational and chronic methamphetamine use.

Methamphetamine, a synthetic psychostimulant, was first synthesised in 1888 by Japanese chemist, Nagayoshi Nagai (Sato, 2008). However, it was not until the 1930s that methamphetamine became available commercially for its use as a pharmaceutical to treat disorders such as narcolepsy and obesity (N.I.H., 2013). In the 1940s, Germany manufactured more than 35 million methamphetamine tablets and distributed them to soldiers during WWII in aid of the "Blitzkreig" or "lightning war" effort to facilitate Germany's rapid invasion of Europe (Shunk, 2015). However, Germany halted production and distribution once methamphetamine's dangerous addictive potential was recognised. Despite this recognition, methamphetamine continued to be available on the global market as a prescribed therapeutic. After several decades of commercial availability it gained popularity as a recreational drug in the 1960s. In more recent times, methamphetamine is readily available in its powder or crystalline form whereby users most commonly smoke or intravenously inject the drug (Cunningham, Liu, & Muramoto, 2008; Matsumoto et al., 2002; Radfar & Rawson, 2014; Simon, Richardson, et

al., 2001). Both routes of administration produce intense acute physical and behavioural effects.

Acute physical and behavioural effects of methamphetamine

Although methamphetamine affects multiple neurotransmitter systems, many of the physical and behavioural effects of methamphetamine come as a result of its powerful action on the brain's dopamine system (Meyer & Quenzer, 2013). Under normal circumstances, once a dopaminergic cell receives enough sensory input to depolarise, an event-related potential will cause the neuron to "fire". Subsequently, vesicles within the pre-synaptic cell will release dopamine into the synaptic cleft where it binds to the dopamine receptors on the post-synaptic cell. Any leftover excess dopamine molecules are either taken back up into the pre-synaptic cell via the dopamine transporter (DAT), a process referred to as "reuptake", for repackaging into synaptic vesicles for future re-release, or they are metabolised to avoid over accumulation (Meyer & Quenzer, 2013).

In contrast, methamphetamine causes dopamine to be released in the absence of an action potential and thus its action is independent of the cell firing. After administration, methamphetamine readily crosses the blood brain barrier due to its high lipid solubility. Once in the central nervous system (CNS), methamphetamine acts as an indirect dopamine receptor agonist, with two key mechanisms of action. Firstly, methamphetamine is taken up by the DAT where it enters the presynaptic cell and causes dopamine release from the vesicles into the synaptic cleft. Secondly, methamphetamine causes the DAT to function in reverse whereby the DAT allows dopamine to be transported out of the pre-synaptic cell. A third mechanism can occur when methamphetamine is administered at high doses, whereby metabolism of

dopamine in the synaptic cleft is blocked via inhibition of monoamine oxidase. The result of these mechanisms is an excessive release of dopamine in the synaptic cleft and thus a supranormal increase in the availability of dopamine in the CNS (Meyer & Quenzer, 2013).

More apparent effects are observed following acute administration of methamphetamine, including changes in the cardiovascular system such as, increased blood pressure, heart rate and respiration (Kirkpatrick, Gunderson, Perez, et al., 2012; N.I.H., 2013). Methamphetamine elevates confidence and mood, making users experience prolonged bouts of euphoria and sociability, making it a popular party drug (Halkitis, Fischgrund, & Parsons, 2005). Increased wakefulness and sustained attention are also observed following acute administration and in turn facilitate an increase in activity (Kirkpatrick, Gunderson, Johanson, et al., 2012). These seemingly positive effects of acute methamphetamine, together with the common routes of administration (i.e. via smoking or intravenous injection) producing a rapid onset, no doubt contribute to the drug's reputed high addictive potential (A.C.C., 2015; C.O.A.G., 2015; Cunningham et al., 2008; Matsumoto et al., 2002; Meyer & Quenzer, 2013; N.I.H., 2013; Radfar & Rawson, 2014; Simon, Richardson, et al., 2001). In contrast, the downside is that methamphetamine can also make individuals feel extremely restless and agitated. Indeed, quite notoriously, some users become extremely violent and some experience psychotic episodes (N.I.H., 2013). However, it is unclear whether these extreme reactions are a result of the sleep deprivation that occurs in the days following acute use, or whether these are due to more chronic, binge-like use. Nevertheless, the violence associated with use and lack of control exhibited by users pose a significant problem that needs to be addressed.

Effects of chronic methamphetamine use on the brain

Understanding the effects chronic methamphetamine use has on the brain is vital, considering its high addictive potential, and the fact that users often fall victim to long term periods of abuse. Using various approaches, researchers have examined the impact of long term methamphetamine use on brain morphology and function. For example, Thompson and colleagues (Thompson et al., 2004) used magnetic resonance imaging (MRI) to assess brain morphology in 22 individuals currently using methamphetamine and compared this group to 21 healthy age-matched controls. The study found there was reduced grey-matter by 11.3% in the cingulate and more modest depletions in the limbic and paralimbic cortices and the hippocampus of participants in the methamphetamine group compared to control participants. Thompson also found increases in white matter in the temporal and occipital lobes. The increases and decreases in grey- and white-matter density are indicative of changes in the structure of the brain as a result of long-term drug use, a phenomena referred to as drug-dependent plasticity. Although such findings are important, changes in brain morphology do not necessarily mean that brain function is impacted.

However, some evidence for changes in brain function at a neurochemical level was identified by Wilson and colleagues (Wilson et al., 1996). This research used post-mortem neurochemical assays on the brains of chronic methamphetamine users (*n*= 12) compared to a healthy control group (*n*= 11). Wilson's work suggests dopamine and DAT depletion in the accumbens, caudate and putamen nerve terminals in the methamphetamine users compared to the control participants. Follow up work coming from the same laboratory on an additional 8 participants yielded the same pattern of results (Moszczynksa et al., 2004). These findings indicate that over time the function of

dopamine within striatum becomes down-regulated, perhaps as a consequence of persistent excess dopamine levels caused by methamphetamine use.

Abnormalities in dopamine function in the striatum of methamphetamine users have also been identified by researchers using positron emission tomography (PET) (McCann et al., 1998; Volkow, Chang, Wang, Fowler, Ding, et al., 2001; Volkow, Chang, Wang, Fowler, Leonido-Yee, et al., 2001; Wang et al., 2004). For example, McCann and colleagues (McCann et al.) compared abstinent methamphetamine users (Cuzen, Koopowitz, Ferrett, Stein, & Yurgelun-Todd) to healthy controls (*n*= 10) and found that those with a history of methamphetamine use had decreased DAT concentration in the caudate and putamen, which the authors suggest are indicative of persistent damage to the axons and axon terminals of dopamine cells in these regions. Other work using PET found that abstinent methamphetamine users (*n*= 15) had fewer D2 receptors compared to controls (*n*= 20) in the caudate (16%) and putamen (10%) (Volkow, Chang, Wang, Fowler, Ding, et al., 2001). In a concurrent piece of work Volkow and colleagues (Volkow, Chang, Wang, Fowler, Leonido-Yee, et al., 2001) found reduced DAT in the caudate (27.8%) and putamen (21.1%) in abstinent methamphetamine abusers (*n*= 15) with an average of 5 months sobriety, compared to controls (*n*= 18). There is also some evidence that these changes in striatal dopamine function persist in the long term because reduced DAT was also found by Wang and colleagues when this research group examined a group of methamphetamine users who had been abstinent for an average period of three years (Wang et al., 2004).

Although much work has focussed on the striatum, there is evidence for functional changes outside this area. For example, Paulus and colleagues (Paulus et al., 2002) used functional magnetic resonance imaging (fMRI) to investigate functional

deficits in individuals with a history of chronic methamphetamine use who were in the early stages of recovery. Methamphetamine users (*n*= 10) were compared to age- and education-matched controls (*n*= 10) and scanned whilst performing a decision-making task. Paulus found that compared to controls, methamphetamine users had significantly less activation of the dorsolateral prefrontal cortex whilst they performed the decision-making task. In addition, the ventromedial part of the prefrontal cortex was activated while the control group performed the task but no activation was found in the same area of those with a history of methamphetamine use.

Although informative, results from studies investigating changes in the structure and function of the human brain need to be interpreted with prudence for several reasons. Firstly, Thompson and colleagues (Thompson et al., 2004) noted that the individuals in the methamphetamine group used in his study also reported use of other substances. Such poly-drug use is common in drug-using populations which makes it impossible to isolate the effect of one drug over another. Moreover, drugs acquired on the black market are adulterated so purity and integrity of street drugs cannot be determined. Secondly, durations and patterns of use vary considerably amongst individuals (Moszczynksa et al., 2004; Paulus et al., 2002; Volkow, Chang, Wang, Fowler, Ding, et al., 2001; Volkow, Chang, Wang, Fowler, Leonido-Yee, et al., 2001; Wang et al., 2004; Wilson et al., 1996) and this makes it difficult to determine how extensive or prolonged use needs to be in order to be considered potentially damaging at a neurological level. Thirdly, these studies rely on self-reported drug use which is subject to bias and general failings of memory over time. Fourth, it is impossible to determine whether any apparent neurological abnormalities were pre-existing and similarly, unless a longitudinal design is adopted, whether any abnormalities improve over time.

So, although such studies give valuable insight, the design of such experiments do not allow causal determinations to be made.

More conclusive evidence for methamphetamine-dependent neurological changes come from studies on laboratory rodents. Early studies using pharmacological and morphological techniques with rats have shed light on the neurotoxic effect methamphetamine has when it is administered at high doses (Davidson, Gow, Lee, & Ellinwood, 2001; Kleven & Seiden, 1992). Strong evidence has now accumulated which demonstrates persistent depletion of dopamine across various brain regions, and like the changes observed in the human literature, these changes are most notable in the striatum (Marek, Vosmer, & Seiden, 1990; Ricaurte, Guillery, Seiden, Schuster, & Moore, 1982; Ricaurte, Schuster, & Seiden, 1980; Schmidt, Ritter, Sonsalla, Hanson, & Gibb, 1985; Wagner, Lucot, Schuster, & Seiden, 1983; Wagner et al., 1980; Wagner, Seiden, & Schuster, 1979). Further investigations have linked these pharmacological findings to changes in brain structure, whereby loss of dopamingeric cells have been identified following administration of methamphetamine at high doses (Brunswick, Benmansour, Tejani-Butt, & Hauptmann, 1992; Eisch, Gaffney, Weihmuller, O'Dell, & Marshall, 1992; O'Dell, Weihmuller, & Marshall, 1991; Ricaurte, Seiden, & Schuster, 1984; Seiden & Vosmer, 1984; Wagner et al., 1983; Wagner et al., 1980). However, these studies generally administer methamphetamine on only a couple of occasions at very high doses, which does not mimic typical human use patterns.

More recently, researchers have adopted different dosing regimens in an effort to better model the binge-crash nature of human use (Davidson et al., 2001). Complementing the human literature, several investigations with rats have also identified abnormalities in the dopamine system following this pattern of

administration of methamphetamine. For example, work by Broening and colleagues (Broening, Pu, & Vorhees, 1997) found evidence for impaired dopamine innervation to the nucleus accumbens core (but not the shell) following four, 10mg/kg s.c. doses of methamphetamine in a 6 hour period. Using a lower dose of methamphetamine at 5mg/kg s.c. administered four times in a six-hour period, Cass and Manning found a reduction in evoked striatal dopamine efflux, and a slower reuptake, for up to one month after exposure (Cass & Manning, 1999). However, one year post-methamphetamine administration, dopamine function returned to levels comparable to control animals.

In an early study examining behavioural sensitization, the characteristic heightened locomotor response of animals given a low dose of a psychostimulant following prior repeated exposure to the same drug, Nishikawa and colleagues (Nishikawa, Mataga, Takashima, & Toru, 1983) found evidence for sensitization even in a group of animals that had only received methamphetamine on one prior occasion, suggesting that sensitization to methamphetamine occurs remarkably quickly. In this study, methamphetamine was administered at 6mg/kg i.p. once a day for either 1, 3, 7 or 14 days. Following a two-week withdrawal period, animals were administered a low dose of methamphetamine (2mg/kg i.p.) and locomotor and biochemical responses were recorded. All rats previously administered methamphetamine showed increased locomotion following the methamphetamine challenge. For those rats that had previous repeated administrations, dysregulation of dopamine was also found. Specifically, compared to control rats, those with a history of repeated methamphetamine administration exhibited higher turnover of dopamine in the striatum following the methamphetamine challenge. This suggests that chronic methamphetamine use disrupts dopamine function by increasing dopaminergic tone in the striatum.

Work with laboratory rodents has also provided good evidence for changes in brain morphology following exposure to methamphetamine. Jedynak and colleagues (Jedynak, Uslaner, Esteban, & Robinson, 2007) exposed rats to methamphetamine five days per week for a period of four weeks, on an escalating dose regimen beginning at 0.5mg/kg and increasing to 6mg/kg for the final four days. After a 3-day withdrawal period, behavioural sensitization to methamphetamine was confirmed by way of a locomotor challenge to a sub-threshold (0.5mg/kg i.p.) dose of the drug, and these animals were then left for three months in their home cages. After this time, Jedynak used GFP immunohistochemistry in order to visualise cell morphology. In comparison to saline-injected controls, rats exposed to methamphetamine showed a significant increase in the density of dendritic spines on medium spiny neurons (MSNs), cells which receive glutamatergic input from the PFC and dopamingeric input from the substantia nigra, in the dorsolateral striatum (DLS). This work also found a significant decrease in spine density on MSNs in the dorsomedial striatum (DMS) in the methamphetamine-treated rats compared to saline control-group animals. This study provides strong evidence of methamphetamine-dependent synaptic plasticity in the dorsal striatum. However, aside from enhanced locomotor response to confirm sensitization, no behavioural or cognitive assays were included in the experiment so the impact these changes have on behaviour require further research.

Does chronic methamphetamine use negatively affect cognition and executive function?

At present it is unclear exactly what the effects of long-term chronic methamphetamine use are, particularly in the domain of cognition. Indeed, expert consensus in the field is lacking (Baicy & London, 2007; Bernheim, See, & Reichel, 2016;

Dean, Groman, Morales, & London, 2013; Hart, Marvin, Silver, & Smith, 2012; Jan, Kydd, & Russell, 2012; Nordahl, Salo, & Leamon, 2003) with three of the most recently published reviews of the effect chronic methamphetamine use has on cognitive function reaching different conclusions (Bernheim et al., 2016; Dean et al., 2013; Hart et al., 2012). For example, following their review of translational models of methamphetamine self-administration is rats, Bernheim and colleagues concluded that chronic use leads to dysregulation in various cognitive domains, such as: attention, set shifting, and recognition memory. However, reviews of laboratory studies of methamphetamine users in the human population published by Hart and colleagues concluded that the cognitive performance of methamphetamine users differed from controls on only a minority of measures and the clinical significance of the differences was limited. Yet, following their review of both animal and human research, Dean and colleagues conclude that there is some evidence for both sides: "findings are mixed with some support for a causal relationship between methamphetamine abuse and cognitive decline, and other findings which suggest there is no relationship" (Dean et al., 2013, p. 259) and add that in instances where dysfunction is apparent, it is unknown how long these deficits persist.

One domain of executive function that has yielded consistent findings of deficits in both rodents (Cheng, Etchegaray, & Meck, 2007; Cox et al., 2016; Furlong, Leavitt, Keefe, & Son, 2016; Izquierdo et al., 2010; Shoblock, Maisonneuve, & Glick, 2003; Son, Kuhn, & Keefe, 2013; White, Minamoto, Odell, Mayhorn, & White, 2009) and humans (Henry et al., 2011; Monterosso, Aron, Cordova, Xu, & London, 2005; Salo et al., 2005; Tolliver et al., 2012) exposed to methamphetamine comes from studies of response inhibition. Researchers employing stop-signal tasks, which require subjects to either respond or inhibit a response depending on distinct cues, have found increased reaction

times for human methamphetamine users on stop trials compared to control subjects, whilst no difference was observed between the groups on the go trials. That this difference in response times is exclusively on stop trials, suggests that the methamphetamine users in this study found it more difficult to cease responding when they were required to do so (Monterosso et al., 2005). Furlong and colleagues (Furlong et al., 2016) used a similar stop-signal procedure in rats that had been exposed to a neurotoxic regimen of methamphetamine. Like Monterosso, Furlong found a specific stop-signal deficit in rats exposed to the neurotoxic regimen compared to rats that were exposed to a non-neurotoxic methamphetamine regimen and a control group of saline-treated rats. However, no differences in performance were found between groups on go-trials. It seems therefore that prolonged exposure to methamphetamine can result in difficulty inhibiting a response when it is inappropriate.

These findings from studies using response inhibition paradigms in the laboratory are in line with anecdotal statements from methamphetamine users who report engaging in bizarre stereotypic behaviour. These stereotypies, colloquially referred to as "punding" or "tweaking" can take a variety of forms (e.g. sorting of objects, assembling and reassembling gadgets) but are generally considered to be the performance of a useless task that is carried out in a compulsive and repetitive manner (Nordahl et al., 2003; Scott et al., 2007). Although users report being aware of these behaviours, they claim they are unable to stop carrying them out, even after dedicating long periods of time to them. Most notable is the prolonged and uncontrollable nature of these bizarre repertoires that mirror the habitual nature of the methamphetamine addiction itself.

Effect of methamphetamine on executive function in humans

Appropriate behaviour in complex situations requires an organism to integrate information in order to withhold elicited but inappropriate responses and thus exhibit "top-down" control flexibly to coordinate behaviour to obtain desired outcomes. Numerous studies have used the Stroop task to assess cognitive function in humans currently using methamphetamine (Farhadian, Akbarfahimi, Hassani Abharian, Hosseini, & Shokri, 2017; Simon et al., 2000a; Simon, Domier, et al., 2001) or during periods of abstinence (Chang et al., 2002; Hekmat, Mehrjerdi, Moradi, Ekhitari, & Bakshi, 2011; King, Alicata, Cloak, & Chang, 2010; Salo, Nordahl, et al., 2009; Salo et al., 2002; Salo, Ravizza, & Fassbender, 2011). Empirical findings from studies comparing performance of people currently using methamphetamine compared to controls have consistently found significant deficits (Farhadian et al., 2017; Simon et al., 2000a; Simon, Domier, et al., 2001). However findings from some studies comparing abstinent methamphetamine users to controls have been more varied; some research has found increased reaction times for methamphetamine users compared to controls (Hekmat et al., 2011; King et al., 2010; Salo et al., 2002) whereas others have found that performance of methamphetamine users was within the normal range for the test (Chang et al., 2002). Farhadian examined how performance on the Stroop task differs between those currently using methamphetamine and those who are abstinent, finding that current users performed worse on the Stroop task compared to those in recovery (Farhadian et al., 2017). Indeed, there is some indication that observed deficits on the Stroop task improve following longer periods of sobriety (Salo, Nordahl, et al., 2009; Salo et al., 2011).

Studies utilising fMRI on methamphetamine users have shown that poorer performance on the Stroop task was associated with hypoactivation of the PFC whilst the task was being completed, compared to control participants (Nestor, Ghahremani, Monterosso, & London, 2011; Salo, Fassbender, Buonocore, & Ursu, 2013; Salo, Ursu, Buonocore, Leamon, & Carter, 2009). Work using Magnetic Resonance Spectroscopy (MRS) an imaging technique which provides a measure of levels of brain chemicals and metabolites, rather than structure (as in MRI) or activation (as in fMRI), found reduced levels of metabolites in the Anterior Cingulate Cortex (ACC) of abstinent methamphetamine users who also exhibited increased Stroop interference (Salo et al., 2007). Differences in the human literature can be due to a number of confounding variables so investigations that allow for better experimental control using laboratory rodents are informative.

Another task used to examine executive function in humans is the Wisconsin Card Sort Test (WCST). In this classic task, the participant is required to sort a deck of cards based on an unknown guiding principle (i.e. either by shape, colour, or number). After each attempt to sort a card, the participant is given feedback by the experimenter as to whether their choice was "correct" (positive feedback) or "incorrect" (negative feedback). Once the participant adopts a consistent sorting strategy (e.g. sort by colour), based on the omission of negative feedback from the experimenter over several trials, the guiding principle is changed and the participant must learn to adopt a new sorting strategy (e.g. sort by shape). Much like the Stroop task, this task requires participants to make use of a rule they are holding in mind (sort by dimension X) to direct their responses according to a particular dimension (colour, shape, number) of a compound cue. In addition, the WCST also requires participants to use feedback to indicate when they should discard one rule, and then generate a new one. An index of

response inhibition is provided by the "perseveration" score, or how many times the participant fails to change their strategy after receiving negative feedback, against the total number of errors made (Aron, Robbins, & Poldrack, 2004). The higher the number of perseveration errors made, the greater the implied impairment. Several studies have used the WCST to investigate deficits in executive function in methamphetamine users.

Several researchers have used the WCST to study methamphetamine users after various periods of abstinence from the drug and findings have again been mixed (Chou et al., 2007; Han et al., 2008; Henry et al., 2011; Hosak et al., 2012; Iudicello et al., 2010; Kim et al., 2006; Kim et al., 2005; Kim, Kwon, & Chang, 2011; Simon et al., 2000b; van der Plas, Crone, van den Wildenberg, Tranel, & Bechara, 2009; Woods et al., 2005). Choosing to focus exclusively on those in early abstinence (i.e. 2 weeks), Chou and colleagues found that participants made fewer total errors compared to baseline performance following a 2-week period of abstinence. The total number of errors made by the participants improved markedly, from 32% to 15%, during these two weeks. However, the sample size was small (n= 5) and four of the participants were experiencing delusions and/ or hallucinations during the baseline test. The study did not incorporate a control group into the experimental design, nor did they discuss the performance of the sample against WCST standardized norms. All of these factors make interpretation of Chou's findings problematic.

Other researchers have focussed less on precise periods of abstinence, by setting a minimum period of sobriety as inclusion criteria, which have ranged anywhere from five days to four weeks as a minimum (Han et al., 2008; Henry et al., 2011; Hosak et al., 2012; Kim et al., 2005; Kim et al., 2011; Woods et al., 2005). For example, Hosak and colleagues administered the WCST to inpatients (n= 43) at a treatment facility who had

been drug free for at least four weeks. Hosak et al. found that the methamphetamine-users performance was significantly poorer compared to a group of healthy control participants (*n*= 52) on all WCST measures. However, although the methamphetamine-users made more errors on the task, this group's performance was still within the normal range based on WCST norms. Work by Han and colleagues also required a minimum of four weeks abstinence from participants in their study. Likewise, Han et al. found that those with a history of methamphetamine use (*n*= 37) made significantly more errors and more perseverative errors compared to healthy controls (*n*= 40). However, unlike Hosak and colleagues, the researchers did not disclose whether or not the performance of the methamphetamine users lay within the test's norms. Although impairments in response inhibition were found in both Hosak's and Han's studies, it is not clear whether these impairments persist in the long-term or whether there could be some recovery of function after longer periods of abstinence.

However, there is some evidence for recovery of function that has come from studies using the WCST that have adopted repeated measures designs (Iudicello et al., 2010; Kim et al., 2006). Work by Iudicello and colleagues examined the effects of long-term abstinence from methamphetamine on executive function. They found that participants who relapsed back into drug use were still impaired on the WCST at a one-year follow-up, but those individuals who remained drug-free were not distinguishable from non-using controls after an average of one year's sobriety. Similar results were found by Kim and colleagues who examined both short- (less than six months) and long-term (more than six months) abstinent methamphetamine users and compared these two groups to non-using controls (*n*= 20). Long-term sober methamphetamine users (*n*= 18) performed more poorly compared to control subjects, however they performed better than short-term abstinent users (*n*= 11). The findings from the independent

studies of Iudicello and Kim do provide some evidence that the impairments found in the early stage of sobriety do improve to some extent over time.

In contrast to studies examining abstinent participants, some studies using the WCST have found no difference in the performance of current methamphetamine users compared to healthy controls (Simon et al., 2000b; van der Plas et al., 2009). For example, Simon and colleagues administered the WCST to individuals currently using methamphetamine (*n*= 65) and did not find any differences between users compared to healthy controls (*n*= 65), even though the methamphetamine group did show significant deficits on other tasks used in the experiment (such as Stroop task, Trail Making, and memory recall). It may indeed be the case that some of the methamphetamine users in this study were under the influence of the drug at the time of testing and the drug could have enhanced their performance by increasing attentional function, making the methamphetamine group perform at levels comparable to controls. However, a study completed by van der Plas and colleagues (van der Plas et al., 2009) utilised a sample of inpatients with minimum periods of abstinence of 15 days, and, like Simon et al., these researchers also failed to find impairments on methamphetamine users' (*n*= 38) WCST performance compared to controls (*n*= 36). Taken together, the findings from these two studies cast some doubt on whether or not acute methamphetamine use has a negative impact on executive function.

Models of Addiction

The nature of substance addiction has been the focus of a plethora of studies since the 1960s. Over subsequent decades, several neuropsychological models have been put forth in an effort to explain the mechanisms involved in the onset and maintenance of this debilitating disorder. The most prominent theories in psychology

stem from the instrumental learning principles of positive and negative reinforcement and how these principles influence behaviour. Positive reinforcement refers to a type of learning that occurs because a response results in a rewarding outcome. Negative reinforcement refers to a type of learning that occurs because a response results in the termination of an aversive situation. These two types of instrumental learning have formed the building blocks for three of the most prominent models of addiction in neuropsychology today.

In the 1980s Wise and Bozarth published their "Psychomotor stimulant theory of addiction" (Wise & Bozarth, 1987). This model posits that all drugs of abuse share a common ability to promote psychomotor activity and approach behaviours and thus must share a common biological mechanism. Working as positive reinforcers, all drugs of abuse, either directly or indirectly, increase dopamine in the mesolimbic system, a brain circuit that starts in the ventral tegmental area and ends in the nucleus accumbens in the ventral striatum. In Wise and Bozarth's opinion the ability of drugs of abuse to act as positive reinforcers by their action on the brain's reward pathway, the mesolimbic system, is the cause of drug dependence. However, this theory fails to explain why drug use continues when it is no longer rewarding, persisting despite adverse consequences that occur as a result of drug use.

Koob and Le Moal (Koob, Caine, Parsons, Markou, & Weiss, 1997; Koob & Le Moal, 1997, 2001, 2008) posit that drug addiction is caused by neuroadaptations which occur as a result of repeated exposure to drugs. The model is centred on the concept of homeostasis; the positively reinforcing aspect of drug taking (i.e. the activation of the brain's reward system) is counteracted by an aversive opponent process (i.e. withdrawal processes) in order to return to a state of equilibrium. Over time, the

withdrawal processes become increasingly more debilitating so that avoiding these negative emotional states becomes the main motivating factor to continue using drugs. So whilst Wise and Bozarth emphasise positive reinforcement, Koob and Le Moal acknowledge both types of reinforcement but consider negative reinforcement, or the desire to alleviate negative emotional states, as the ultimate driving force. However, the problem with this theory is that not all addictive drugs produce debilitating withdrawal symptoms (e.g. psychostimulants).

A model put forth by Everitt and Robbins (Everitt & Robbins, 2005, 2016) also emphasises the role that positive reinforcement plays in addiction whilst accounting for the fact that drug use continues despite negative consequences. Rooted in contemporary learning theory, this model posits that positive reinforcement produces two types of instrumental behaviour. The first, termed "goal-directed" behaviour or "A-O associations" characterises instrumental actions (A) that are deliberately carried out in order to obtain a desired outcome (O). For example, a drug user deliberately makes contact (A) with their drug dealer in order to obtain the drug (O). The second type of behaviour, termed "S-R habits," characterises instrumental responses (R) that are more rigid, and carried out automatically when they are elicited by discrete or contextual stimuli (S) previously present during training. For example, a sober methamphetamine user may run into a friend who they used to take drugs with (S) and find themselves seeking out (R) and taking drugs despite a desire to stay sober.

Like the model proposed by Wise and Bozarth, Everitt and Robbins argue that dopamine plays a key role in disrupting the balance between the neural systems that coordinate actions and habits. In this model, drug addiction is viewed as an aberrant form of S-R habit learning caused by plastic changes on neural pathways involved in

actions and habits, via drug-dependent long-term potentiation and long-term depression. So over time, voluntary control of behaviour is diminished and habits dominate. This model has good face validity, in the first stages of substance use disorders, drug taking begins with a deliberate, conscious decision to take the drug, with the goal in mind to experience the drug's desired effects. In other words, initially, the behaviour is goal-directed. However, as addiction takes hold, the individual finds it progressively more difficult to control their drug-intake and the pursuit of drugs dominates their life, often despite a goal to get, or to stay, abstinent. At this point, the addict has lost executive control over their drug taking; the pursuit of drugs is habitual, compulsive, and persists despite adverse consequences.

There is now considerable empirical evidence mapping A-O and S-R onto distinct neural systems. Goal-directed behaviour depends on communication between the DMS and medial prefrontal cortex (mPFC) and S-R habits recruit the DLS and most likely the motor cortex (Everitt & Robbins, 2016). Specifically, the model posits that in the early stages of voluntary recreational drug use, these networks function normally and the systems are balanced. However, repeated drug-use leads to the formation of compulsive habits because of a shift in dominance in the control of behaviour from circuits centred on the PFC to those centred on the DLS. Also occurring is a shift from activity elicited by the rewarding aspect of Pavlovian unconditioned stimuli (US) within the nucleus accumbens in the ventral striatum, to activity within the DLS. Everitt and Robbins suggest it is the changes in the relative importance of these areas in behavioural control that results in the dominance of habits and diminished goal-directed, top-down control.

The concept of drug-dependent neural plasticity and its influence on behaviour and cognition is currently a focal topic in neuroscience (Goldstein & Volkow, 2011; Hester, Lubman, & Yucel, 2010; Jonkman & Kenny, 2013; Robinson & Kolb, 1999; Taylor, Lewis, & Olive, 2013; Yucel & Lubman, 2007) and for psychostimulants in particular, there is growing empirical evidence for drug-dependent neural plasticity (Ferrario et al., 2005; Li, Acerbo, & Robinson, 2004; Robinson, Gorny, Mitton, & Kolb, 2001). As mentioned previously, Jedynak and colleagues (Jedynak et al., 2007) found evidence of morphological changes in the dorsal striatum following exposure to methamphetamine. Visualisation of cell morphology using the Golgi-Cox staining method found good evidence that exposure to amphetamine, a psychostimulant similar to methamphetamine, causes increased branching and spine density on MSNs in the striatum and pyramidal cells in the PFC (Li, Kolb, & Robinson, 2003; Robinson & Kolb, 1997, 1999). These findings provide some support to the model of addiction posited by Everitt and Robbins. However, because these studies did not include assessments of goal-directed or habitual behaviour in these studies, the impact of these drug-dependent changes on behaviour can only be hypothesised. There are well-established behavioural protocols in rats that can probe goal-directed action and habits in rats and that have provided much insight over the last few decades.

Empirical investigations of goal-directed actions and S-R habits in rats

Researchers using instrumental learning paradigms have identified behaviour in rats that model goal-directed actions and habits in humans, and have allowed for the empirical examination of the associative structure underlying these behaviours (Adams, 1982; Adams & Dickinson, 1981). Typically, studies of instrumental learning involve training a rat to make a response, for example a lever press, in order to obtain a

particular outcome, such as a food reward. During this training, the animal learns that there is a causal relationship between their behaviour (the instrumental action) and the delivery of food (the instrumental outcome). Following acquisition of the instrumental action, the action-outcome association can be examined in two ways. Firstly, the value of the instrumental outcome can be reduced, by making the outcome no longer desirable (Adams & Dickinson, 1981). Secondly, the contingent relationship between the response and the outcome can be degraded, by delivering the reinforcer in the absence of the instrumental action (Adams, 1982; Adams & Dickinson, 1981). The logic behind these procedures is that an animal that is goal-directed, and thus guided by action-outcome associations, should reduce their rate of performing the response if the outcome is no longer desirable, or if the response no longer causes the outcome to occur (Dickinson, 1985). In contrast, an animal that is habitual, and thus guided by stimulus-response associations, will not reduce their rate of performing the response because the value of the outcome, and the action-outcome contingency, do not guide their behaviour, rather the response is elicited by stimuli associated with prior instrumental reinforcement (Dickinson, 1985).

One common method used by researchers to achieve outcome devaluation is a procedure that pairs the instrumental reinforcer with lithium chloride-induced nausea. Following this procedure, instrumental performance is then tested in extinction, where delivery of the reward no longer occurs when the animal performs the instrumental response. Testing the behaviour in extinction allows for the animal's integration of the knowledge gained during training and the outcome devaluation procedures to be probed, and thus assays the memory of the association in the absence of new learning (Adams, 1982). Accordingly, an animal that reduces its rate of performing the instrumental response following devaluation of the outcome is behaving in a goal-

directed manner, whereas, an animal that continues to perform the instrumental response despite the outcome no longer being desirable, is behaving habitually (Dickinson, 1985, 1994). Thus, in studies of instrumental learning, goal-directed behaviour can be indexed by sensitivity to outcome devaluation procedures. On the other-hand, habits can be indexed by insensitivity to changes in the value of the outcome (Dickinson, 1985, 1994).

Outcome devaluation and contingency degradation procedures have provided researchers with methods allowing them to examine the mechanisms leading to behaviour that is controlled by stimulus-response habits. For example, Adams (1982) examined sensitivity to outcome devaluation following two different levels of instrumental training. One group of rats underwent a low level of training, where 100 instrumental responses were reinforced, whilst another group of rats underwent a more extensive instrumental training regimen, where 500 responses were reinforced. Following training, half of the rats in each training group had the instrumental reinforcer devalued by way of lithium chloride-induced nausea, whilst the remainder of the rats served as devaluation controls and received injections of saline. Adams found that rats who underwent a low level of training showed an effect of devaluation, whereby these rats significantly reduced performance of the instrumental response compared to rats that did not have the instrumental reinforcer devalued. However, this devaluation effect was not observed in rats that underwent extensive training. Over-trained rats that had the instrumental reinforcer devalued continued to perform the instrumental response at rates comparable to the non-devalued control group. To ensure that the devaluation procedure had succeeded in producing a taste aversion to the reinforcer, all rats underwent a reacquisition test, where performance of the instrumental response once again resulted in delivery of the reinforcer. Results from

the reacquisition test confirmed that the over-trained rats had acquired an aversion to the reinforcer, because, they significantly reduced their rate of responding compared to the non-devalued control group when they were punished with the presentation of the now "poisonous" food. These results show that goal-directed behaviour is evident in the early stages of acquisition, but behaviour comes under habitual control following extensive training (Adams, 1982; Balleine & Dickinson, 1998; Dickinson, Balleine, Watt, Gonzalez, & Boakes, 1995). This effect mirrors the development of habits in humans for tasks that are performed repeatedly.

Knowing that over-training results in habits provides a framework to investigate the neural underpinnings of goal-directed actions and habits. Consequently, there is now strong evidence that goal-directed behaviour and habits are dependent on distinct brain areas (Balleine, Delgado, & Hikosaka, 2007; Corbit & Balleine, 2003; Coutureau & Killcross, 2003; Killcross & Coutureau, 2003; Yin, Ostlund, Knowlton, & Balleine, 2005). For example, Yin and colleagues found that pre- and post-training lesions to, and temporary inactivation of, the dorsomedial striatum (DMS) resulted in performance that was insensitive to outcome devaluation after only limited training, suggesting that this area is vital for both the acquisition and expression of goal-directed actions (Yin et al., 2005). It has also been found that pre-training lesions applied to the prelimbic (PL) region of the medial prefrontal cortex (mPFC) prevent rats from acquiring goal-directed performance, even when they have received only limited training (Killcross & Coutureau, 2003), suggesting that the PL area is crucial for learning goal-directed actions. Together, these findings provide strong evidence that goal-directed actions involve both the DMS and the PL.

In contrast, habit based performance appears to depend on the integrity of the DLS (Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999; Killcross & Coutureau, 2003; Tang, Pawlak, Prokopenko, & West, 2007; Yin, Knowlton, & Balleine, 2004). For example, pre-training lesions to the dorsolateral striatum (DLS) have been shown to maintain sensitivity to outcome devaluation following habitual performance produced by over-training (Yin et al., 2004). The findings of Yin indicate that the DLS is crucial for habit formation, but also indicate that goal-directed behaviour can be expressed when the habit pathway is disrupted. More recently, Yin and colleagues (Yin, Knowlton, & Balleine, 2006) found that temporary inactivation of the DLS enabled overtrained rats to be sensitive to a contingency degradation procedure compared to saline-infused controls. Also, consistent with a role of the DLS in habit acquisition, is Tang and colleagues' finding from cell recordings taken whilst rats underwent extensive training of a motor task (Tang et al., 2007). Tang found that, when performance of a motor task became habitual, the majority of neurons in the striatum decreased activity, except for a group of neurons located in the DLS which increased activity.

Along with the DLS, another area involved in habits is the infralimbic (IL) region of the PFC (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003; Smith, Virkud, Deisseroth, & Graybiel, 2012). Killcross and Coutureau demonstrated that lesioning the IL prior to extensive training renders an animal unable to develop habits following outcome devaluation whilst sham-lesioned animals having experienced the same level of training were insensitive to devaluation of the outcome (Killcross & Coutureau, 2003). This is supported by the finding that post-training inactivation of the IL allowed goal-directed behaviour to be expressed when the response had been overtrained (Coutureau & Killcross, 2003). This demonstrates that goal-directed responding can be reinstated even in instances where habits dominate, and therefore that goal-directed

and habitual learning occur in parallel with performance being dictated by whichever system dominates at the time of test. Lesion and inactivation studies have provided good evidence for the roles of specific brain regions in goal-directed behaviour and habits.

Effects of drugs of abuse on the development of S-R habits in rats

Studies using similar paradigms have indicated that instrumental reward learning is affected by exposure to drugs of abuse. For example, Nelson and Killcross (Nelson & Killcross, 2006) found that repeated exposure to amphetamine, a drug similar to methamphetamine, prior to a limited amount of lever press training for food, accelerated habit-based instrumental performance. In this experiment, one group of rats were exposed to a 2mg/kg dose of amphetamine once per day for seven consecutive days, whilst a second group of rats was injected with an equivalent volume of saline vehicle and served as a control group. Following a low level of training, half of the rats in each group had the instrumental reinforcer devalued (and the remaining animals did not) and sensitivity to outcome devaluation was then probed in an extinction test. Nelson and Killcross found that rats pre-exposed to amphetamine continued to perform the instrumental response at rates comparable to rats that did not receive devaluation of the instrumental reinforcer, suggesting that they were insensitive to the value of the outcome. In comparison, and as is usual, rats in the control group showed a significant effect of devaluation, reducing their lever press performance accordingly. A subsequent reacquisition test confirmed that the outcome devaluation procedure successfully produced a taste aversion to the food in all animals. This result suggests that the transition from goal-directed performance to behaviour controlled by

habits occurred more rapidly than usual following sensitisation of the dopaminergic system by way of repeated amphetamine exposure.

Extending their findings in a subsequent study, Nelson and Killcross (Nelson & Killcross, 2013) found that systemic administration of the D1 antagonist, SCH23390, prior to each instrumental training session reversed the accelerated dominance of habits in amphetamine-sensitised rats. The procedure mirrored that of their 2006 study with the addition of groups receiving systemic administration of either SCH23390, the D2 antagonist eticlopride, or the non-specific dopamine antagonist alpha-flupenthixol before each training session. Nelson and Killcross found that amphetamine-sensitized animals that received SCH23390 or alpha-flupenthixol before each training session remained sensitive to outcome devaluation at test. In contrast, amphetamine-sensitised animals receiving eticlopride prior to training sessions were habitual at test and also during a reacquisition test, indicating compulsive responding even in the presence of the devalued reward. These experiments show that accelerated habit formation is modulated by the activity of distinct dopamine-receptor subtypes during instrumental training.

Accelerated habit formation has also been found in rats pre-exposed to cocaine (Corbit, Chieng, & Balleine, 2014). Following low levels of training for a food reward and outcome devaluation by specific satiety, cocaine exposed rats showed insensitivity to outcome devaluation compared to saline control animals. Using whole cell patch clamp electrophysiology, Corbit also found that cocaine exposure increased glutamate release in the DMS, but not the DLS. In an attempt to regulate glutamatergic input in cocaine exposed animals, Corbit subsequently co-administered N-acetylcysteine (NAC), a compound that normalises glutamate homeostasis, and cocaine, prior to instrumental

training and outcome devaluation. At test, rats that received co-administration of cocaine and NAC at 60mg/kg or 120 mg/kg were goal-directed compared to rats that received only cocaine, which again showed habitual performance. The findings of Nelson and Killcross (Nelson & Killcross, 2006, 2013) and Corbit and colleagues (Corbit, Chieng, et al., 2014) together provide good evidence that pre-exposure to the psychostimulants amphetamine and cocaine promote the rapid expression of habitual responding, and that dopamine and glutamate are involved in this process. However, whether or not methamphetamine has a similar habit-promoting effect is as yet unknown.

Evidence for the role of dopamine in habits has also come from studies directly looking at drug self-administration. In a series of experiments, Corbit et al. (Corbit, Nie, & Janak, 2012) demonstrated that extended instrumental training for an ethanol reward was insensitive to devaluation by specific-satiety compared to sucrose. Responding for ethanol remained sensitive to devaluation after 1 and 2 weeks of training, however after 4 and 8 weeks, no devaluation effect for ethanol was observed (whereas responding for sucrose remained goal-directed). Corbit also found that inactivating the DMS during training in the first and second of training weeks attenuated responding, whereas inactivation of the DLS across the 4- and 8-week periods resulted in animals showing sensitivity to devaluation of the ethanol. These studies provide good support to Everitt and Robbins' model of addiction. Initially alcohol consumption was goal-directed, however following sustained access, instrumental responding for ethanol was insensitive to devaluation and thus controlled by habits. Also, the transition from goal-directed action to habits coincided with a shift in dependence from the DMS to the DLS, demonstrated by temporary inactivation of these areas. However, it is not clear from

this study whether ethanol affects the learning or the performance (i.e. behavioural expression at test) of the habits observed.

In order to assess the neurochemical underpinnings of habitual alcohol seeking, Corbit followed up on these initial findings. In this work, Corbit and colleagues (Corbit, Nie, & Janak, 2014) used the same paradigm of instrumental training for ethanol for 8 weeks but prior to test she infused either an AMPA receptor antagonist to reduce the action of the neurochemical glutamate, a D2-receptor antagonist raclopride, or saline vehicle into the DLS and compared performance following devaluation. Corbit found that animals in the control group were insensitive to devaluation as expected because these animals had received extended training for the ethanol outcome. However, those who had AMPA-receptor antagonist or D2 antagonism in the DLS prior to test showed sensitivity to devaluation. These data suggest that habitual responding for alcohol can revert to being goal-directed if either glutamatergic inputs to the DLS, or D2 receptors within the DLS, are inactive.

Other investigations of the effects of alcohol on the brain also provide support for the Everitt and Robbins model. For example, in a study involving mice, DePoy and colleagues (DePoy et al., 2013) found increases in dendritic length and branching of DLS neurons in mice that had been exposed to alcohol compared to controls. Using in-vivo single unit recordings of cells in the DLS, DePoy et al. also found that the DLS cells were more active in the alcohol exposed mice during learning compared to the activation of DLS cells in the controls. Evidence for over-activation of the DLS during learning has also been found in humans with an alcohol addiction. Sjoerds and colleagues (Sjoerds et al., 2013) used fMRI to assess activation of the DLS and DMS in alcoholics whilst they completed an instrumental learning task. In this experiment, an overreliance on habit

learning was accompanied by increased activation of the DLS and decreased activation of the DMS in humans with alcohol dependence compared to healthy control participants.

The transition from goal-directed to habitual instrumental responding has also been investigated in rats responding for nicotine (Clemens, Castino, Cornish, Goodchild, & Holmes, 2014). Clemens and colleagues found rats who had experienced extended training for nicotine were insensitive to devaluation whereas those who underwent brief instrumental training for nicotine appeared goal-directed, reducing responding following reinforcer devaluation. Using immunohistochemistry, Clemens et al. also found that the DLS was activated only in those rats who had undergone extensive training, not those who had received brief training. However, Clemens and colleagues also found that the DMS was activated in the extended trained group, which contrasts with some other findings in the literature (Corbit & Janak, 2010; Corbit et al., 2012; Faure, Haberland, Conde, & El Massioui, 2005; Yin et al., 2005). It was suggested that the DMS activation Clemens observed may be the result of differences in experimental procedures or an effect exclusive to nicotine's effect on the brain.

Cocaine is another drug of abuse that has been shown to be resistant to devaluation (Miles, Everitt, & Dickinson, 2003; Zapata, Minney, & Shippenberg, 2010). For example, Zapata and colleagues (Zapata et al., 2010) demonstrated that animals given extended instrumental training for cocaine showed insensitivity to outcome devaluation compared to early on in training. Mirroring the findings of Corbit et al. (Corbit, Nie, et al., 2014), Zapata and colleagues also found that inactivating the DLS abolished habits and reinstated goal-directed performance. Along similar lines, Ito et al. (Ito, Dalley, Robbins, & Everitt, 2002) found increased activation of the dorsal striatum

during cocaine-seeking in the presence of a cocaine-paired cue. Also mirroring the findings of Corbit and colleagues, several studies have shown that antagonism of AMPA and dopamine receptors within the DLS reduces cocaine-seeking (Belin & Everitt, 2008; Murray, Belin, & Everitt, 2012; Vanderschuren, Di Ciano, & Everitt, 2005). These studies provide good empirical evidence for Everitt and Robbins' (Everitt & Robbins, 2005, 2016) model that drug use is initially goal-directed but over time the behaviour becomes habitual, and that these transitions in behaviour map onto activity in brain regions involved in actions and habits.

Contextual control of behaviour

Context can exert a powerful influence on behaviour. Contextual cues provide background settings that act as occasion setters or discriminative stimuli for how one behaves in particular situations. The ability of contexts and cues previously paired with drug use to induce craving and relapse is well documented (Crombag, Bossert, Koya, & Shaham, 2008; Garavan et al., 2000; Shaham, Shalev, Lu, De Wit, & Stewart, 2003). Extensive literature also documents the role of drug-paired contexts in behavioural sensitisation and conditioned-place preference (CPP) (Steketee & Kalivas, 2011; Tzschentke, 2007). Contextual cues associated with psychostimulants have also been shown to reduce dopamine activity in the PFC (Lin, Pan, & Yeh, 2007) and modulate activity in the striatum (Uslaner et al., 2001). More recently, the ability of drug-paired contexts to influence goal-directed choice for natural rewards has been explored.

Influence of drug-paired contexts on goal-directed choice

In a novel procedure, Ostlund and colleagues (Ostlund, Maidment, & Balleine, 2010) examined whether a context previously paired with ethanol influenced the ability to choose between two instrumental responses when the outcome associated with one

of two choice responses had been devalued. In the first phase of the experiment, Ostlund exposed rats to two distinct contexts, one paired with ethanol administration, the other with saline control treatment. Following context conditioning, Ostlund then trained the rats on two instrumental responses for different outcomes (i.e. a left lever press resulted in a grain pellet and a right lever press resulted in a sucrose pellet) in a third, distinct context. In the next phase, Ostlund devalued one of the outcomes via specific satiety and then tested the animals under extinction conditions in either the ethanol-paired context or the saline-paired context. The procedure was reversed on the following day so that each animal was tested in both contexts. Ostlund found that when animals were tested in the saline-paired context, choice was goal-directed; rats reduced responding for the devalued reinforcer but continued to respond for the valued alternative. However, when rats were tested in the ethanol-paired context, choice was not guided by the value of the outcome; rats did not show a preference between the two alternatives. This demonstrates that goal-directed decision-making can be disrupted by a context previously paired with intoxication by alcohol.

In recently published work, Furlong et al. replicated Ostlund's findings using methamphetamine-paired contexts (Furlong, Supit, Corbit, Killcross, & Balleine, 2015). Furlong used the same procedure of exposing rats to methamphetamine- and saline-paired contexts interchanging over two weeks and subsequent instrumental choice training in a third context. Following devaluation by specific satiety, at test, rats demonstrated goal-directed choice when tested in the saline-paired context, responding for the valued outcome significantly more than the devalued outcome. However, when tests were conducted in the methamphetamine-paired context, decision-making was impaired whereby no preference for the valued outcome over the devalued outcome was shown. This impairment in the methamphetamine-paired context continued even

when negative feedback was provided by presentation of the devalued outcome (paralleling the findings of Nelson and Killcross, (2013) following amphetamine sensitization and systemic D2 antagonism). Furlong also found that cFos activation was reduced in D1-expressing cells in the DMS, but not the DLS, following exposure to the methamphetamine context, suggesting that goal-directed systems were inhibited by exposure to the methamphetamine-paired context. In an effort to restore the relative balance of activation of D1 and D2 neurons in the DMS, which have opposing influences on performance (Gerfen & Surmeier, 2011; Surmeier, Carrillo-Reid, & Bargas, 2011), Furlong infused the adenosine 2A (A_{2A}) receptor antagonist (ZM241385) into the DMS prior to a devaluation test and reacquisition test. Antagonising the A_{2A} receptor specifically, should restore the balance of D1 and D2 activity within the DMS by reducing activity of D2 expressing neurons only (Lovinger, 2010), thereby increasing the relative activity of the D1 neurons in the DMS. Interestingly, Furlong and colleagues found that this infusion restored goal-directed choice in the methamphetamine-paired context but only during the reacquisition test, not during the devaluation test.

The findings of Furlong and colleagues (Furlong et al., 2015) are significant because they provide evidence that exposure to a context previously paired with the administration of methamphetamine can disrupt goal-directed choice whilst the same animals are capable of goal-directed choice whilst they occupy a "neutral" or saline-paired context. Thus, under certain circumstance, methamphetamine exposed animals are capable of goal-directed choice. Furlong et al.'s study also provides evidence that this dominance of habits over goal-directed choice seen in the methamphetamine-context is likely to occur because of an imbalance of activity of D1 and D2 within the DMS specifically. This has echoes of the Nelson and Killcross (2013) findings whereby D2 antagonism in chronic amphetamine animals rendered these animals insensitive to

negative feedback during reacquisition testing. Thus, the context specificity of the Furlong and colleagues' finding shows that goal-directed choice can be observed in methamphetamine-contexts when animals are faced with negative feedback if the D2 activating influence of the methamphetamine-paired context is diminished by restoring D1/D2 balance in the DMS.

Contextual resolution of response conflict in rats

Contexts can also influence executive function particularly when an organism is faced with conflicting information and needs to behave in a situationally appropriate manner. A novel behavioural paradigm, has recently been used to investigate the neural basis of these processes in rats (George, Jenkins, & Killcross, 2011; Haddon, George, & Killcross, 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007, 2011a, 2011b; Marquis, Killcross, & Haddon, 2007; Reichelt, Good, & Killcross, 2013). The top half of Table 1 summarises the experimental design of the biconditional discrimination task designed by Killcross and colleagues (Haddon & Killcross, 2005) to probe executive function in rats.

Table 1. Experimental design of Haddon and Killcross' biconditional discrimination task and relationship to the classic Stroop Task.

Context	Biconditional training stimuli		Compound stimuli at test	
			Congruent	Incongruent
C1	A1: LP1-> O1	A2: LP2 -> O1	A1V1, A2V2	A1V2, A2V1
C2	V1: LP1 -> O2	V2: LP2 -> O2	A1V1, A2V2	A1V2, A2,V1

Context	Training stimuli		Compound stimuli at test	
	"Red"	"Green"	Congruent	Incongruent
Colour naming			RED, GREEN	RED, GREEN
Word reading	RED	GREEN	RED, GREEN	RED, GREEN

Note. C1/C2, O1/O2, LP1/LP2, A1/A2, and V1/V2 refer to the different experimental chambers (contexts), reward outcomes, auditory and visual stimuli, respectively.

The response conflict task used by Killcross and colleagues was designed to mirror the response conflict that arises in the classic human Stroop task (Stroop, 1935). As the bottom half of Table 1 details, the Stroop conflict occurs when incongruent colour-word compounds are presented to participants and they are required to either read the word (ignoring the conflicting colour of the ink) or to name the colour of the ink the word is written in (and ignoring the conflicting word itself). Which response is appropriate on a given trial is determined by the context of the current task instructions (read word vs. name colour). Research using the novel rodent procedure has shown that under normal conditions, rats are also able to disambiguate cues that elicit conflicting responses and behave in a context-appropriate manner (Haddon et al., 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007, 2011a, 2011b; Marquis et al., 2007; Reichelt et al., 2013). As depicted in the top-half of Table 1, in this procedure rats initially undergo simultaneous training on two discriminations, one

auditory (A1 -> LP1; A2 -> LP2; where A1 is a tone and A2 is a clicker) and one visual (V1 -> LP1; V2 -> LP2; where V1 is a steady light and V2 is a flashing light), in two distinct contexts (C1 and C2; operant chambers with distinct visual, olfactory and tactile elements). For example, in the auditory context when one of the training stimuli (A1 or A2) is presented, both levers (LP1 and LP2) are inserted into the chamber and signal that an outcome (O1 or O2; grain pellet or sucrose pellet) can be earned by pressing one of the levers. However, reward only occurs when the rats presses the correct lever for the stimulus presented. Across several sessions of training in that context, rats learn that during A1, a response on LP1 is reinforced and during A2, a response on LP2 is reinforced. The training in the alternative context uses the visual stimuli to indicate which lever press is rewarded. At test, audiovisual compounds of the training stimuli are presented in each of the two contexts. The audiovisual compounds are either congruent, comprising elements that signal that the same lever press response is correct regardless of context (e.g. A1V1; A2V2) or they are incongruent whereby the elements signal that different lever press responses would lead to reward in the two different contexts (e.g., A1V2; A2V1). The appropriate response in such situations is determined by the test context. When tested in the context in which the auditory discrimination was learned, the appropriate response is that dictated by the auditory cues, whereas when tested in the visual context, the appropriate response is that dictated by whichever visual cue is present in the compound. Hence, in order to resolve the response conflict elicited by incongruent compounds, animals must use the background contextual information to guide their responding.

In the original demonstration of the Stroop effect, as adult humans have more day-to-day experience in word reading, rather than colour naming, the word-reading response tends to be dominant. That is, the effect of incongruent compound

presentations is asymmetrical; participants can readily read the word when word and colour are in conflict, but require more time and effort (and make more mistakes) when required to name the colour when this conflicts with the word itself. Although the original demonstration of response conflict in rats was conducted in a symmetrical procedure (rats had equal experience of both discriminations – akin to some demonstrations of Stroop-like effects in humans using artificial stimulus dimensions, see MacLeod for a review (MacLeod, 1991), Haddon and colleagues (Haddon et al., 2008) was also able to mirror the asymmetry of word-reading and colour naming seen in humans by manipulating the degree of training in the rodent task. In this version of the task, one discrimination is "over-trained" compared to the other, "under-trained" discrimination. Quite simply, one discrimination (either auditory or visual) received three times as many training trials as the other. At test rats were able to respond in a context-appropriate manner to incongruent compounds when they occurred the "over-trained" context (akin to humans being asked to read the word) but were unable to respond appropriately in the under-trained context (mirroring the increased difficulty seem when humans are asked to name the colour of the ink in which a conflicting word is written). As such, this procedure appears to provide a valid rodent model of response conflict, akin to that seen in many human response conflict tasks

Studies probing the neuroanatomical underpinnings of this response conflict behaviour have revealed selective effects on the ability of rats to disambiguate these incongruent compound cues. For example, rats with extensive lesions to the mPFC have deficits on incongruent trials compared to sham lesioned control rats (Haddon & Killcross, 2005). Further investigations characterised more precisely the role of the mPFC in this task. Reversible inactivation of the PL region of the mPFC rendered animals unable to perform the context appropriate response when faced with

incongruent compound cues that has received equivalent amounts of training. In contrast, inactivation of the IL had no effect on performance (Marquis et al., 2007). These data indicate that the PL cortex is central to the ability of animals to make use of contextual cues to guide responding in the face of conflicting cues. Using the asymmetrical training procedure Haddon and colleagues have also found evidence for a role of the IL region of the mPFC in the dominance over responding acquired by more extensively trained cues. Following asymmetrical training, animals were tested with an incongruent compound (comprising an over-trained and an under-trained cue) in the context where the appropriate response was dictated by the under-trained cue. As indicated above, in normal circumstances rats cannot resolve this conflict as performance is dominated by the response elicited by the over-trained cue, overriding the ability of the context to guide responding towards the under-trained cue. However, inactivation of the IL at test allowed the context appropriate under-trained response to be expressed (Haddon & Killcross, 2011a), suggesting that inactivation of this region suppressed the dominant stimulus-response elicited by the over-trained cue, and allowed the subordinate response elicited by the under-trained cue, in combination with context-informed control of responding, to emerge. These studies highlight the important yet distinct roles the PL and IL play in contextual control of behaviour in situations of response conflict.

Studies using this procedure have also shed some light on the neurochemical underpinnings of contextual control of response conflict. For example, using microdialysis George and colleagues (George et al., 2011) found higher mPFC dopamine levels in rats following test sessions when the biconditional discrimination paradigm was used compared to control rats who underwent a simple discrimination test. George et al. also found lower levels of dopamine in the nucleus accumbens of rats performing

the biconditional discrimination compared to those in the simple discrimination control condition. These findings indicate that both PFC and striatal dopaminergic systems are involved during complex tasks that require conflict resolution.

Complementing these findings, Haddon and Killcross (Haddon & Killcross, 2011b) found that altering dopaminergic tone in the PL via direct infusion of the D1 receptor agonist SKF38393 resulted in improved performance on incongruent compound cue trials. However, on congruent compound cue trials performance was overall impaired following infusion of the D1 agonist. Further analysis revealed that animals with low baseline performance performed better on both congruent and incongruent trials at test following infusion of SKK38393 into the PL, whereas those rats who performed best at baseline performed worse following the increase in D1 tone via the infusion. Haddon and Killcross interpreted this as indicating that when performance is suboptimal increasing D1 tone improves performance. However, when performance is already at an optimal level, increasing D1 tone is counterproductive.

Using the same procedure, Reichelt and colleagues examined whether alteration of dopaminergic tone by way of acute administration of amphetamine (1.5 mg/kg) influenced performance on this task (Reichelt et al., 2013). Reichelt et al. found a selective deficit in performance on incongruent trials following acute systemic administration of amphetamine, whilst performance on the congruent trials remained intact. This deficit on the incongruent trials was abolished when amphetamine was co-administered with the atypical antipsychotic clozapine. However, when amphetamine was co-administered with alpha-flupenthixol, the deficit on incongruent trials observed with amphetamine alone remained. Clozapine is thought to restore balance to dopamine across prefrontal and striatal regions, therefore suggesting that homeostatic

balance of dopamine is required in order to exert appropriate cognitive control to resolve conflict. The effect of other drugs of abuse that disrupt dopamine, such as methamphetamine, on performance of this conflict resolution task is not yet known.

Outline of this thesis

As discussed, methamphetamine use is a global public health concern and at present there is a lack of expert consensus on whether long term methamphetamine use causes significant deficits to cognition and executive function. The overall aim of this thesis is to provide clarity on this contentious issue. Several findings from the literature reviewed here are of particular interest. First, in line with Everitt and Robbins model, chronic exposure to several drugs of abuse have been found to promote the early dominance of S-R habits. However, it is not yet known whether chronic exposure to methamphetamine will have a similar effect. Second, it has also been demonstrated that methamphetamine-paired contexts can impact goal-directed choice. This raises the question of whether a methamphetamine paired context can bias S-R habits using a single lever procedure. Third, disrupting dopaminergic tone prior to a rodent task of executive function has been shown to impair performance. This provides an opportunity to examine the influence of acute and chronic methamphetamine administration on a task designed to mirror the human Stroop task. Lastly, several investigations have found evidence of drug-dependent neural plasticity on brain areas of key importance to S-R habits and executive function. However, the impact these changes to neural architecture may have on S-R habits and executive function is not clear.

Therefore, this thesis had three main aims. First, to examine the influence of chronic methamphetamine and methamphetamine-paired contexts on S-R habits.

Second, to investigate whether acute or chronically administered methamphetamine has a detrimental impact on performance in a task that models human executive function. Third, to employ identical chronic methamphetamine dosing regimens to those used in Chapters 2 and 3 in order to draw analogies between methamphetamine-dependent changes to neural morphology to the behaviours observed in the experiments of Chapters 2 and 3.

Chapter 2

Effect of methamphetamine and methamphetamine-paired contexts on the acquisition and expression of S-R habits in rats.

As outlined in Chapter 1, there is strong evidence that exposure to the psychostimulant amphetamine causes an early dominance of S-R habits over goal-directed behaviour in rats who have undergone minimal instrumental training (Nelson & Killcross, 2006, 2013). However, the effect methamphetamine has on the goal-directed status of instrumental performance is yet to be determined. There is some evidence for an early dominance of S-R habits if rats are tested in a context previously paired with methamphetamine (Furlong et al., 2015) however, this study utilized a two-lever procedure designed to examine the effect on instrumental choice decisions, unlike the single-lever procedure used in the amphetamine studies (Nelson & Killcross, 2006, 2013) that are explicitly designed to assess the acquisition of S-R habits and their dominance over goal-directed control of performance. Therefore, the current experiments had two aims: 1) to examine whether, like amphetamine, pre-training methamphetamine exposure facilitates the early expression of habit-dominated instrumental performance; and 2), whether exposure to methamphetamine-paired contexts causes behaviour to be come under habitual control, using a single-lever procedure.

Experiment 1 Aim

Experiment 1investigated the role of context on goal-directed instrumental performance in methamphetamine exposed rats receiving low levels of training typically associated with expression of goal-directed performance (following the study

of Furlong et al., 2015). Specifically, this experiment sought to examine whether previous exposure to two distinct contexts, one paired with methamphetamine administration and another paired with saline control injections, differentially affect an animal's ability to behave in a goal-directed manner. All animals in this experiment were administered methamphetamine and saline in distinct contexts and then underwent a low level of instrumental training in which presses on a single lever led to reward. Subsequently, the instrumental reinforcer was devalued for half of the animals by way of lithium chloride-induced nausea whilst the remaining rats received saline control injections (non-devalued group). Rats were then tested in either the methamphetamine- or saline-paired context for sensitivity to devaluation of the outcome. If context is able to differentially influence the performance of goal-directed instrumental behaviour then these 'undertrained' rats tested in the saline-paired context should exhibit sensitivity to devaluation of the outcome whereas those tested in the methamphetamine context will not appropriately reduce their rate of lever pressing.

Method

Design

This study employed a two (Context at test: methamphetamine/saline) X two (Devaluation: Devalued/Non-devalued) between subjects factorial design. The dependent variables were lever press responses and magazine entries per minute.

Subjects

Thirty-two naïve, male, Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 247 to 366g at the start of the experiment. Subjects were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All

experimental procedures were carried out during the light phase of the cycle. Each rat was handled individually by the researcher prior to commencement of the experiment. Prior to behavioural training, rats were placed on food restriction. During food restriction, each rat received 15g lab chow per day, and were kept within 85% of their free feeding weight. Rats remained on food restriction until after completion of the reacquisition test. Water was available *ad libitum* for the duration of the experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered at a dose of 1 mg/kg. This dose was used in Furlong et al.'s study and showed that a context previously paired with methamphetamine at this dose disrupted goal-directed choice (Furlong et al., 2015). Methamphetamine was administered intraperitoneally (i.p.) in a volume of 1 ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine. All rats used in this experiment were administered methamphetamine on seven occasions.

Apparatus

Eight identical operant chambers (30 cm x 24cm x 22 cm; Med Associates, VT) which were individually enclosed in sound- and light- attenuating cabinets were used in the experiment. Each chamber consisted of left- and right-hand aluminium walls and clear Perspex roof, back wall, and front door. The floor was comprised of 19 steel bars (3.8 mm diameter, spaced 1.6 cm apart), which were secured over a tray of corncob

bedding. At the top of the left-hand wall a 3-W house light was positioned and this was illuminated at the commencement of every session and turned off once the session was completed. On the right-hand wall of the chambers a retractable lever was positioned to the left of a recessed food magazine located at bottom centre of the wall. Food grain pellets (45mg; Bio-Serv) could be delivered as rewards, at specific times via a pellet dispenser, into the food magazine. Entries into the magazine were measured by infrared detectors at the entry of the recess. A computer equipped with Med-PC software (Med Associates Inc.) controlled the equipment and recorded data.

The behavioural training and devaluation procedures were carried out in the bare operant chambers, however the sensory (olfactory, tactile, and visual) properties of the chambers were altered to create two distinct contexts (A and B) for the context conditioning, tests, and reacquisition procedures. In context A, 1mL of 10% rose essence (Queen Fine Foods) was placed into the corncob bedding, an insert covered in rough gritted tape was placed over the steel bar floor, and black and white striped wallpapers were applied to the walls of the operant chambers. In context B, 1mL of 10% peppermint essence (Queen Fine Foods) was placed into the corncob bedding, a smooth Perspex insert covered the steel bar floor, and polka dot wallpapers were applied to the walls of the operant chambers.

Procedure

Table 2 provides a summary of the key stages of the experimental procedure.

Table 2. Key stages of Experiment 1.

1	2	3	4	5	6
Context conditioning	Rest	Behavioural training	Devaluation	Test	Reacquisition

Context conditioning.

During this phase, all rats were administered methamphetamine, via i.p. injection, every other day for fourteen consecutive days. On alternate days, rats received an injection of the equivalent volume of saline. During this procedure all subjects were exposed to contexts A and B. For half of the subjects methamphetamine administration was paired with context A and saline was paired with context B. For the other half of the subjects saline was paired with context A and methamphetamine with context B. The order of methamphetamine/saline administration was counterbalanced across subjects.

Each day, rats were taken from the holding room to the laboratory where they were individually weighed. After weights were recorded, syringes were loaded with the appropriate volume of methamphetamine or saline. Drug-context pairings were carried out using the same procedure employed by Furlong and colleagues (2015), whereby each rat was taken from the home cage and placed in context A or B for ten minutes. Timing began once the first rat from each squad was placed into the context. After ten minutes, each rat was taken out of the context chamber, injected with methamphetamine or saline, and then placed back into context for thirty minutes. The purpose of giving rats 10 minutes in the context prior to methamphetamine treatment is to ensure that the novel context of the chamber is associated with methamphetamine administration, rather than the novel laboratory environment. After thirty minutes, rats were taken out of the context and returned to their home cages.

Rest.

Upon completion of context conditioning, rats were rested for seven consecutive days to ensure that methamphetamine had been eliminated from the system and any acute withdrawal effects had subsided.

Magazine training.

In order to train rats to collect food pellets from the magazine, each rat underwent four, thirty-minute sessions of magazine training, which were conducted over three consecutive days. During these sessions, food pellets were delivered to the magazine in the operant chamber on a random-time (RT) 60-sec schedule, whereby a food pellet was dispensed on average, every sixty seconds. Magazine entries were recorded during these training sessions. After 30 minutes the house light turned off to signal the end of the session, rats were taken out of the chambers and returned to the home cages.

Lever-press training.

Following successful completion of magazine training, all rats underwent training on the left lever. Initially, each rat received two sessions, conducted over two days, of lever press training on a continuous reinforcement schedule, whereby each lever-press was rewarded with a food pellet. Each session began with the house-light being illuminated and insertion of the left lever. Each session ended once twenty-five rewards had been earned, whereby the house-light would turn off and the lever would retract. Following successful completion of these two sessions, rats then underwent three further sessions of training, conducted over three days. In these three sessions, rewards were delivered on a random-interval (RI) 30-sec schedule, whereby pellets were available, on average, every thirty seconds and delivered following the next lever press. Previous studies have demonstrated that this amount of rewards and schedule of

reinforcement have been shown to produce stable rates of responding whilst maintaining sensitivity to devaluation of the reward (Dickinson et al., 1995). Each session began with the house-light being illuminated and insertion of the left lever. Once forty rewards had been earned, the house-light would turn off and the lever would retract, signalling the end of the session. Therefore, each animal earned a total of one hundred and twenty rewards on this schedule.

Devaluation.

Devaluation of the instrumental reinforcer was carried out over three sessions (Adams & Dickinson, 1981; Nelson & Killcross, 2006). Each twenty-minute session took place in the operant chambers where rats were given free access to the food rewards, which were placed in a glass ramekin in the corner of the chamber. After this time, rats were taken out of the boxes and injected intraperitoneally with either 0.15M, lithium chloride at a volume of 15ml/kg (Devalued group, *n*= 16) or the equivalent volume of saline (Non-devalued controls, *n*= 16). Following the injection on each devaluation day rats were placed back into their home cages and returned to the holding room.

Test.

Twenty-four hours after the last devaluation session, sensitivity of lever press performance to devaluation of the food reward was tested in the next phase of the experiment. Half of the rats were tested in the methamphetamine (*n*= 16) context and the other half were tested in the saline (*n*= 16) context. During the test, rats were placed into the appropriate context and illumination of the house light and insertion of the lever signalled the beginning of the session. The test was conducted under extinction conditions for a period of 8 minutes, whereby lever pressing did not result in the delivery of the food reward so that the subjects' integration of their previous

experience was tested in the absence of further learning. Lever responses and magazine entries were recorded during the test.

Reacquisition.

To confirm that the devaluation was successful in producing a taste aversion to the food reward a reacquisition test was carried out. During the 20-min reacquisition session, lever pressing once again resulted in delivery of the food reward on an RI30 schedule of reinforcement. For each rat, the reacquisition session was conducted in the same context that was used for the test session.

Results

Between subjects ANOVA (via GLM using SPSS) was used to investigate the influence and any interactions of the two independent variables, Context (methamphetamine or saline) and Devaluation (devalued or non-devalued), on the dependent variables. The dependent variables, lever press and magazine entry rates per minute, were analysed separately. An alpha level of 0.05 was used for all statistical tests.

Instrumental Training

On the final day of instrumental training there was a significant difference in the rate of lever pressing (presses per minute) between rats that were to be tested in the methamphetamine (M = 13.87, SD = 5.11) and saline contexts (M = 20.71, SD = 11.32) whereby rats to be tested in the saline context lever pressed at significantly higher rates than those to be tested in the methamphetamine context ($F_{(1,26)} = 4.44$, $p < .05$). However, there were no significant differences between the to-be-devalued group (M = 17.05, SD = 7.51) and the to-be-non devalued group (M = 17.07, SD = 10.75) ($F < 1$) and there was no significant interaction between the two factors ($F < 1$) (data not shown). For magazine entry rates there were no significant differences between rats that were

to be tested in the methamphetamine (M = 11.53, SD = 3.34) and saline (M = 10.52, SD = 6.24) contexts (F < 1), and no significant differences between the to-be-devalued group (M = 10.24, SD = 4.00) and the to-be-non devalued group (M = 11.87, SD = 5.52) (F < 1). There was also no significant interaction between the two factors on magazine entries rates (F < 1) (data not shown).

Test

Levenne's test for homogeneity of variance was significant (p = .03) therefore this assumption was violated. In order to meet this assumption, the lever press rates were square root transformed (Howell, 2002). This transformation corrected the violation and subsequently homogeneity of variance was assumed. Response rates from two rats were greater than two standard deviations from the mean, thus data from these animals were excluded from all analyses.

The mean response rates per minute made during the test are presented in Figure 1. Rats tested in the saline context reduced responding for the devalued outcome compared to non-devalued control animals. However, rats tested in the methamphetamine context did not reduce responding for the devalued outcome, lever pressing at rates comparable to non-devalued controls. Statistical analysis confirmed this observation. ANOVA yielded a significant main effect of Context ($F_{(1,26)}$ = 5.72, p = .02), Devaluation ($F_{(1,26)}$ = 14.98, p < .01), and a significant interaction between the two factors ($F_{(1,26)}$ = 4.35, p < .05). Simple effects analysis indicated that response rates of devalued and non-devalued rats were significantly different for rats tested in the saline context ($F_{(1,14)}$ = 16.34, p< .01), but this was not the case for rats tested in the methamphetamine context ($F_{(1,14)}$ = 1.70, p = .20). It is acknowledged that there is a low rate of responding at test compared to training response rates, however this is expected in non-devalued animals because the test is conducted under extinction and in a context

where the lever is novel. Context-dependent decrements in responding is well documented in cases where the instrumental response is switched from the training context to a novel context (Bouton & Todd, 2014; Bouton, Todd, & Leon, 2014; Thrailkill & Bouton, 2015; Trask, Thrailkill, & Bouton, 2017).

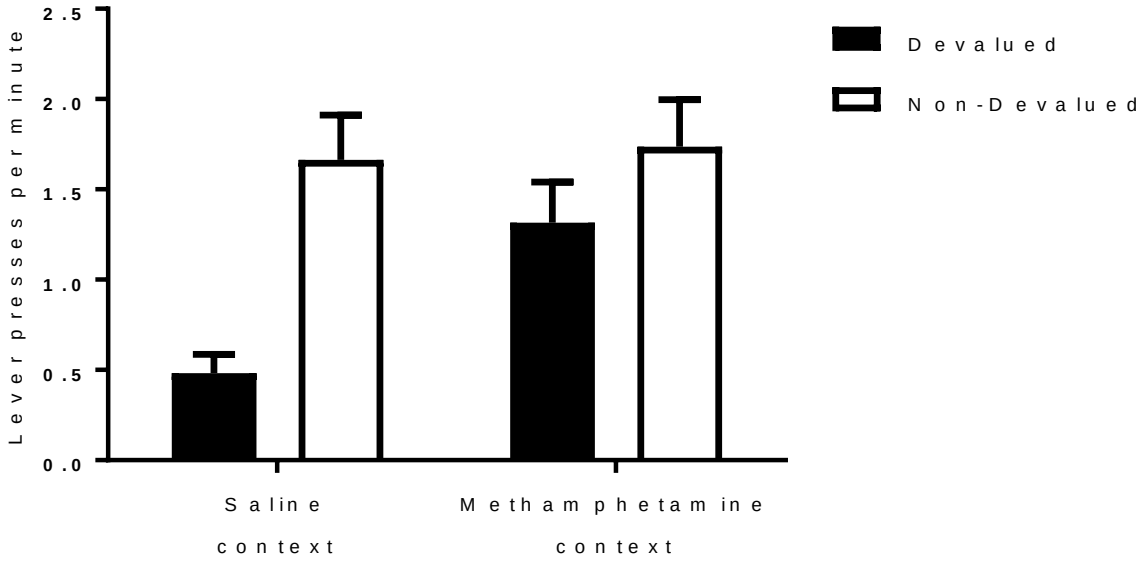

Figure 1. Mean lever presses per minute during test in either the methamphetamine or saline context following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; Non-devalued, white bars).

In contrast to lever press behaviour in the methamphetamine-paired context, magazine entry behaviour appeared to show sensitivity to devaluation in both the methamphetamine- and saline-paired contexts. The mean magazine entry rates per minute made during the test are presented in Figure 2. Statistical analysis revealed a main effect of Devaluation ($F_{(1,26)} = 8.74$, $p < .01$), but no effect of Context ($F_{(1,26)} = 2.31$, $p = .14$), and no interaction ($F_{(1,26)} = 2.61$, $p = .12$).

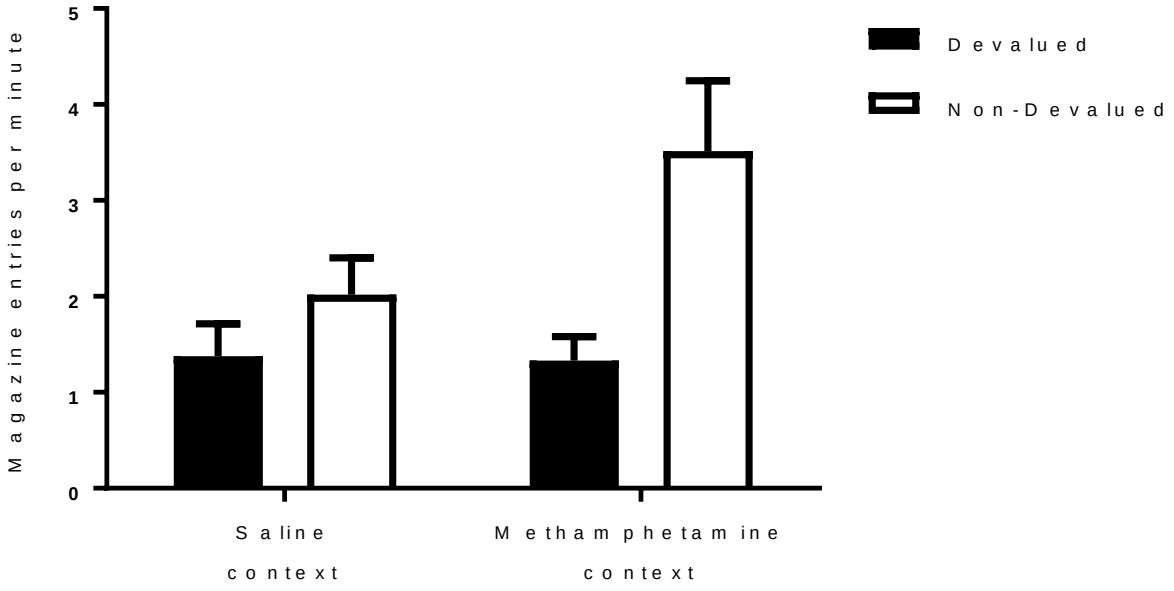

Figure 2. Mean magazine entries per minute during test in either the methamphetamine or saline context following devaluation of the reward. Error bars depict +SEM. (Devalued, black bars; Non-devalued, white bars).

Reacquisition

The mean response rates per minute made during the reacquisition test are presented in Figure 3, which shows that, regardless of whether the test took place in the methamphetamine or saline context, rats in the devalued group lever pressed at significantly lower rates compared to their non-devalued counterparts. This observation was confirmed by ANOVA which indicated there was a significant main effect of Devaluation ($F_{(1,26)} = 8.86$, $p = .01$). There was also a significant main effect of Context ($F_{(1,26)} = 6.06$, $p = .02$) whereby animals in the saline context lever press more overall, but no significant interaction between the two factors ($F < 1$). A similar pattern of results was found for magazine entry rates. Mean magazine entry rates were:

methamphetamine context devalued = 3.66 (*SD* = 1.49); methamphetamine context non-devalued = 11.53 (*SD* = 5.20); saline context devalued = 5.39 (*SD* = 3.84); saline context non-devalued = 8.31 (*SD* = 3.95). However, the assumption of homogeneity of variance was violated as indicated by Levene's test (p = .01). Thus, a square root transformation was conducted which corrected the violation (p = .20). A between subjects ANOVA was performed on the transformed magazine entry rate which confirmed that there was a significant main effect of Devaluation ($F_{(1,26)}$ = 16.07, p < .01), but no effect of Context (F < 1), and no interaction between the two independent variables ($F_{(1,26)}$ = 2.02, p = .15) (data not shown).

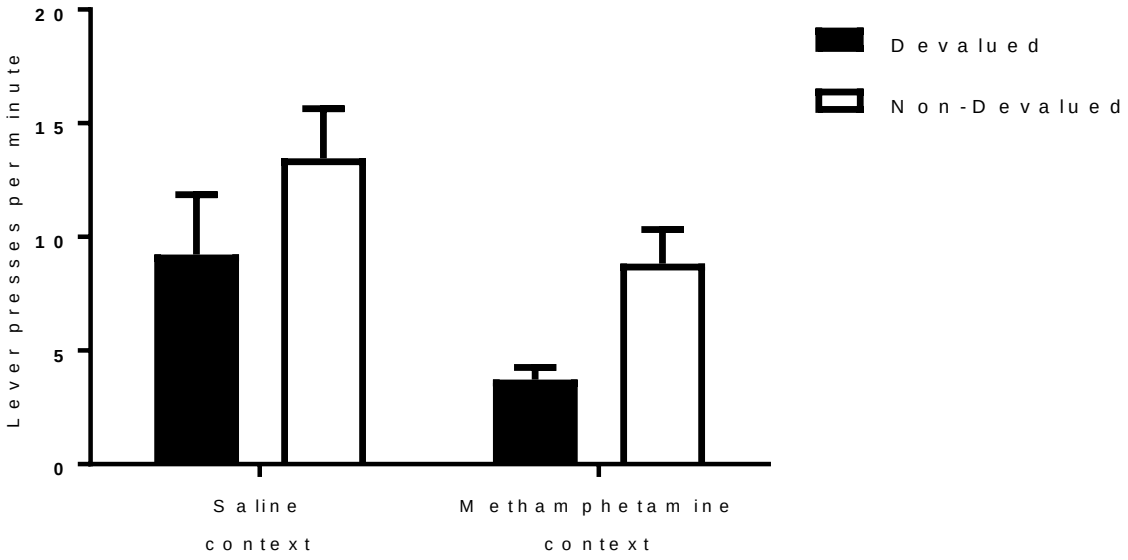

Figure 3. Mean lever presses per minute during the reacquisition test in the methamphetamine or saline context following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

Discussion Experiment 1

The results obtained in Experiment 1 suggest that exposure to a methamphetamine-paired context causes instrumental behaviour to come under the

control of S-R habits whereby devalued animals respond at rates comparable to non-devalued controls. However, if instrumental performance is tested in a context previously paired with saline, devalued animals demonstrate goal-directed behaviour whereby they appropriately reduce their rates of responding compared to a non-devalued control group. However, one caveat to this experiment is that all animals had been repeatedly exposed to methamphetamine so it is unclear whether the observed effect is purely due to context alone, or an interaction between repeated methamphetamine and context. Experiment 2 aimed to address this caveat by assessing whether the same differential effects of methamphetamine- and saline-paired contexts is observed when experience of methamphetamine is limited to one occasion. It has been demonstrated previously that methamphetamine context place preference is observed following one drug-context pairing (Herrold et al., 2009). Therefore, this provides an opportunity to examine the role of a methamphetamine-paired context in the absence of repeated experience with methamphetamine. If the effect observed in Experiment 1 is purely caused by the methamphetamine-paired context and not by repeated experience with the drug itself, then we might expect that rats tested in the methamphetamine context would not exhibit sensitivity to devaluation of the instrumental outcome because the context itself promotes S-R habits over goal-directed behaviour.

Method Experiment 2

Design

This experiment employed a two (Context: Methamphetamine/Saline) X two (Devaluation: Devalued/Non-devalued) between subjects factorial design. The dependent variables were lever press responses and magazine entries per minute.

Subjects

Thirty-two naïve, male, Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 265g and 359g at the start of the experiment. Subjects were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All experimental procedures were carried out during the light phase of the cycle. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 15g lab chow per day, and were kept at 85% of their free feeding weight. Rats remained on food restriction for the duration of the experiment. Water was available *ad libitum* in the home cage for the duration of the experiment. Each rat was handled individually prior to commencement of this experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine. All rats used in this experiment were administered methamphetamine on one occasion.

Apparatus

The Apparatus used in Experiment 2 was identical to that used in Experiment 1 with the exception of a minor alteration to where the devaluation took place. The first

two sessions of the devaluation procedure were carried out in 8 individual feeding cages (33 cm x 18 cm x 14 cm). All 8 cages were identical and consisted of opaque Perspex walls and floor. Each was fitted with a stainless steel cage top and a glass ramekin to hold grain pellet rewards. The final session of devaluation was carried out in the bare operant chamber with food rewards available in the magazine.

Procedure

Table 3 provides a summary of the key stages of the experimental procedure.

Table 3. Key stages of Experiment 2.

1	2	3	4	5
Context conditioning	Rest	Behavioural training	Devaluation	Test

Context conditioning.

On each day of this phase, rats were taken from the holding room to the laboratory where they were individually weighed. After weights were recorded, syringes were loaded with the appropriate volume of methamphetamine or saline. Rats received one treatment of methamphetamine, via i.p. injection, and exposed to a distinct context (A). During another session, rats received an injection of the equivalent volume of saline, and were exposed to a second context (B). The order of methamphetamine/saline administration was counterbalanced across subjects. Drug-context pairings were achieved by taking each rat from the home cage and placing it in context A or B for ten minutes. Timing began once the first rat from each squad was placed into the context. After ten minutes, each rat was taken out, injected with

methamphetamine or saline, and placed back into context for thirty minutes. Once thirty minutes had passed, rats were taken out returned to their home cage.

Rest.

Following context conditioning, rats were rested for two days. Methamphetamine has an average half-life of 10 hours so this two day rest would be sufficient to allow the drug to be cleared from the system (Cruickshank & Dyer, 2009).

Magazine training.

In a third "neutral" context, rats were trained to collect food pellets from the magazine, each rat underwent one thirty-minute session of magazine training. During this session, food rewards were delivered to the magazine in the operant chamber on a random-time (RT) sixty second schedule, whereby a food pellet was dispensed on average, every sixty seconds. Magazine entries were recorded during these training sessions. After 30 minutes the house light turned off to signal the end of the session, rats were taken out of the chambers and returned to the home cage.

Lever-press training.

Following successful completion of magazine training, all rats underwent training on the left lever. Each session began with the house-light being illuminated and insertion of the left lever, and each session ended with the house light switching off and the lever retracting. Initially rats learnt to lever press on a continuous reinforcement schedule whereby every lever-press was rewarded with a food pellet. Once twenty-five rewards had been earned the session ended, the rat was removed from the chamber and returned to the home cage. Following successful completion of this initial training, rats underwent three further training sessions, conducted over three days. During these three sessions, rewards were delivered on increasing random-interval (RI) schedules, namely fifteen, thirty, and sixty seconds, whereby pellets were

available, on average, every fifteen, thirty or sixty seconds, and delivered following the next lever press. During these RI sessions rats earned forty rewards and following this, the session ended. Therefore, each animal earned a total of one hundred and twenty rewards during training on the interval schedules. Note, in order to establish more stable response rates, the interval of reinforcement used in Experiment 2 and subsequent experiments in this Chapter, differs to that used in Experiment 1. It is unlikely that this difference would impact the results because as Adams (1982) demonstrated, it is the number of reinforced responses that dictates whether an animal is sensitive to devaluation of the instrumental reinforcer, not the time of the interval of reinforcement used in training. Importantly, the number of reinforced responses in all Chapter 2 experiments were the same (i.e. 120; undertrained).

Devaluation.

Devaluation was carried out over three days. The first two twenty-minute sessions took place in the individual feeding cages where rats were given access to 20g of the reinforcer via a glass ramekin. After this time, rats were taken out of the boxes and injected i.p. with either 0.15M, 15ml/kg lithium chloride (Devalued group, n= 16), or the equivalent volume of saline (Non-devalued control group, n= 16). The final devaluation session took place in the operant chambers where ten food rewards were available inside the magazine. During this session, each rat remained inside the operant chamber for a period of ten minutes whilst magazine entries were recorded. Following ten minutes, rats were removed from the Skinner boxes, injected, and placed back into their home cages.

Test.

Twenty-four hours after the last session of devaluation, sensitivity to devaluation of the food reward was tested in the next phase of the experiment. Half of the rats were

tested in the methamphetamine (Methamphetamine group, *n*= 16) context and the other half were tested in the saline (Saline group, *n*= 16) context. During the test, rats were placed into the context and illumination of the house light and insertion of the lever signalled the beginning of the session. As with the previous experiment, the test was conducted over eight minutes under extinction conditions. Lever responses and magazine entries were recorded during the test.

Results

Between subjects ANOVA (via GLM using SPSS) was used to investigate the influence and any interactions of the two independent variables, test context (methamphetamine or saline) and devaluation (devalued or non-devalued), on the dependent variables. The dependent variables, lever press and magazine entry rates per minute, were analysed separately. Two rats were excluded from all analyses due to experimenter error at the time of testing. An alpha level of 0.05 was used for all statistical tests.

Instrumental Training

On the final day of instrumental training there were no differences in lever press rates (per min) between intended groups. Mean response rates were: methamphetamine context to-be-devalued = 11.88 (*SD* = 2.31), methamphetamine context to-be-non devalued = 12.13 (*SD* = 1.27), saline context to-be-devalued = 11.57 (*SD* = 2.53), and saline context to-be-non devalued = 12.26 (*SD* = 2.46). Between subjects ANOVA supported this observation, whereby there were no significant main effects of Context ($F < 1$), Devaluation ($F < 1$), and no interaction ($F < 1$) between the two factors. For magazine entry rates there were also no differences between intended groups. Mean magazine entry rates were: methamphetamine context to-be-devalued =

7.57 (*SD* = 2.69), methamphetamine context to-be-non devalued = 7.90 (*SD* = 3.39), saline context to-be-devalued = 7.94 (*SD* = 1.79), and saline context to-be-non devalued = 9.93 (*SD* = 2.44). Again this observation was supported by ANOVA, whereby there were no significant main effects of Context ($F_{(1,26)} = 1.52$, $p = .23$), Devaluation ($F_{(1,26)} = 1.41$, $p = .25$), and no interaction ($F < 1$) between the two factors.

Test

The mean lever press rates per minute made during the test are presented in Figure 4. Rats tested in both the methamphetamine and saline contexts reduced responding for the devalued outcome compared to non-devalued control animals. Statistical analysis confirmed this observation. ANOVA yielded a significant main effect of Devaluation ($F_{(1,26)} = 9.30$, $p = .01$), but there was no main effect of Context ($F_{(1,26)} = 1.06$, $p = .31$), and no significant interaction between the two factors ($F < 1$).

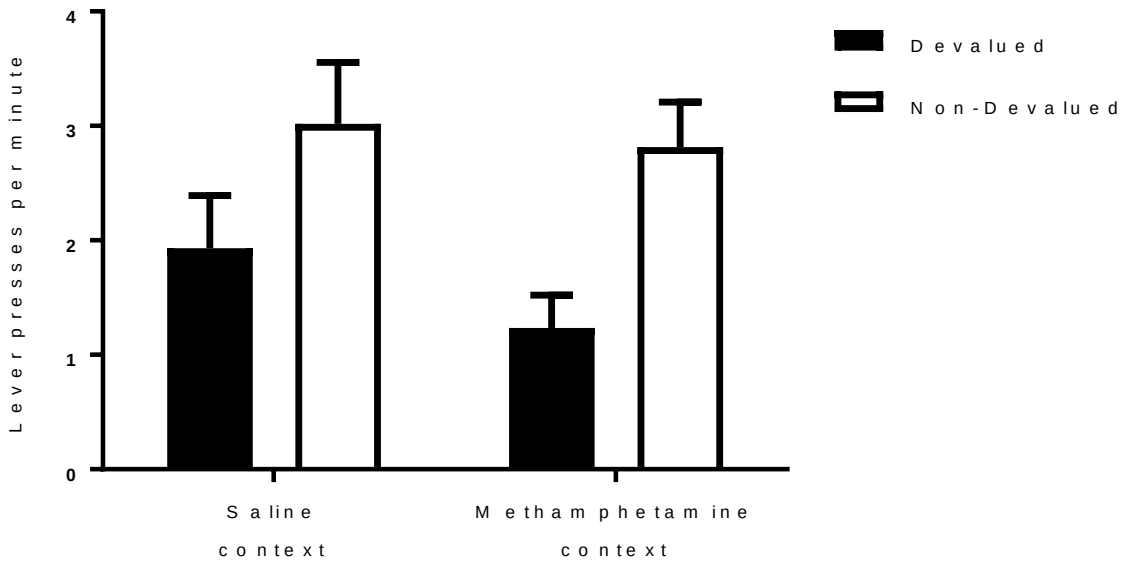

Figure 4. Mean lever presses per minute during the test in either the methamphetamine or saline context following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

The mean magazine entry rates per minute made during the test are presented in Figure 5, which shows that all devalued animals, regardless of which context the test took place in, reduce their magazine entry rates compared to non-devalued controls. Statistical analysis revealed a significant main effect of Devaluation ($F_{(1,26)} = 17.93$, $p < .01$) but there was no effect of Context ($F < 1$), and no interaction ($F < 1$).

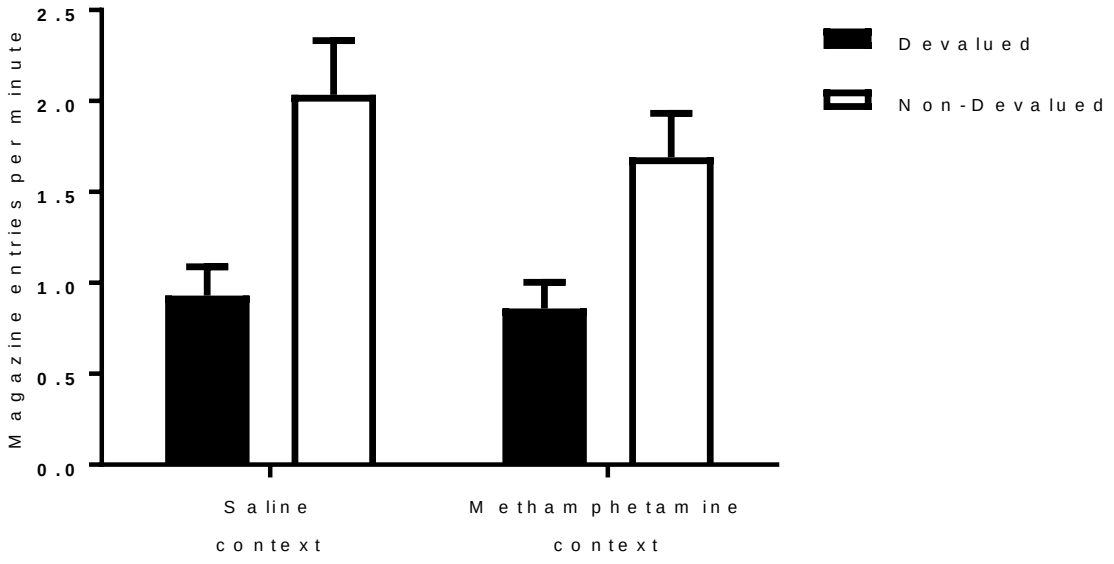

Figure 5. Mean magazine entries per minute during the test in either the methamphetamine or saline context following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

Discussion Experiment 2

The results from Experiment 2 reveal that devalued rats tested in the methamphetamine-paired context show sensitivity to devaluation of the outcome whereby they significantly reduce their rate of responding for the reward compared to non-devalued controls. This effect mirrors that of devalued animals who were tested in

the saline-paired context. These results are in contrast to the effect of the differential effect of methamphetamine- and saline-paired contexts in Experiment 1. Taken together, these findings indicate that the context alone is not responsible for the effect observed in Experiment 1, but rather, an interaction between the role of context conditioning (i.e. a psychological process) and repeated exposure to methamphetamine (i.e. a physical process) caused this effect. Previous research (Nelson & Killcross, 2006, 2013) provides strong evidence that repeated exposure to amphetamine, a drug similar to methamphetamine, causes expression of S-R habits in undertrained rats. Although such findings provide the basis of the assumption of methamphetamine-induced habits here, some researchers have found distinct psychological and neuropharmacological differences between amphetamine and methamphetamine (Shoblock, Maisonneuve, et al., 2003; Shoblock, Sullivan, Maisonneuve, & Glick, 2003). Thus, the aim of Experiment 3 was to ascertain whether simple repeated exposure to methamphetamine causes behaviour of undertrained rats to come under the control of S-R habits.

Method Experiment 3

Design

This study employed a two (Drug: Methamphetamine/Saline control) X two (Devaluation: Devalued/Non-devalued) between subjects factorial design. The dependent variables were lever press responses and magazine entries per minute.

Subjects

Thirty-two naïve, male, Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 318g and 398g at the start of the experiment. Animals were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All

experimental procedures were carried out during the light phase of the cycle. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 15g lab chow per day, and kept to 85% of their free feeding weight for the duration of the experiment. Rats had *ad libitum* access to water at all times in the home cage. Each rat was individually handled by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine.

Apparatus

The behavioural training, devaluation procedure (final day only), test, and reacquisition sessions were conducted in 8 identical operant chambers (30 cm x 24 cm x 22 cm; Med Associates, VT) which were individually enclosed in sound- and light-attenuating cabinets. Each chamber consisted of left- and right-hand aluminium walls and clear Perspex roof, back wall, and front door. The floor was comprised of 19 steel bars (3.8 mm diameter, spaced 1.6 cm apart), which were secured over a tray of corncob bedding. At the top of the left-hand wall a 3-W house light was positioned and this was illuminated at the commencement of every session and turned off once the session was completed. On the right-hand wall of the chambers a retractable lever was

positioned to the left of a recessed food magazine located at bottom centre of the wall. Food grain pellets (45mg; Bio-Serv) could be delivered as rewards, at specific times via a pellet dispenser, into the food magazine. Entries into the magazine were measured by infrared detectors at the entry of the recess. A computer equipped with Med-PC software (Med Associates Inc.) controlled the equipment and recorded data.

The first two sessions of the devaluation procedure were carried out in 8 individual feeding cages (30 cm x 18 cm x 14 cm). All 8 cages were identical and consisted of clear Perspex walls and floor. Each was fitted with a stainless steel cage top and a glass ramekin to hold grain pellet rewards.

Procedure

Table 4 summarises the key stages of the experimental procedure.

Table 4. Key stages of Experiment 3.

1	2	3	4	5	6
Drug exposure	Rest	Behavioural training	Devaluation	Test	Reacquisition

Drug exposure.

Rats received methamphetamine (*n*= 16) or control vehicle (*n*= 16) injections i.p. every other day for fourteen consecutive days. On each injection day, rats were taken from the holding room to the laboratory where they were individually weighed. After weights were recorded, syringes were loaded with the appropriate volume of methamphetamine or saline, and each rat was injected according to their group allocation. Following the injection rats were placed back into their home cage and returned to the holding room.

Rest.

Following drug exposure, rats were rested for seven consecutive days as outlined in Experiment 1.

Magazine training.

In order to train rats to collect food pellets from the magazine, each rat underwent a thirty-minute session of magazine training. Rats were taken from the holding room, transferred to the laboratory, and each was placed inside an operant chamber. During magazine training, food pellets were delivered to the magazine in the operant chamber on a random-time (RT) sixty second schedule, whereby a food pellet was dispensed on average, every sixty seconds. After 30 minutes the house light turned off to signal the end of the training session, rats were taken out of the chambers and returned to the holding room.

Lever training.

Following successful completion of magazine training, all rats underwent training on the left lever. Each session began with the house-light being illuminated and insertion of the left lever, and each session ended with the house light switching off and the lever retracting. Initially rats learnt to lever press on a continuous reinforcement schedule whereby every lever-press was rewarded with a food pellet. Once twenty-five rewards had been earned the session ended, the rat was removed from the chamber and returned to the home cage. Following successful completion of this initial training, rats underwent three further training sessions, conducted over three days. During these three sessions, rewards were delivered on increasing random-interval (RI) schedules, namely fifteen, thirty, and sixty seconds, whereby pellets were available, on average, every fifteen, thirty or sixty seconds, and delivered following the next lever press. During these RI sessions rats earned forty rewards and following this,

the session ended. Therefore, each animal earned a total of one hundred and twenty rewards during training on the interval schedules. Lever press responses and magazine entries were recorded during each lever training session.

Devaluation.

Devaluation of the food reward was carried out over three sessions. The first two sessions took place in the feeding cages where rats were given free access to the food rewards for twenty minutes. After this time, rats were taken out of the cages and injected intraperitoneally with either 0.15M, 15ml/kg lithium chloride (Devalued group, *n*= 16) or the equivalent volume of saline (Non-devalued control group, *n*= 16). The final devaluation session took place inside the operant chambers. During this final session, rats were placed into their chamber where 10 food rewards were placed inside the magazine where the rats were able to consume them over a period of 10 minutes. Following the injection on each devaluation day rats were placed back into their home cages and returned to the holding room.

Test.

As with previous experiments, sensitivity to devaluation of the food reward was tested twenty-four hours later. During the 8-minute test, rats were placed into the operant chambers and illumination of the house light and insertion of the lever signalled the beginning of the session. Lever responses and magazine entries were recorded during the test.

Reacquisition.

To confirm that the devaluation was successful in producing a taste aversion to the food reward a reacquisition test was carried out. During the 20-minute reacquisition session, lever pressing once again resulted in delivery of the food reward on an RI sixty-second schedule of reinforcement.

Results

Between subjects ANOVA (via GLM using SPSS) was used to investigate the influence and any interactions of the two independent variables, Drug (methamphetamine or saline) and Devaluation (devalued versus non-devalued), on the dependent variables. The dependent variables, lever press and magazine entry rates per minute, were analysed separately. One rat was excluded from all analyses due to equipment failure at the time of testing. An alpha level of 0.05 was used for all statistical tests.

Instrumental Training

On the final day of instrumental training there were no differences in lever press rates between intended groups. Mean response rates were: methamphetamine to-be-devalued = 11.69 (SD = 3.01), methamphetamine to-be-non devalued = 11.60 (SD = 2.19), saline to-be-devalued = 10.36 (SD = 1.58), and saline to-be-non devalued = 11.55 (SD = 2.58). Between subjects ANOVA indicated there were no significant main effects of Drug ($F < 1$), Devaluation ($F < 1$), and no interaction ($F < 1$) between the two factors. For magazine entry rates there were also no differences between intended groups. Mean response rates were: methamphetamine to-be-devalued = 6.90 (SD = 2.80), methamphetamine to-be-non devalued = 10.17 (SD = 4.87), saline to-be-devalued = 7.34 (SD = 1.55), and saline to-be-non devalued = 6.52 (SD = 2.03). Again this observation was supported by ANOVA, whereby there were no significant main effects of Drug ($F(1,27) = 2.03$, $p = .17$), Devaluation ($F(1,27) = 1.18$, $p = .29$), and no interaction ($F(1,27) = 3.28$, $p = .08$) between the two factors were found.

Test

The mean lever press rates per minute made during the test are presented in Figure 6. Following reward devaluation, saline rats reduced responding for the reward compared to non-devalued control animals. However, this was not the case for methamphetamine animals that had the reward devalued. These animals continued to lever press at rates comparable to their non-devalued counterparts. Statistical analysis confirmed this observation. ANOVA yielded a significant main effect of: Drug ($F_{(1,27)}$ = 6.11, p = .02), Devaluation ($F_{(1,27)}$ = 6.92, p = .01) and a significant interaction between the two factors ($F_{(1,27)}$ = 6.67, p = .02). Simple effects analysis revealed that lever press rates of devalued and non-devalued groups were significantly different for saline control treated rats ($F_{(1,14)}$ = 13.14, p < .01), but this was not the case for rats administered chronic methamphetamine (F < 1).

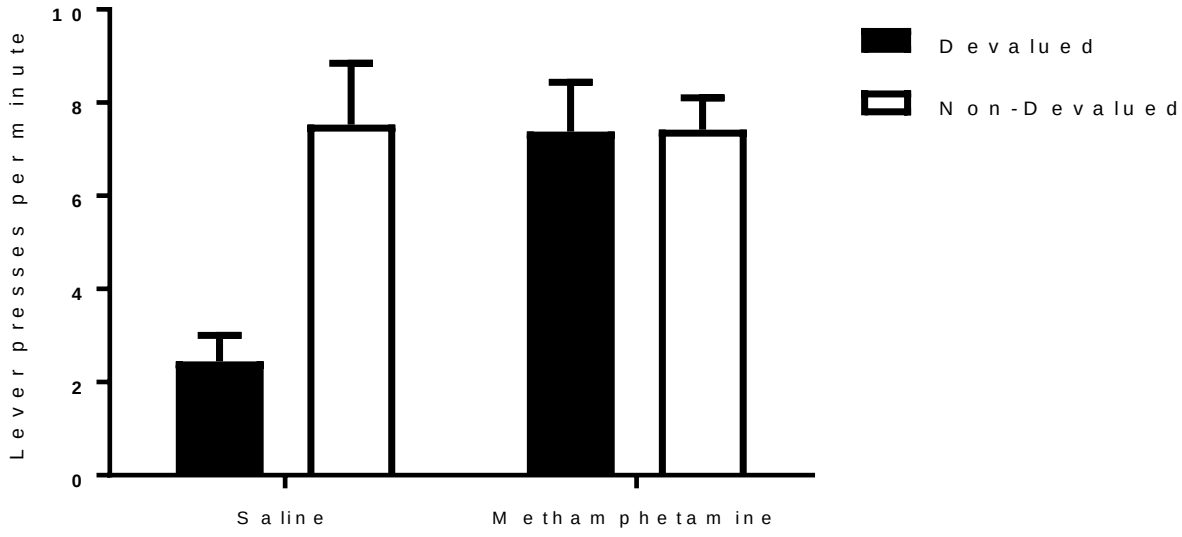

Figure 6. Mean lever presses per minute for methamphetamine and saline rats during the test following devaluation of the reward. Error bars depict +SEM. (Devalued, black bars; No devaluation, white bars).

The mean magazine entry rates per minute made during the test are presented in Figure 7. Unlike lever presses, all devalued rats, regardless of drug history, reduced their magazine entries compared to non-devalued controls. Statistical analysis revealed a main effect of: Drug ($F_{(1,27)} = 6.19$, $p = .02$), Devaluation ($F_{(1,27)} = 10.64$, $p = .01$) but there was no significant interaction between the two factors ($F < 1$).

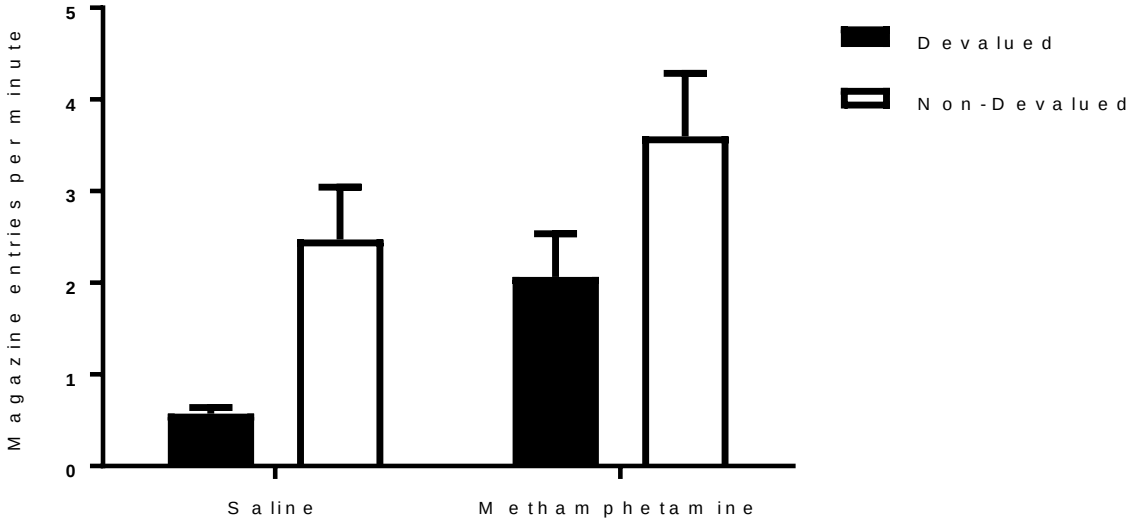

Figure 7. Mean magazine entries per minute by methamphetamine and saline rats during the test following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

Reacquisition

The mean lever press response rates per minute made during the reacquisition test are presented in Figure 8. Homogeneity of variance assumption was violated (Levene's test $p < .01$), so a square root transformation on lever rates during

reacquisition was performed. However, following transformation, Levene's test was still significant (p = .02) so a more conservative alpha level of .02 was adopted for this analysis. ANOVA indicated there was a very significant main effect of Devaluation ($F_{(1,27)}$ = 169.93, p < .01). There was no significant main effect of Drug ($F_{(1,27)}$ = 2.52, p = .12), and no significant interaction between the two factors ($F_{(1,27)}$ = 1.76, p = .20). For magazine entries, homogeneity of variance assumption was also violated (Levene's test p = .04), so a square root transformation was performed on the magazine entry rates which corrected this violation (p = .43). Mean magazine entry rates were: methamphetamine devalued = 1.57 (SD = 0.75); methamphetamine non-devalued = 5.78 (SD = 1.51); saline devalued = 0.64 (SD = 0.25); saline non-devalued = 4.52 (SD = 1.23). ANOVA confirmed that there was a significant main effect of Drug ($F_{(1,27)}$ = 12.59, p < .01), Devaluation ($F_{(1,27)}$ = 154.92, p < .01), but no interaction between the two independent variables (F < 1).

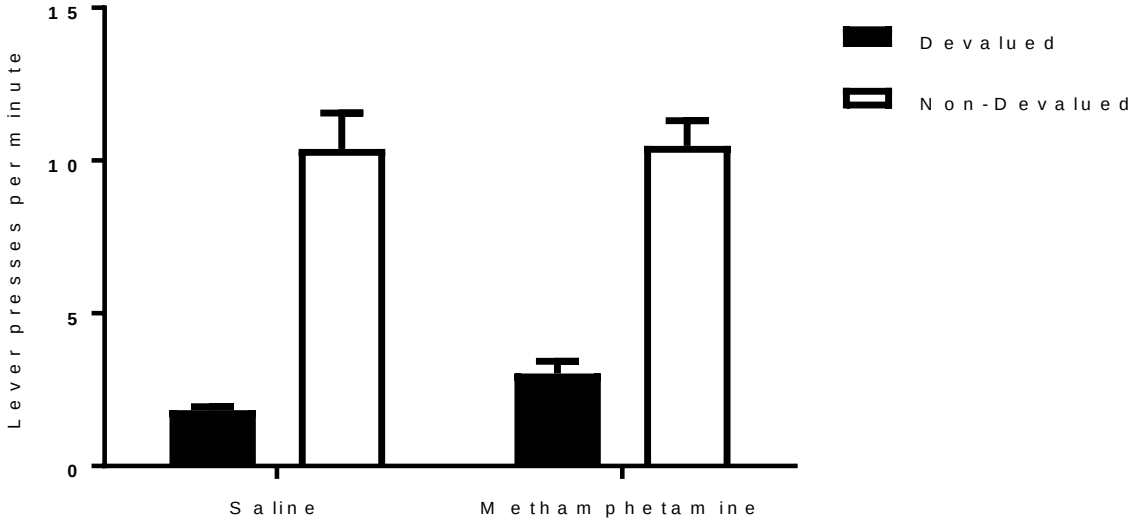

Figure 8. Mean lever presses per minute made by methamphetamine and saline rats during the reacquisition test following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

Discussion Experiment 3

The results from Experiment 3 show that rats which are repeatedly exposed to methamphetamine show insensitivity to outcome devaluation compared to saline control treated animals. This result mirrors the effect found by Nelson and Killcross in their studies of rats exposed repeatedly to amphetamine (Nelson and Killcross 2006, 2013). This suggests that methamphetamine causes neurochemical or neurostructural changes that promote the early development of S-R habits. The finding from Experiment 3 also support the notion that the influence of the methamphetamine-paired context observed in Experiment 1, is the result of an interaction between the context and repeated methamphetamine exposure. In experiment 4 we aimed to examine whether the effect of methamphetamine on the early expression of S-R habits is observed long-term. To explore this question we introduced a delay of 6 weeks between methamphetamine administration and instrumental training.

Method Experiment 4

Design

This study employed a two (Drug: Methamphetamine/Saline control) X two (Devaluation: Devalued/Non-devalued) between subjects factorial design. The dependent variables were lever press responses and magazine entries per minute.

Subjects

Thirty-two naïve, male, Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 370 to 461g at the start of the experiment. Animals were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All

experimental procedures were carried out during the light phase of the cycle. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 15g lab chow per day, and kept to 85% of their free feeding weight for the duration of the experiment. Rats had *ad libitum* access to water at all times in the home cage. Each rat was handled individually by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine.

Apparatus

The behavioural training, devaluation procedure (final day only), test, and reacquisition sessions were conducted in 8 identical operant chambers (30 cm x 24 cm x 22 cm; Med Associates, VT) which were individually enclosed in sound- and light-attenuating cabinets. Each chamber consisted of left- and right-hand aluminium walls and clear Perspex roof, back wall, and front door. The floor was comprised of 19 steel bars (3.8 mm diameter, spaced 1.6 cm apart), which were secured over a tray of corncob bedding. At the top of the left-hand wall a 3-W house light was positioned and this was illuminated at the commencement of every session and turned off once the session was completed. On the right-hand wall of the chambers a retractable lever was

positioned to the left of a recessed food magazine located at bottom centre of the wall. Food grain pellets (45mg; Bio-Serv) could be delivered as rewards, at specific times via a pellet dispenser, into the food magazine. Entries into the magazine were measured by infrared detectors at the entry of the recess. A computer equipped with Med-PC software (Med Associates Inc.) controlled the equipment and recorded data.

For the first two sessions of the devaluation procedure were carried out in 8 individual feeding cages (30 cm x 24 cm x 22 cm). All 8 cages were identical and consisted of clear Perspex walls and floor. Each was fitted with a stainless steel cage top and a glass ramekin to hold grain pellet rewards.

Procedure

Table 5 provides a summary of the key stages of the experimental procedure.

Table 5. Key stages of Experiment 4.

1	2	3	4	5	6
Drug exposure	Rest	Behavioural training	Devaluation	Test	Activity assay

Drug exposure.

Rats received methamphetamine (*n*= 16) or control (*n*= 16) injections i.p. every other day for fourteen consecutive days. The procedure for drug exposure was identical to the drug exposure procedure described in Experiment 3.

Rest.

Following drug exposure, rats were rested for forty-two consecutive days.

Magazine training.

In order to train rats to collect food pellets from the magazine, each rat underwent a thirty-minute session of magazine training. Rats were taken from the

holding room, transferred to the laboratory, and each was placed inside an operant chamber. During magazine training, food pellets were delivered to the magazine in the operant chamber on a random-time (RT) sixty second schedule, whereby a food pellet was dispensed on average, every sixty seconds. Magazine entries were recorded during this training session. After 30 minutes the house light turned off to signal the end of the training session, rats were taken out of the chambers and returned to the holding room.

Lever training.

Following successful completion of magazine training, all rats underwent training on the left lever. Each session began with the house-light being illuminated and insertion of the left lever, and each session ended with the house light switching off and the lever retracting. Initially rats learnt to lever press on a continuous reinforcement schedule whereby every lever-press was rewarded with a food pellet. Once twenty-five rewards had been earned the session ended, the rat was removed from the chamber and returned to the home cage. Following successful completion of this initial training, rats underwent three further training sessions, conducted over three days. During these three sessions, rewards were delivered on increasing random-interval (RI) schedules, namely fifteen, thirty, and sixty seconds, whereby pellets were available, on average, every fifteen, thirty or sixty seconds, and delivered following the next lever press. During these RI sessions rats earned forty rewards and following this, the session ended. Therefore, each animal earned a total of one hundred and twenty rewards during training on the interval schedules. Lever press responses and magazine entries were recorded during each lever training session.

Devaluation.

Devaluation of the food reward was carried out over three sessions. The first two sessions took place in the feeding cages where rats were given free access to the

food rewards for twenty minutes. After this time, rats were taken out of the cages and injected intraperitoneally with either 0.15M, 15ml/kg lithium chloride (Devalued group, *n*= 16) or the equivalent volume of saline (Non-devalued control group, *n*= 16). The final devaluation session took place inside the operant chambers. During this final session, rats were placed into their chamber where 10 food rewards were placed inside the magazine where the rats were able to consume them over a period of 10 minutes. Following the injection on each devaluation day rats were placed back into their home cages and returned to the holding room.

Test.

Following the devaluation procedure rats were rested for twenty four hours. Sensitivity to devaluation of the food reward was tested in the next phase of the experiment. During the test, rats were placed into the operant chambers and illumination of the house light and insertion of the lever signalled the beginning of the session. Lever responses and magazine entries were recorded during the eight minute test conducted under extinction.

Activity Assay.

This final experimental procedure took place to confirm whether the chronic regimen of methamphetamine resulted in sensitisation, an assessment of locomotor activity following a challenge injection of methamphetamine (1mg/ml/kg i.p.) was carried out. Although this dose of methamphetamine is not sub-threshold, chronic methamphetamine animals should exhibit the typical heightened locomotor response when administered methamphetamine (at any dose) relative to drug-naïve animals. This assay utilised a 2x2 between subjects design, with independent variables of drug history (methamphetamine or saline) and drug challenge (methamphetamine or saline), and any interaction between these factors were assessed for influence on the dependent

variable distance travelled in one hour following drug administration (cm). Distance travelled was measured in centimetres and calculated by the detection of beam breaks of infrared beam strips located on the periphery of the activity chambers. These beam breaks determine the animals' movement and position within the X and Y planes of the chamber. Activity was monitored in four chambers (43cm x 43cm x 30cm) that were housed inside sound- and light-attenuating cabinets. The activity chamber walls were made of clear Perspex with a removable clear Perspex roof. Each chamber contained a house light and an exhaust fan which were turned on during the session. Activity was recorded by way of 16 evenly spaced infrared transmitters and receivers positioned around the sides of each chamber. Infrared beam strips were situated 10 cm apart and 4 cm off the floor of the chamber. These beams were connected to a control box which was monitored by a computer equipped with Med PC Activity Monitor software. Beam breaks were summed for each animal to give an index of total activity for that session. Locomotor activity was measured for 60 minutes post injection. Rats were injected and then immediately placed inside the activity chambers to commence the session. Animals were counterbalanced as to whether they came from the devalued or non-devalued groups.

Results

Between subjects ANOVA (via GLM using SPSS) were used to investigate the influence and any interactions of the two independent variables, Drug (methamphetamine or saline) and Devaluation (devalued or non-devalued), on the dependent variables. The dependent variables, lever press and magazine entry rates per minute, were analysed separately. For the activity assay, a between subjects ANOVA (via GLM using SPSS) were used to investigate the influence and any interactions of the

two independent variables, Challenge drug (methamphetamine or saline) and Drug history (methamphetamine or saline) on the dependent variable distance travelled in one hour following drug challenge (in cm). Two rats were excluded from all analyses due to their lever press rates being two standard deviations above the mean on the final day of instrumental training. An alpha level of 0.05 was used for all statistical tests.

Instrumental Training

On the final day of instrumental training there were no differences between intended groups. Mean response rates were: methamphetamine to-be-devalued = 10.31 (*SD* = 1.91), methamphetamine to-be-non devalued = 10.99 (*SD* = 2.30), saline to-be-devalued = 11.79 (*SD* = 3.29), and saline to-be-non devalued = 11.42 (*SD* = 2.52). Between subjects ANOVA indicated there were no significant main effects of Drug ($F_{(1,26)}$ = 1.02, p = .32), Devaluation ($F < 1$), and no interaction ($F < 1$) between the two factors. For magazine entry rates there were also no differences between intended groups. Mean response rates were: methamphetamine to-be-devalued = 9.13 (*SD* = 3.47), methamphetamine to-be-non devalued = 8.57 (*SD* = 5.86), saline to-be-devalued = 7.44 (*SD* = 1.62), and saline to-be-non devalued = 8.88 (*SD* = 4.94). Again this observation was supported by ANOVA, whereby there were no significant main effects of Drug ($F < 1$), Devaluation ($F < 1$), and no interaction ($F < 1$) between the two factors were found.

Test

The mean lever press rates per minute made during the test are presented in Figure 9. Both methamphetamine and saline rats reduced responding for the devalued outcome compared to non-devalued control animals. Statistical analysis confirmed this observation. ANOVA yielded a significant main effect of Devaluation ($F_{(1,26)}$ = 9.52, p =

.01), but there was no main effect of Drug ($F < 1$), and no significant interaction between the two factors ($F < 1$).

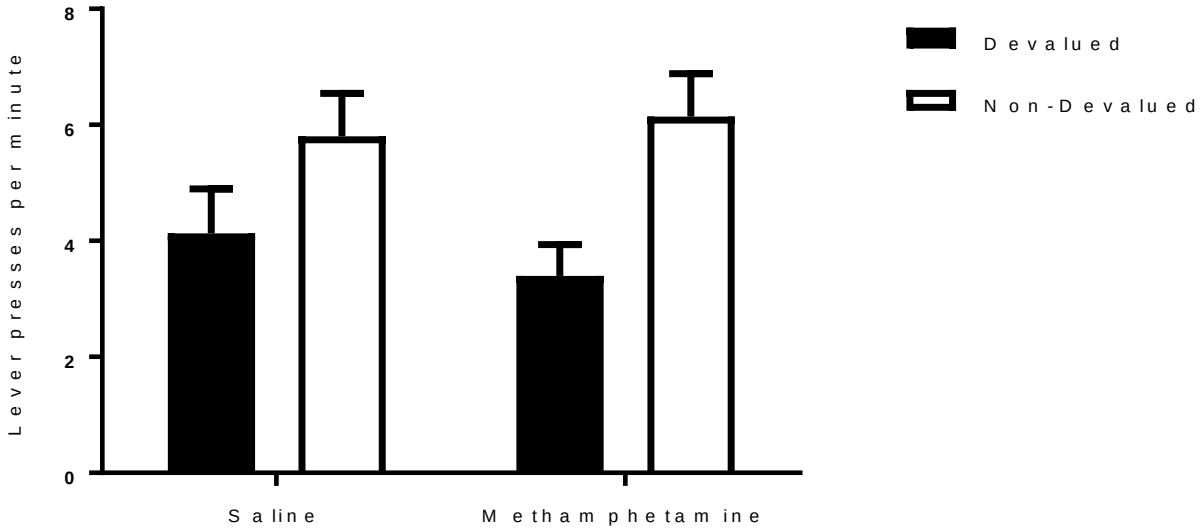

Figure 9. Mean lever presses per minute made by methamphetamine and saline rats during the test following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

The mean magazine entry rates per minute made during the test are presented in Figure 10. Statistical analysis revealed no significant main effect of Drug ($F_{(1,26)}$ = 1.22, p = .28), Devaluation ($F < 1$), and no interaction ($F < 1$).

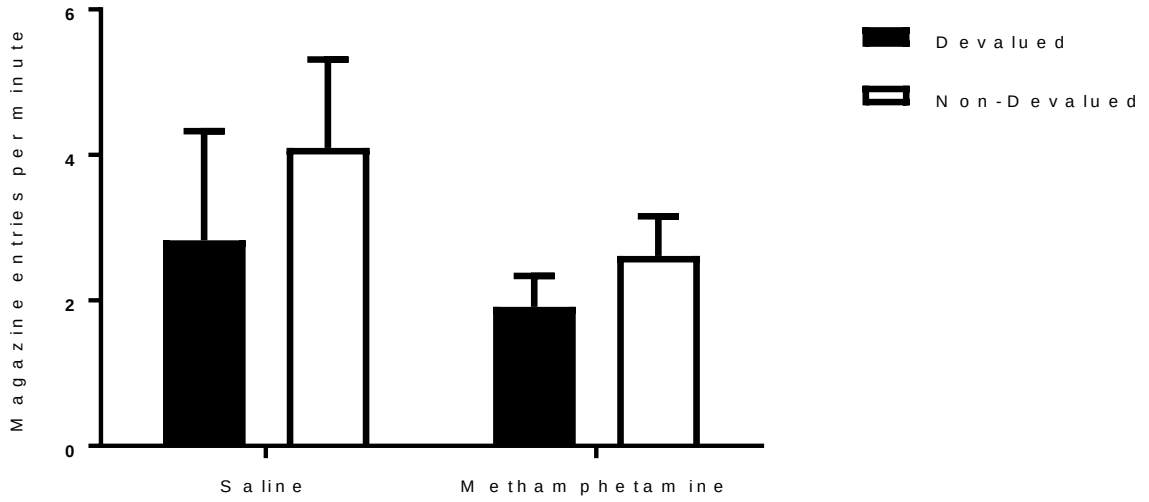

Figure 10. Mean magazine entries per minute made by methamphetamine and saline rats during the test following devaluation of the reward. Error bars depict +SEM (Devalued, black bars; No devaluation, white bars).

Activity Assay

The mean total distance travelled (cm) by rats in the locomotor activity chambers during the one hour assay following acute administration of methamphetamine or saline challenge are presented in Figure 11. Both chronic methamphetamine treated rats and saline control treated rat increased locomotor activity following a challenge dose of methamphetamine, however this was more pronounced in the chronic methamphetamine animals compared to the saline controls. Statistical analysis via ANOVA confirmed that there was no significant main effect of Drug ($F < 1$), but there was a significant main effect of Challenge ($F_{(1,26)} = 70.90$, $p < .01$), and a significant interaction between the two factors ($F_{(1,26)} = 4.98$, $p = .04$). Simple effects analysis revealed that the total distance travelled by chronic methamphetamine treated and saline control rats was significantly different for rats given a

methamphetamine challenge ($F_{(1,13)} = 4.16$, $p < .05$), but this was not the case for rats given the saline challenge ($F_{(1,13)} = 1.29$, $p = .27$).

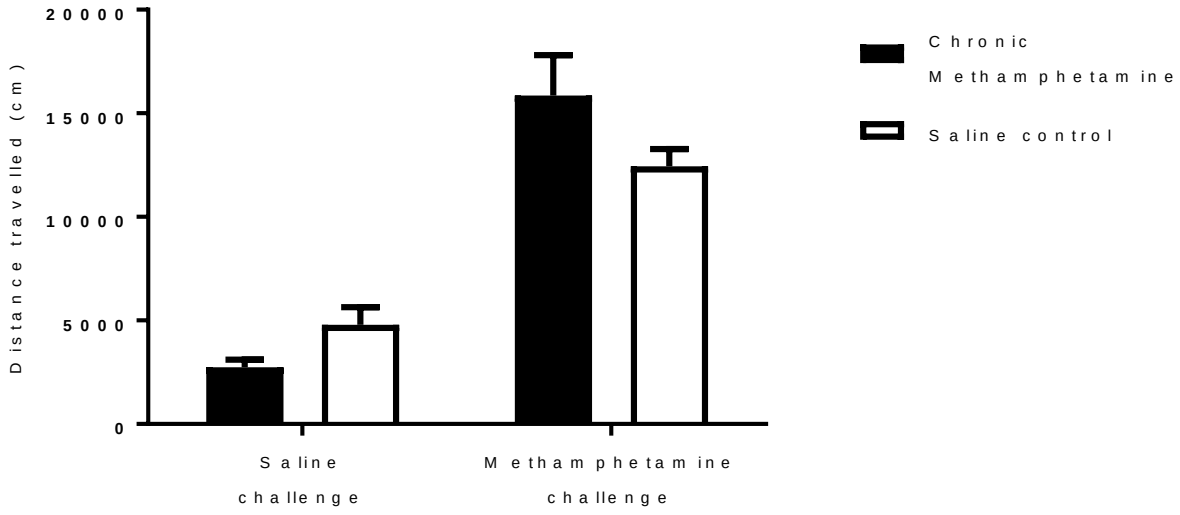

Figure 11. Mean distance travelled during the one hour activity assay by chronic methamphetamine rats and saline controls following methamphetamine challenge or saline control injections. Error bars depict +SEM. Chronic methamphetamine (black bars), Saline control (white bars).

Discussion Experiment 4

The results from Experiment 4 show that rats exposed to repeated methamphetamine 6 weeks prior to test show the same sensitivity to outcome devaluation as their saline control counterparts. This finding stands in contrast to the results from Experiment 3, whereby animals repeatedly exposed to methamphetamine show early expression of S-R habit dominated instrumental behaviour when tested only 1 week following drug exposure. Taken together, these findings suggest that repeated methamphetamine can cause the early observation of S-R habits but this is only the case

for instrumental responses that are learned relatively soon (i.e. 1 week) after drug exposure, whereas instrumental responses that are learned following a longer period of abstinence (i.e. 6 weeks) remain goal-directed. Importantly, this indicates some recovery of normal behaviour over time. Indeed, as discussed in Chapter 1, the human literature reflects the finding here whereby people with histories of chronic methamphetamine use who remain sober for relatively long periods of time improve performance on both the Stroop task (Salo, Nordahl, et al., 2009; Salo et al., 2011) and the WCST (Iudicello et al., 2010; Kim et al., 2006) . Interestingly, although chronic methamphetamine treated animals show goal-directed behaviour when there is a relatively long delay between drug exposure and instrumental training, suggesting some recovery from the impact of methamphetamine treatment during this time, these same animals still showed a hyper-locomotion response to a challenge dose of methamphetamine in the subsequent activity assay. There is good evidence (Fujiwara, Kazahaya, Nakashima, Sato, & Otsuki, 1987; Jedynak et al., 2007; Nishikawa et al., 1983; Nishioku, Shimazoe, Yamamoto, Nakanishi, & Watanabe, 1999; Shoblock, Sullivan, et al., 2003; Thanos et al., 2016; Vanderschuren et al., 1999) that sensitisation to psychostimulants, indexed by hyper-locomotion, persists for long after periods of abstinence. These results from this experiment indicate that some effects of methamphetamine-sensitisation remains, despite the animals demonstrating normal goal-directed behaviour.

Although these findings suggest that methamphetamine influences the expression of goal-directed responding, the designs of the experiments reported so far all feature methamphetamine exposure prior to instrumental training; that is, instrumental responses were learned following exposure to the drug. As such, these experiments do not allow one to determine whether or not the drug treatment is

influencing the acquisition of habits or goal-directed responding during training, or the dominance of one or other form of behaviour during test. To distinguish potential effects of performance at test from an impact on learning during training, a direct comparison needs to be made between an instrumental response learned prior to methamphetamine exposure, to an alternative response learned after drug exposure. Thus, Experiment 5 aimed to investigate whether instrumental responses acquired prior to methamphetamine exposure were sensitive to outcome devaluation during a test conducted after drug exposure, and whether a second response (leading to an alternative outcome) acquired *after* methamphetamine exposure and tested in a second devaluation test, still shows sensitivity to devaluation of this second outcome.

Method Experiment 5

Design

This study employed a two (Response: Response 1, Response 2) within subjects X two (Drug: Methamphetamine or Saline) X two Devaluation (Devalued or Non-devalued) mixed design. The dependent variables were responses and magazine entries per minute.

Subjects

Sixty-four naïve, male, Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 301g and 444g at the start of the experiment. Animals were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All experimental procedures were carried out during the light phase of the cycle. Prior to behavioural training rats were placed on food restriction. During food restriction, each

rat received 15g lab chow per day, and were kept at 85% of their free feeding weight. Rats remained on food restriction for the duration of the experiment. Water was available *ad libitum* in the home cage for the duration of the experiment. Each rat was handled individually by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine.

Apparatus

The apparatus used in this experiment was identical to that used in the previous experiments except for the addition of a second food reward and manipulanda. In this experiment two food rewards were used, a food grain pellet (45mg; Bio-Serv) that was delivered at specific times via a pellet dispenser and 20% sucrose solution with 5% lemon flavour added that was delivered at specific times via a peristaltic pump, into the magazine. The second manipulanda used in this experiment was a chain that was positioned on the opposite side of the magazine to that which the lever was located. The chain was 12 cm long, with 11 links, and hung 2 cm from the front wall and 3 cm from the side wall of the chamber. Applying downward force to the chain activated a microswitch which recorded each chainpull. The left/right position of the lever and chain was counterbalanced across the operant chambers. All apparatus used for the

devaluation procedure was identical to that described previously, however, the sucrose reward was delivered via plastic water bottles in the individual chambers and the pellets were available in ramekins.

Procedure

Table 6 provides a summary of the key experimental stages.

Table 6. Key stages of Experiment 5.

1	2	3	4	5	6	7
Response 1 training	Drug exposure	Reward 1 devaluation	Test 1	Response 2 training	Reward 2 devaluation	Test 2

Response 1 magazine training.

In order to train rats to collect the first food reward from the magazine, each rat underwent a thirty-minute session of magazine training. All procedures for the magazine training were identical to those used in the previous experiment with exception that for half of the rats the reward was grain pellets and for the other 32 rats the reward was sucrose.

Response 1 training.

Following successful completion of magazine training, all rats underwent instrumental training for response one. All training procedures were identical to those used previously, with the exception that half of the rats learned to lever press for their respective reward and the remaining half learned to chain-pull for their reward. For those rats learning to chain pull, the chain was already present in the chamber when the animals was placed in it; this is not the case for those learning to lever press. However, the illumination of the house light signalled that the session had commenced and the

reward was available. At the end of the session, the house light turned off, signalling that the reward was unavailable and the session had terminated.

As with previous experiments, rats underwent an initial session of response training whereby each response was rewarded on a continuous reinforcement schedule. Following successful acquisition of the response, rats were trained on the RI schedule over 3 sessions for a total of 120 rewards. Responses (lever press or chain pulls) and magazine entries were recorded during each training session.

Drug exposure.

Rats received methamphetamine (*n*= 32) or saline control (*n*= 32) injections i.p. every other day for fourteen consecutive days. The procedure for drug exposure was identical to the procedure described in Experiment 3.

Rest.

Following drug exposure, rats were rested for 7 consecutive days.

Reward 1 devaluation.

Devaluation of the first reward was carried out in exactly the same manner as the previous experiment however on the last day of devaluation, as appropriate to group, 10mL sucrose was made available in the magazine dispensed by via a syringe prior to the session so that the reward was freely available. Rats were allocated to the Devalued group (*n*= 32) or Non-devalued control group (*n*= 32).

Test 1.

Twenty-four hours later, sensitivity to devaluation of the food reward was tested in the next phase of the experiment using the same procedures that were used in the previous experiment. Responses (lever press and chain pulls) and magazine entries were recorded during the 8 minute test.

Response 2 Magazine training.

Three days after Test 1, rats were trained to collect their alternate reward from the magazine in exactly the same manner as described previously. The second reward was different to that earned during the first instrumental training sessions, i.e. if rats earned grain pellets for their first response, they received magazine training with sucrose and would earn sucrose in subsequent instrumental training.

Response 2 training.

Following successful completion of magazine training, rats proceeded to instrumental training with their alternate, second response. The training procedure for this response was identical to that used previously. During these sessions rats learnt the response they had not acquired previously, i.e. if rats lever pressed during the first phase of training, they learned to chain pull during this second training phase (and vice versa). Responses (lever presses or chain pulls) and magazine entries were recorded during each training session.

Reward 2 devaluation.

Devaluation of the second reward was carried out in the same manner as the previous session and allocation to devaluation groups remained the same (i.e. those who had the first reward devalued also had the second devalued and those rats receiving saline control injections during phase 1 devaluation, received saline control injections following exposure to the second reward).

Test 2.

Sensitivity to devaluation of the second reward was tested using the same procedures that were used in test 1. Responses (lever press or chain pulls) and

magazine entries were recorded during the 8 minute test performed under extinction conditions.

Reacquisition.

To confirm that the devaluation was successful in producing a taste aversion to the second food reward a reacquisition test was carried out. Unlike response 1 where all animals showed devaluation, this was required for response 2 due to a failure of the methamphetamine animals to show sensitivity to reinforcer devaluation for response two. During the 20-minute reacquisition session, responses (i.e. lever pressing or chain-pulling) once again resulted in delivery of the reward on an RI sixty second schedule of reinforcement.

Results

Mixed within- and between-subjects ANOVA (via GLM using SPSS) was used to investigate the main effects and any interactions of the within-subjects variable Response (response 1, response 2) and the two between-subjects variables, Drug (methamphetamine or saline) and Devaluation (devalued or non-devalued), on the dependent variables. The dependent variables, instrumental response rates (lever press or chain pull) and magazine entry rates per minute, were analysed separately. One rat was excluded from all analyses due to an experimenter error at the time of testing and one rat was excluded due to a failure to acquire response 1. On the final day of instrumental training for response 1, two rats response rates were two standard deviations above the mean, thus their data were excluded from all analyses. Three rats were excluded on the same basis for being outliers on magazine entries for response 1. On the final day of instrumental training for response 2, three rats were excluded for being outliers on response 2 and three rats were excluded for being outliers on

magazine entries. Thus the final number of animals per group for phase 1 and phase 2 of the experiment were: methamphetamine devalued (*n*= 12), methamphetamine non-devalued (*n*= 14), saline devalued (*n*= 11) and saline non-devalued (*n*= 14). An alpha level of 0.05 was used for all statistical tests.

Instrumental Training

As can be seen in Figure 12 on the final day of training for both Response 1 and Response 2 there were no differences in instrumental response rates between intended devaluation groups. ANOVA indicated there were no significant main effects of Drug ($F_{(1,47)}$ = 2.17, p = .15), Devaluation (F < 1), and Response ($F_{(1,47)}$ = 2.39, p = .14). There was also no significant interaction between any of the factors (all *Fs* < 1).

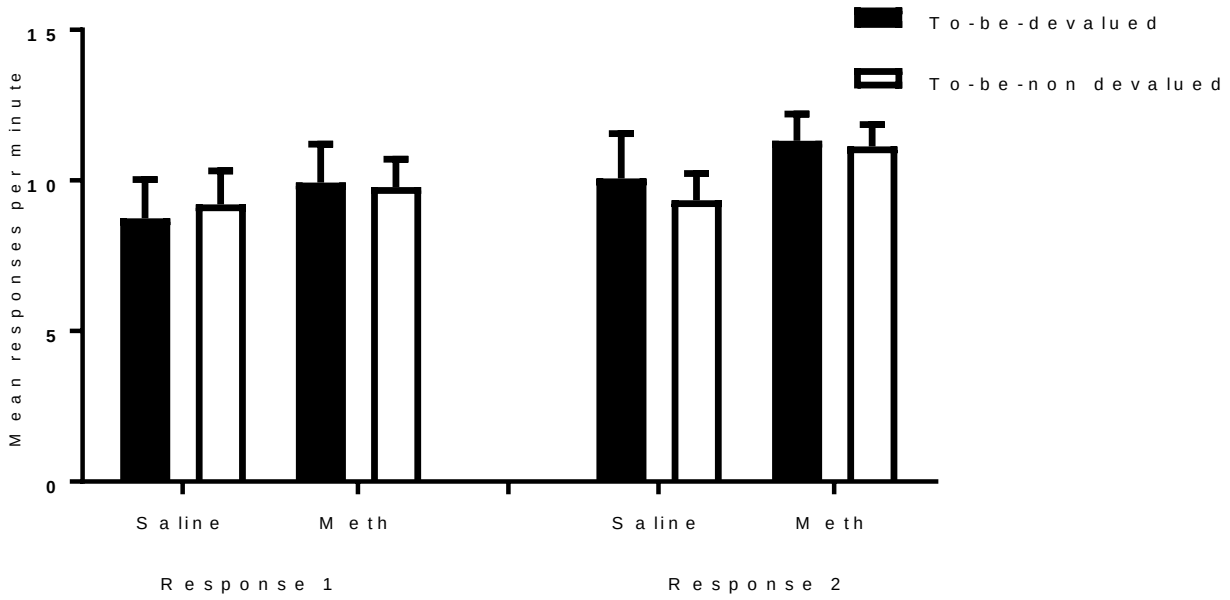

Figure 12. Mean responses per minute on the final day of training for chronic methamphetamine-treated and saline control-treated rats on response 1 and response 2 prior to devaluation of the rewards. Error bars depict +SEM. To-be-devalued (black bars) or no devaluation (to-be-non devalued- white bars).

As Figure 13 shows there were also no differences between intended devaluation groups on magazine entry rates. ANOVA confirmed this whereby there were no significant main effects of Drug ($F_{(1,47)} = 1.41$, $p = .24$), Devaluation ($F < 1$), and Response ($F < 1$). There was also no significant interaction between any of the factors: Response and Drug ($F < 1$), Response and Devaluation ($F < 1$), Drug and Devaluation ($F < 1$), and Response, Drug, and Devaluation ($F < 1$).

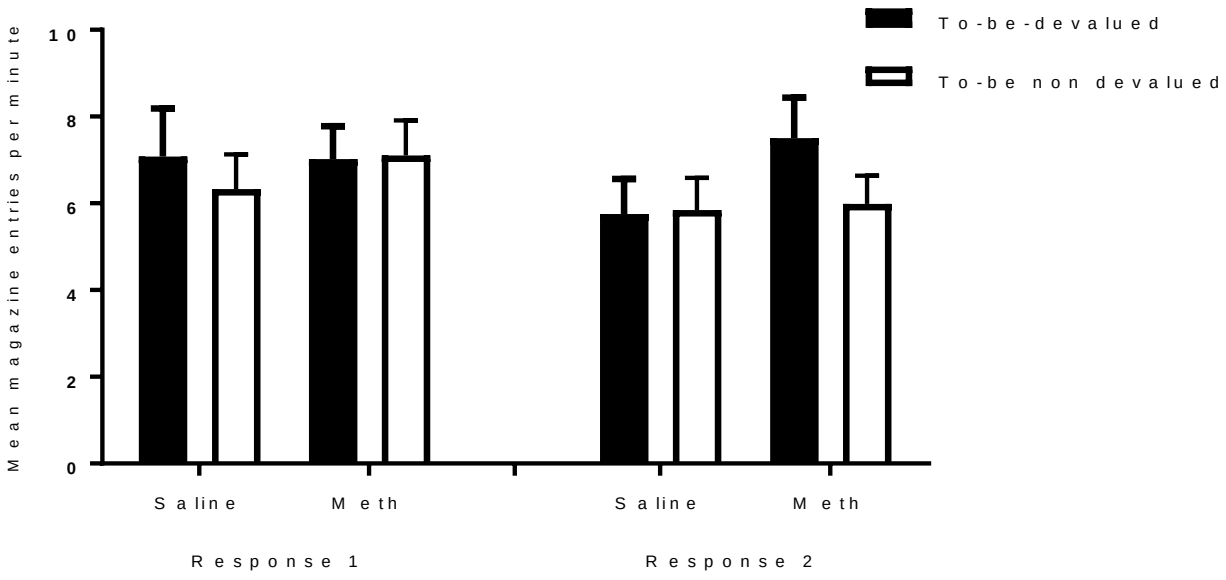

Figure 13. Mean magazine entries per minute on the final day of training for chronic methamphetamine-treated and saline control-treated rats for response 1 and response 2 prior to devaluation of the rewards. To-be-devalued (black bars) or no devaluation (to-be-non devalued- white bars). Error bars depict +SEM.

Test

The assumption of homogeneity of variance was violated for response rates in both tests (Levene's *p*'s < .05) so to correct this assumption response rates were square root transformed which corrected the violations (Levene's *p*'s > .05). The mean response rates per minute made during the tests are presented in Figure 14. During tests of devaluation for Response 1 and Response 2, saline rats reduced responding for the devalued reward compared to non-devalued control animals. This was also the case for methamphetamine animals during the test of response 1, which was acquired *before* drug exposure, but for which reward devaluation and testing occurred *after* drug exposure. However, during test of devaluation of Response 2, which was acquired *after* drug exposure, the methamphetamine-treated animals in the devalued group continued responding at rates comparable to their non-devalued counterparts. Statistical analysis confirmed this observation. ANOVA yielded a significant main effect of: Response ($F_{(1,47)}$ = 5.76, p = .02), Devaluation ($F_{(1,47)}$ = 37.47, p < .01), Response and Drug interaction ($F_{(1,47)}$ = 8.21, p = .01), Response and Devaluation interaction ($F_{(1,47)}$ = 12.53, p< .01), and importantly, a significant three-way interaction between Response, Drug, and Devaluation ($F_{(1,47)}$ = 4.95, p = .03). There was no main effect of Drug ($F_{(1,47)}$ = 1.05, p = .31) and no interaction between Drug and Devaluation ($F_{(1,47)}$ = 3.10, p = .09).

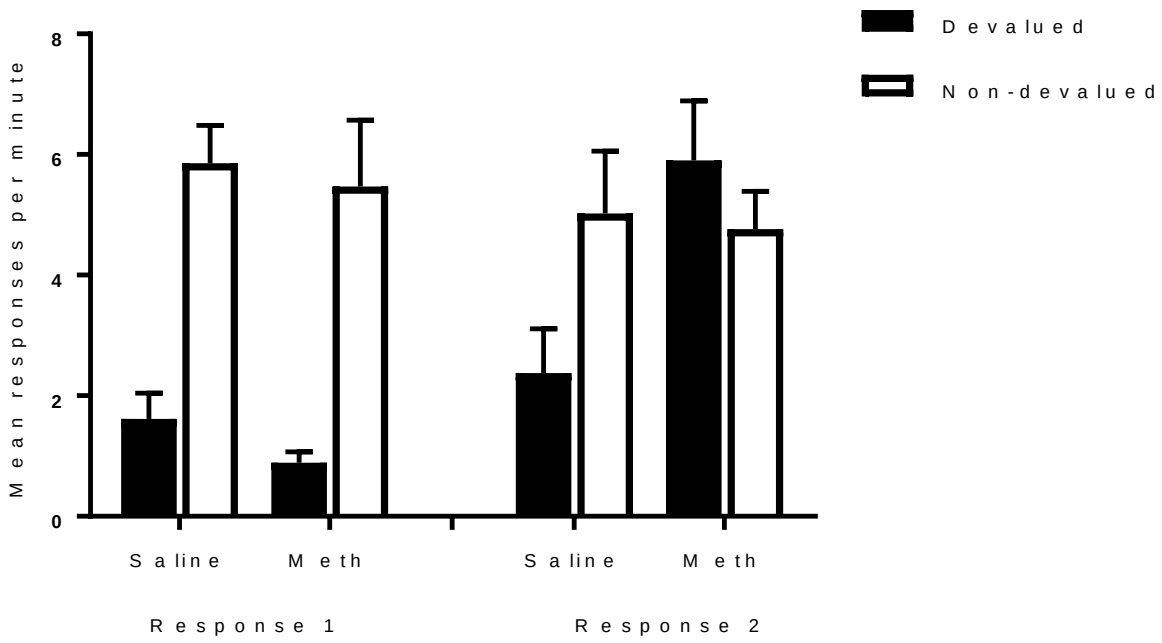

Figure 14. Mean responses per minute for methamphetamine- and saline-treated rats during test for sensitivity to devaluation of Response 1 and Response 2 following devaluation of rewards. Devalued (black bars) and no devaluation (white bars) . Error bars depict +SEM.

Simple effects analysis for response 1 revealed that regardless of subsequent drug exposure, response rates of devalued and non-devalued groups during test were sensitive to devaluation of reward. ANOVA yielded a significant main effect of Devaluation ($F_{(1,47)} = 54.60$, $p < .01$). There was no main effect of Drug ($F_{(1,47)} = 1.94$, $p = .17$), and no interaction between Drug and Devaluation ($F < 1$).

Analysis of the simple effects for response 2 revealed that rats receiving saline injections prior to response acquisition were once again sensitive to devaluation, whereby they reduced responding compared to their non-devalued control group. However, animals chronically exposed to methamphetamine before learning response 2

did not show sensitivity to devaluation of reward, continuing to respond at similar rates to their non-devalued counterparts. Supporting this observation, ANOVA yielded a significant main effect of Drug ($F_{(1,47)} = 6.58$, $p = .02$), and a significant Drug and Devaluation interaction ($F_{(1,47)} = 6.95$, $p = .01$). There was no main effect of Devaluation ($F_{(1,47)} = 3.03$, $p = .09$). Saline-treated animals that underwent reward devaluation responded at a significantly lower rate during test 2 compared to the non-devalued group ($F_{(1,25)} = 9.36$, $p = .01$). However, methamphetamine-treated rats that underwent devaluation did not significantly reduce responding for the reward during test 2, responding at similar rates to non-devalued animals ($F < 1$).

The mean magazine entry rates per minute made during the test are presented in Figure 15. The assumption of homogeneity of variance was violated for magazine entry rates in response 1 (Levene's $p < .01$) so both magazine response rates were square root transformed to correct this assumption the violation (Levene's p's $> .05$). Statistical analysis revealed a main effect of: Response ($F_{(1,47)} = 20.09$, $p < .01$), Devaluation ($F_{(1,47)} = 56.17$, $p < .01$), and a significant interaction between Response and Devaluation ($F_{(1,47)} = 7.27$, $p = .01$). There was no main effect of Drug ($F < 1$), no interaction between Response and Drug ($F < 1$), no interaction between Drug and Devaluation ($F < 1$), and no interaction between Response, Drug and Devaluation ($F < 1$). Analysis of the simple effects indicated that across tests of both response 1 ($F_{(1,25)} = 81.49$, $p < .01$) and response 2 ($F_{(1,25)} = 10.99$, $p < .01$) devalued animals entered the magazine significantly less often compared to non-devalued animals. However, overall magazine entry rates were higher for devalued animals during the test of response 2 compared to the test of response 1 ($F_{(1,25)} = 24.30$, $p < .01$).

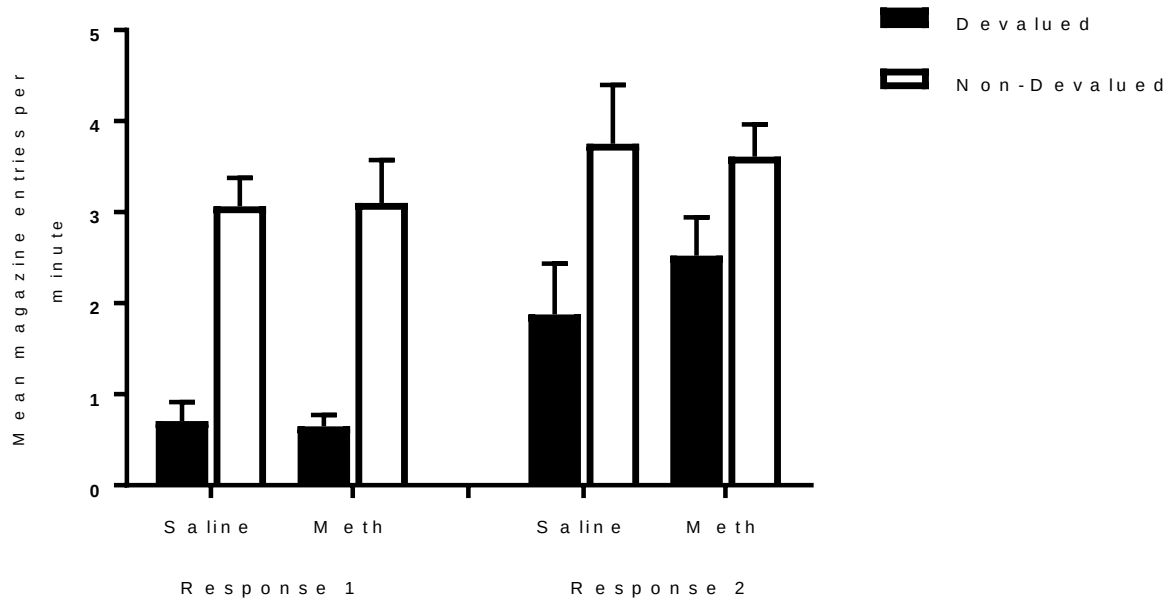

Figure 15. Mean magazine entries per minute made by methamphetamine- and saline-treated rats during test of Response 1 and test of Response 2, following devaluation of rewards. Devalued (black bars) or no devaluation (white bars). Error bars depict +SEM.

Reacquisition

The mean response rates per minute made during the reacquisition test of Response 2 are presented in Figure 16, which shows that rats in the devalued group lever pressed at significantly lower rates compared to their non-devalued counterparts, regardless of drug history. This observation was confirmed by ANOVA which indicated there was a significant main effect of Devaluation ($F_{(1,47)} = 18.79$, $p < .01$). There was no significant main effect of Drug ($F_{(1,47)} = 2.07$, $p = .16$), and no significant interaction between the two factors ($F_{(1,47)} = 3.67$, $p = .06$). Mean magazine entry rates were: methamphetamine devalued = 3.30 (SD = 2.33); methamphetamine non-devalued = 5.66 (SD = 1.94); saline devalued = 1.74 (SD = 0.99); saline non-devalued = 6.12 (SD = 2.04).

However, homogeneity of variance assumption was violated (Levene's $p = .04$) so magazine entry data was square root transformed which corrected the violation (Levene's $p > .05$). ANOVA confirmed that there was a significant main effect of Devaluation ($F_{(1,47)} = 46.05$, $p < .01$), but there was no main effect of Drug ($F_{(1,47)} = 1.75$, $p = .19$). However, there was a significant interaction between the two factors ($F_{(1,47)} = 4.17$, $p < .05$).

Simple effects analysis on the Drug x Devaluation interaction revealed that methamphetamine animals who were devalued performed significantly fewer magazine entries per minute compared to the non-devalued methamphetamine animals ($F_{(1,47)} = 11.53$, $p < .01$). Likewise, saline controls who were devalued performed significantly fewer magazine entries per minute compared to non-devalued saline animals ($F_{(1,47)} = 38.05$, $p < .01$). Looking at the difference of Drug on Devaluation, methamphetamine devalued animals performed significantly more magazine entries per minute compared to saline devalued animals ($F_{(1,47)} = 5.15$, $p = .03$). However, there were no significant difference between non-devalued methamphetamine and saline animals ($F < 1$). So, despite the significant Drug x Devaluation interaction, methamphetamine animals still displayed sensitivity to devaluation compared to non-devalued methamphetamine animals.

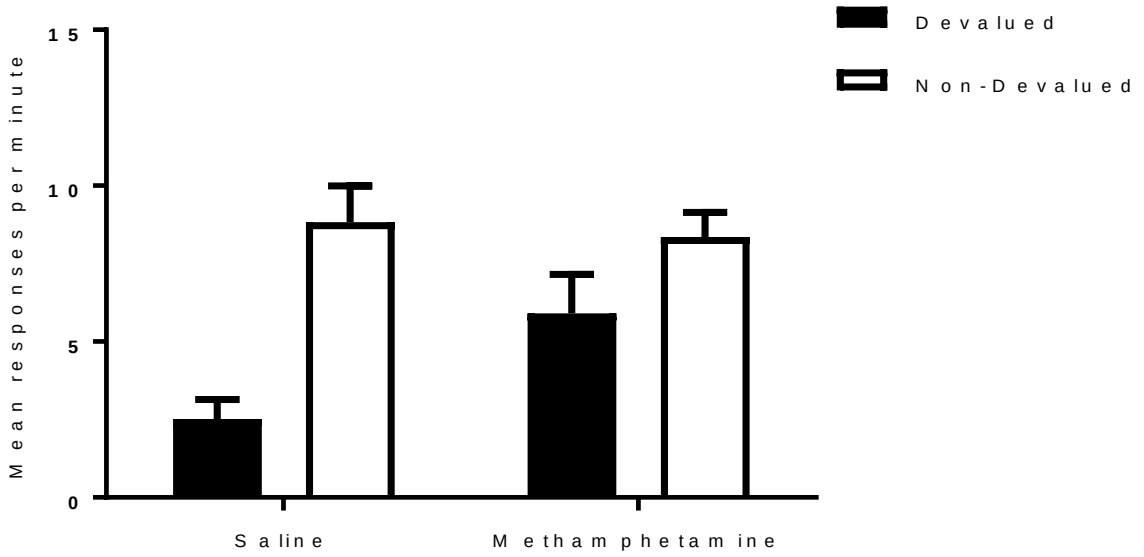

Figure 16. Mean responses per minute made by methamphetamine- and saline-treated rats during the reacquisition test following devaluation of the reward associated with response 2. Devalued (black bars) or no devaluation (white bars). Error bars depict +SEM.

Discussion Experiment 5

The results from Experiment 5 provide strong evidence that an instrumental response acquired with limited training prior to methamphetamine administration remains goal-directed even when tested after drug exposure, whereas a similarly trained instrumental response acquired after animals are exposed to repeated methamphetamine is predominantly controlled by S-R habit processes. These findings suggest that methamphetamine-exposed animals are capable of goal-directed performance but habits dominate when instrumental responses are acquired after methamphetamine exposure. It therefore seems that the habits observed in these

experiments are the result of methamphetamine affecting acquisition phase of instrumental responses, as opposed to an effect on the ability of goal-directed responding to be expressed at test.

Under normal circumstances, when animals are initially learning an instrumental response, both the goal-directed systems and S-R habit systems are engaged, but in this early stage the goal-directed system has behavioural control. Over time, S-R habits come to dominate performance of a task once it is well-learned; adaptively reducing cognitive load. This dual process model of instrumental learning posits that although S-R habits and goal-directed actions are distinct, there is interplay between the two whereby one system dominates performance over the other under certain circumstances (Daw, Niv, & Dayan, 2005; Dolan & Dayan, 2013). Coutureau and Killcross (2003) elegantly demonstrated this when overtrained animals showed sensitivity to reinforcer devaluation (thus goal-directed dominated performance) when the infralimbic region of the mPFC was temporarily inactivated whilst control animals displayed S-R habit dominated performance as would be expected with extended training. Similarly, inactivation of the DMS in the early stages of instrumental training leads to insensitivity to outcome devaluation (S-R habit dominated performance) whilst control animals demonstrate sensitivity to outcome devaluation as would be expected with limited training (Yin et al., 2005). Thus although a shift in dominance from one system to the other occurs under certain circumstance, the two systems are not mutually exclusive.

The results from Experiment 5 show that methamphetamine does not influence the ability of the goal-directed system to dominate after limited training because the instrumental response acquired prior to chronic methamphetamine but prior to test is

sensitive to reward devaluation. However, when a second instrumental response is acquired with only limited training but shortly after a period of chronic methamphetamine exposure, S-R habits dominate. This rapid acquisition of habits caused by methamphetamine could be due to the drug boosting neurochemical activation or causing neurostructural changes in the DLS which then biases behavioural control in favour of S-R habits. Or, it may be that the goal-directed system is impaired by methamphetamine-induced neurochemical or structural changes which thus bias S-R habits control, or indeed a combination of both processes could be involved in this early shift in dominance of S-R habits.

Chapter 2 Discussion

These experiments examined the effect of acute and chronic methamphetamine administered pre- or post-training on the sensitivity of instrumental responding to reward devaluation after limited training. These experiments also examined the influence of methamphetamine-paired contexts on goal-directed action. There are several findings from these experiments that warrant further comment in order to elucidate the specificity of the habit-dominated performance observed in methamphetamine-exposed animals.

Experiment 1 replicated the Furlong et al. (2015) finding that a methamphetamine-paired context can disrupt goal-directed performance. We found that S-R habits are observed when animals are tested in methamphetamine-paired contexts, but the performance of methamphetamine-treated animals when they were tested in the saline context was starkly different. In this instance, the saline-paired context was able to allow goal-directed behaviour to be expressed. The ability of methamphetamine-exposed animals to demonstrate goal-directed performance

suggests that the methamphetamine-paired context was the catalyst of the dominance of S-R habits observed in Experiment 1. However, the findings from Experiment 2 do not provide additional support for this idea.

In Experiment 2 the early dominance of habits was not observed in the methamphetamine-paired context when methamphetamine exposure was limited to a single occasion. In this experiment context did not appear to exert the same influence over behavioural control. However, one might note with caution that the degree of contextual control may also be moderated by the level of contextual conditioning. Whilst a conditioned place preference can be found following a single pairing of context and methamphetamine, it does not necessarily follow that contextual control of instrumental responding is as rapidly acquired. Unfortunately there is no logical way to disentangle repeated contextual conditioning sessions from repeated drug exposure in that same context. Nevertheless, it remains likely that an interplay between psychological (context) and physiological (drug-dependent neural changes) factors must underlie the early transition to habit-dominated behaviour observed in Experiment 1.

Experiment 3 demonstrated that simple exposure to methamphetamine prior to instrumental training can lead to changes in the control of instrumental performance. In line with the findings of Nelson and Killcross' (2006; 2013) studies using amphetamine, animals with a history of methamphetamine administration demonstrated an early dominance of S-R habits, and no sensitivity to the outcome devaluation procedure was evident.. As such, whilst contextual control of S-R and A-O dominance in instrumental performance was observed following a similar regime of drug exposure in Experiment 1 in which animals received context-drug discrimination training, Experiment 3 provided

evidence that this effect was also present where no contextual manipulation was used. This lends support for the role of drug-induced neural changes in the effect of methamphetamine on instrumental performance, but leaves open the question of the clear role of contextual conditioning processes observed in Experiment 1. In light of this finding, and those of Furlong et al. (2015), it seems likely there are additional context-modulated processes that come into play when animals are faced with contextual discrimination and goal-directed choice procedures.

Experiment 4 extended the findings from Experiment 3 and showed that following a long period of abstinence prior to learning an instrumental response, goal-directed behaviour can be restored in animals chronically exposed to methamphetamine. Here, regardless of drug treatment, all animals showed sensitivity to devaluation of the outcome at test and reduced their rates of instrumental responding compared to non-devalued animals. However, this return to goal-directed action cannot be due to a failure of the chronic methamphetamine regimen used to cause long-lasting effects. Findings from the activity assay of these animals following a methamphetamine challenge confirmed the dosing regimen produced long-term sensitisation. Taken together, this suggests that sensitisation is required to promote the early dominance of acquired habits (because Experiment 2 failed to cause early S-R habit performance following only one dose of the drug). However, animals can remain sensitised to the locomotor activating effects of methamphetamine over an extended period of abstinence whilst the impact on the rapid dominance of S-R habits over instrumental performance recovers over this same timeframe. This suggests a dissociation between these two effects of drug exposure, and potentially to different underpinning neural systems (for example effects on nucleus accumbens-based

processes underpinning locomotor effects, compared to dorsal striatal or prefrontal systems involved in the coordination of actions and habits).

Experiment 5 showed that instrumental behaviours acquired before methamphetamine exposure but prior to test, are sensitive to outcome devaluation and therefore, goal-directed. However, when the same animals acquired a different instrumental response *after* repeated methamphetamine administration, rats were again insensitive to devaluation of the outcome despite low training levels. Thus chronic methamphetamine exposure does not entirely abolish an animal's ability to be goal-directed. Rather, chronic methamphetamine facilitates a rapid shift in dominance that biases the S-R habit system over the goal-directed system, which is likely due to methamphetamine-induced dopamine imbalance.

Chapter 3

Effect of acute and chronic methamphetamine on contextual resolution of response conflict.

As discussed in Chapter 1, there is strong evidence that acute exposure to the psychostimulant amphetamine and alteration of dopaminergic tone in mesofrontal systems by way of direct D1 agonist infusion, both affect the ability of animals to use contextual cues to resolve conflict and respond in a context appropriate manner. However, it is not clear whether methamphetamine will cause similar effects. However, based on the effects of methamphetamine on tasks dependent executive function in humans (Farhadian et al., 2017; Han et al., 2008; Henry et al., 2011; Hosak et al., 2012; Monterosso et al., 2005; Nestor et al., 2011; Salo et al., 2013; Salo, Nordahl, et al., 2009; Salo et al., 2005; Simon et al., 2000b; Simon, Domier, et al., 2001; Tolliver et al., 2012), it seems likely that, as the procedure of contextual resolution of response conflict was explicitly developed to model human executive functioning, methamphetamine may well have an impact in this task. Having established that, like amphetamine, methamphetamine promotes the rapid acquisition of S-R habits with simple tasks, these experiments aimed to investigate the influence of acute and chronic methamphetamine on more complex tasks, such as the conflict resolution task developed by Haddon and Killcross. Therefore, the following experiments aim to examine whether, like amphetamine (Reichelt et al. 2013), methamphetamine abolishes the ability to resolve instances of response conflict by utilizing background contextual information. Of note, however, would be the fact that, following the results in Chapter 2 of this thesis, we will examine the role of prior repeated administration of methamphetamine on the response conflict task, as well as the effects of acute drug administration prior to test

(as in Reichelt et al., 2013). This paradigm provides the opportunity to examine the effect that methamphetamine has on higher level executive function and conflict resolution, similar to the classic human Stroop task, as outlined in Chapter 1. In order to remain consistent with Chapter 2 experiments and Furlong and colleagues (Furlong et al., 2015), the same low dose of methamphetamine (i.e. 1mg/kg) will be used as the main comparison dose in Chapter 3 experiments.

Experiment 6 Aim

Experiment 6 investigated the effect of pre-training repeated methamphetamine administration on the ability to use contextual information to resolve response conflict arising from ambiguous audiovisual compound cues. If repeated methamphetamine exposure selectively disrupts the ability to perform this task, then there should not be a significant difference between response rates on correct and incorrect levers during incongruent compound cue trials, whereas acquisition and performance directed towards single element cues should be unimpaired

Method

Design

This study employed a 2x2x2 mixed between- (Drug: methamphetamine or saline) and within-subjects (Lever: correct, incorrect; Probe: single element, Incongruent compound) factorial design. The dependent variable was lever press response rate during the first 10s of stimulus presentation.

Subjects

Thirty-two naïve, male, Wistar rats (Laboratory Animal Services, University of Adelaide, Victoria, Australia) were used in the experiment. Due to supply issues, the

strain of rats used in Chapter 2 differ to the strain used in Chapter 3, however, both strains have been used in previous methamphetamine research (da-Rosa et al., 2012; Friedman, Castaneda, & Hodge, 1998; Fukami et al., 2004; Furlong et al., 2015; Hser, Huang, Brecht, Li, & Evans, 2008). The rats weighed between 253g and 411g at the start of the experiment. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 17.5g lab chow per day, and were kept at 85% of their free feeding weight. Rats remained on food restriction for the duration of the experiment. Water was available *ad libitum* in the home cage for the duration of the experiment. Each rat was handled individually by the researcher prior to commencement of the experiment. Animals were housed in groups of four in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All experimental procedures were carried out during the light phase of the cycle. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine.

Apparatus

Eight operant chambers (30 cm x 24cm x 22 cm; Med Associates, VT) individually enclosed in sound- and light- attenuating cabinets were used in the experiment. Each chamber consisted of left- and right-hand aluminium walls and clear

Perspex roof, back wall, and front door. The floor was comprised of 19 steel bars (3.8 mm diameter, spaced 1.6 cm apart), which were secured over a tray of corncob bedding. At the top of the left-hand wall a 3-W house light was positioned and on the right-hand wall two panel lights (diameter 2 cm) were positioned. Also on the right-hand wall of the chambers were two retractable levers positioned to the left- and right-hand side of a recessed food magazine located at bottom centre of the wall. Food pellets could be delivered as rewards into the magazine at specific times via a pellet dispenser. Entries into the magazine were measured by infrared detectors at the entry of the recess and a magazine light was installed in the magazine. Individual sound cards in the control panel for each chamber generated auditory stimuli to a speaker located in the left-hand wall. A computer equipped with Med-PC software (Med Associates Inc.) controlled the equipment and recorded data.

The sensory (olfactory, tactile, and gastronomic) attributes of these chambers were modified to create two distinct contexts ("Peppermint" and "Rose"; C1 and C2). In context Peppermint (C1), 1mL of 10% peppermint essence (Queen Fine Foods) was placed into the corncob bedding, a smooth Perspex insert covered the steel bar floor and the left hand side wall of the chamber, and mixed composition grain-sucrose pellets (45mg; Bio-Serv) were available as rewards. In context Rose (C2), 1mL of 10% rose essence (Queen Fine Foods) was placed into the corncob bedding, a Perspex insert covered in rough grip tape was placed over the left-hand side wall, the steel bar floor was left uncovered, and grain pellets (45mg; Bio-Serv) were available as rewards. Two distinct auditory cues were used as stimuli and these consisted of a 2-kHz tone ("Tone" condition) and a 10-Hz train of clicks ("Click" condition). Two distinct visual cues were also used and these consisted of a flashing house-light ("Flash" condition) and a steady

illumination of both left and right panel lights together with the magazine light ("Steady" condition).

Procedure

Table 7 provides a summary of the key stages of the experimental procedure.

Table 7. Key stages of Experiment 6.

1	2	3	4	5	6
Drug exposure	Rest	Magazine training	Lever press acquisition	Biconditional discrimination training	Test

Drug exposure.

Rats received methamphetamine (*n*= 16) or saline control (*n*= 16) every other day for fourteen consecutive days. The procedure for drug exposure was identical to the procedure described in Experiment 3.

Rest.

Following drug exposure, rats were rested for seven consecutive days.

Magazine training.

In order to train rats to collect food pellets from the magazine, each animal underwent two, thirty-min sessions of magazine training, one in each context (Peppermint and Rose; C1 and C2). Rats were taken from the holding room, transferred to the laboratory, and each was placed inside an operant chamber. During magazine training the house-light was illuminated to signal the start of the session and reward pellets (either grain or sucrose-grain, O1 or O2) were delivered to the magazine in the operant chamber on a random-time (RT) sixty second schedule, whereby a reward was

dispensed on average, every sixty seconds. Magazine entries were recorded during this training session. After 30 minutes the house light turned off to signal the end of the session, rats were taken out of the chambers and returned to the holding room.

Lever-press acquisition.

Following magazine training, all rats underwent lever-press acquisition. Initially, each rat received two sessions (one in each context) of lever press training on a continuous reinforcement schedule, whereby each lever-press was rewarded with a pellet. Each session began with the house-light being illuminated. During the session the left- and right-hand levers were presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials. Rats received O1 in one context, and O2 in the second context, counterbalanced. On the following day, rats then underwent one further session of training in each context. In these sessions, rewards were delivered on a random-interval (RI) fifteen second schedule, whereby pellets became available, on average, every fifteen seconds and delivered following the next lever press. During these sessions the left- and right-hand levers were once again presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials.

Biconditional discrimination training.

A summary of the experimental design for this phase of the experiment is shown in Table 8. During this phase of the experiment rats learned different biconditional discriminations in each context, an auditory discrimination in one context (C1) and a visual discrimination in the other context (C2). For the auditory discrimination, for example, rats were required to learn to press the left-lever (LP1) in the presence of the tone (A1) stimulus and to press the right-lever (LP2) in the presence of the click (A2). For the visual discrimination, rats were required to learn to press the left-lever (LP1) in

the presence of the flash (V1) stimulus and to press the right-lever (LP2) in the presence of the steady stimulus (V2). All cue and lever allocations were counterbalanced across animals. When the correct response was made rats were rewarded with the pellet type (O1 or O2) available in that particular context (C1 or C2) on a RI15 second schedule of reinforcement, such that rewards were available on average every fifteen seconds and delivered following the next correct lever press.

Table 8. Summary of biconditional training and test procedures for Experiment 6.

		Test Probe Stimuli	
Context	Biconditional training	Single element	Incongruent compounds
	Experimental design for all animals*		
C1	A1: LP1 → O1	A1	A1V2
	A2: LP2 → O1	A2	A2V1
C2	V1: LP1 → O2	V1	A1V2
	V2: LP2 → O2	V2	A2V1
	Example of the experimental design		
Peppermint	Tone: Left → Grain	Tone	Tone and Steady
	Click: Right → Grain	Click	Click and Flash
Rose	Flash: Left → Mixed	Flash	Tone and Steady
	Steady: Right → Mixed	Steady	Click and Flash

* C1/C2, O1/O2, LP1/LP2, A1/A2, and V1/V2 refer to the different experimental chambers (contexts), reward outcomes, auditory and visual stimuli, respectively. These parameters were counterbalanced across animals.

Each biconditional training session consisted of 24 trials (12, 60-s presentations of each discrimination stimulus). During each trial the stimulus was presented and simultaneously the left- and right-hand levers would be inserted into the chamber. During the first 10 s of the stimulus presentation rewards were unavailable. However, during the final 50 s of the stimulus presentation rewards were available on the RI 15 s schedule of reinforcement. Following the 60-s presentation of the stimulus, both levers retracted and remained in that state for the duration of the inter-trial interval (Halkitis et al.) which has an average duration of 60 s. Therefore, the duration of each session was 48 mins. The house-light remained off during all sessions and was only illuminated to provide the cue in the "Flash" condition. Correct and incorrect lever press responses were recorded during each trial. Rats underwent 18 training sessions in each context.

Test.

All rats completed two test sessions, one in each context. Rats underwent one test session per day, half of the rats were tested in the auditory context first and visual context second, and the order was reversed for the remaining half of the rats, counterbalanced across groups. Each test consisted of two probe types: single element cues and incongruent compound cues. The duration of each trial was 60 s with an average 60-s ITI duration. Therefore each test session was 32 minutes long. During these trials stimulus presentations occurred in the presence of both levers but outcomes were not available during the first 10 seconds of trials. Correct lever pressing was rewarded only in the final 50 seconds of trials in order to prevent extinction. Each test session included 16 trials, presented in random order, which consisted of 12 single element probes and 4 incongruent compound probes. Again, correct and incorrect lever press responses were recorded for the first 10 seconds of trials when rewards were not

available in order to probe executive function, not reward seeking (i.e. so that correct or incorrect responding is not influenced by delivery of the reward).

Test stimuli.

The stimuli used for the test trials consisted of single element non reinforced probes and incongruent compound probes (see Table 8).

Incongruent probe compounds. Incongruent stimulus probe trials consisted of the compound presentation of one auditory and one visual stimulus that had been trained to elicit different lever press responses during biconditional discrimination acquisition. For the example in Table 8, compound stimuli A1V2 (Tone and Steady) and A2V1 (Click and Flash) signalled that different lever press responses were required (LP1 for A1 and LP2 for V2, LP2 for A2 and LP1 for V1). During these trials animals were required to use the context (Peppermint or Rose; C1 or C2) to disambiguate the incongruent compound and respond correctly. Consequently, if the test session occurred in the context where the auditory discrimination had been trained (C1), then the auditory stimulus element of the incongruent compound was the relevant cue, whereas if the test session occurred in the context where the visual discrimination had been trained (C2), then the visual stimulus element of the incongruent compound was the relevant cue. Using the example in Table 8, if an animal was presented with the incongruent probe compound A1V2 (Tone and Steady) in context C1 (Peppermint), then the correct response would be LP1 (because C1 was the context in which auditory discrimination training had taken place and LP1 had been associated with A1 in that context).

Single element probe. These trials consisted of presentation of single auditory or visual stimulus elements, depending on the context. For example, if the test was being conducted in the auditory context (Peppermint; C1) then A1 or A2 were

presented along with the left and right levers, mirroring the biconditional training but in the absence of reward.

Results

A mixed between- and within-subjects ANOVA (via GLM using SPSS) was used to investigate the main effects and any interactions of the three independent variables, Drug (methamphetamine or saline), Lever (Correct, Incorrect), and Probe (Single, Incongruent) on the dependent variable, lever response rate. One rat was excluded from analyses for failing to acquire the biconditional discrimination.

Magazine training and lever press acquisition

During these pre-training phases, all rats were successful in learning to collect both types of the food rewards from the magazines and press both left- and right-hand levers in each of the two contexts.

Biconditional discrimination training

All rats acquired the biconditional discriminations in each context over 18 sessions. On the final day of discrimination training there were no differences between the drug groups. Mean response rates on correct and incorrect levers during the first 10s of each trial (where rewards were not available and therefore did not guide correct responding) were: methamphetamine correct = 7.28 (SD = 2.05), methamphetamine incorrect = 4.10 (SD = 1.51), saline correct = 6.48 (SD = 1.18), and saline incorrect = 3.09 (SD = 0.75). A mixed between- and within-subjects ANOVA confirmed there was a significant main effect of Lever ($F_{(1,29)} = 220.99$, $p < .01$). There was no significant main effect of Drug ($F < 1$) and no significant Drug x Lever interaction ($F < 1$).

Test

Figure 17 shows the effect of prior repeated methamphetamine or saline vehicle injections on correct and incorrect responding during single element and incongruent stimulus compound presentations at test. A 2x2x2 mixed between- and within-subjects ANOVA with a between-subjects factor of Drug (methamphetamine or saline), and within-subjects factors of Lever (correct, incorrect) and Probe (single element or incongruent compound) revealed a significant main effect of Lever ($F_{(1,29)} = 7.70$, $p = 0.01$), but no main effect of Drug ($F_{(1,29)} = 2.19$, $p > .05$) and no main effect of Probe ($F < 1$). Significant interactions were observed between Lever x Probe ($F_{(1,29)} = 12.71$, $p < .01$) and a significant three-way interaction between Drug x Lever x Probe ($F_{(1,29)} = 5.48$, $p = 0.03$. No significant interactions were observed between Drug x Lever ($F < 1$) and Drug x Probe ($F < 1$). In order to explore the significant three-way interaction, the methamphetamine and saline groups were analysed separately in 2x2 within-subjects ANOVAs.

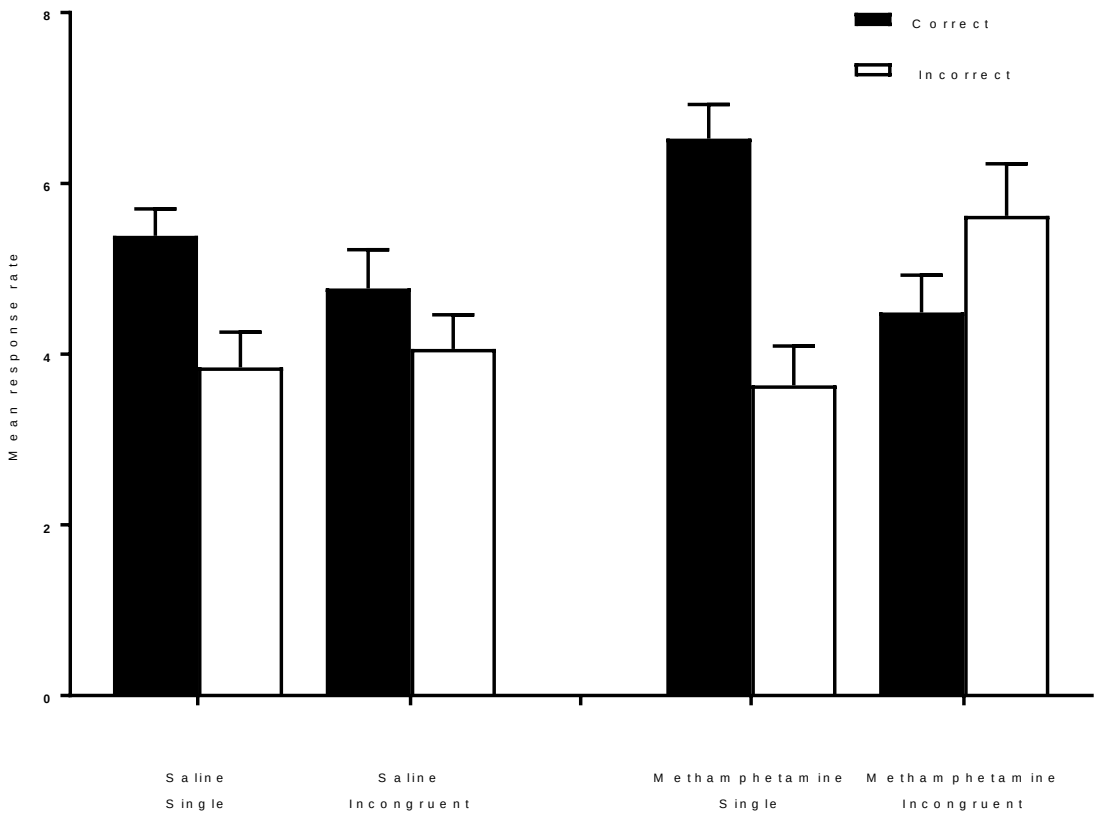

Figure 17. Effect of chronic methamphetamine or saline control injections on correct and incorrect levers during single element or audiovisual incongruent compound stimuli probes during the first 10s of trials. Correct responses (black bars) and Incorrect responses (white bars). Error bars represent S.E.M.

For the saline control group, a 2x2 within-subjects ANOVA with factors of Lever (correct, incorrect) and Probe (single element, incongruent compound) revealed a significant main effect of Lever ($F_{(1,14)} = 10.38$, $p < .01$) but no significant main effect of Probe ($F_{(1,14)} = 1.53$, $p = .24$) or Lever x Probe interaction ($F < 1$). Therefore, saline control animals made significantly more correct responses compared to incorrect responses during the first 10s of each stimulus trial, regardless of the probe type.

For the methamphetamine group, a 2x2 within-subjects ANOVA was conducted with factors of Lever (correct, incorrect) and Probe (single element, incongruent

compound). No significant main effects were observed for Lever ($F_{(1,15)}$ = 2.03, p = .18) or Probe (F < 1). However, there was a significant Lever x Probe interaction ($F_{(1,15)}$ = 22.15, p < .01). Simple effects analysis of the Lever x Probe interaction revealed that methamphetamine animals performed significantly more correct than incorrect responses on the single element trials ($F_{(1,15)}$ = 22.70, p < .01) but no significant differences between correct and incorrect responses were found on the incongruent compound trials ($F_{(1,15)}$ = 1.70, p = .35). Therefore, although methamphetamine animals were able to perform appropriately on the single element probe trials, unlike the saline control animals, rats repeatedly exposed to methamphetamine were unable to use the contextual information to disambiguate incongruent audiovisual compound cues in order to make the correct response.

Discussion Experiment 6

Experiment 6 provides the first evidence of chronic methamphetamine disrupting the ability to perform in a context appropriate manner using the paradigm developed by Haddon and Killcross (2005). Due to methamphetamine's potent effect as an indirect dopamine agonist, it is likely that this effect is due to methamphetamine causing significant imbalance in the dopaminergic system. Indeed, disruption of dopamine by acute systemic injection of amphetamine (Reichelt et al., 2013) and by way of D1 agonist infusion by (Haddon & Killcross, 2011b) provide support for this claim. Thus, the findings of Experiment 1 complements this body of work. However, the animals used in Experiment 1 were all chronically exposed to methamphetamine, whereas the previous studies mentioned have been based on acute modulation of dopaminergic systems. Therefore the aim of Experiment 7 was to examine whether

methamphetamine administered at the doses of 1mg/kg and 2mg/kg but acutely, prior to test, had any impact on performance of this task.

Method Experiment 7

Design

This study employed a 3x2x2 mixed between- (Drug: methamphetamine 1mg/kg, methamphetamine 2mg/kg, or saline) and within-subjects (Lever: correct, incorrect; Probe: single element, incongruent compound) factorial design. The dependent variable was lever press response rate.

Subjects

Twenty four, male, Wistar rats (Laboratory Animal Services, University of Adelaide, Victoria, Australia) were used in the experiment. The rats weighed between 314g and 453g at the start of the experiment. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 17.5g lab chow per day, and were kept at 85% of their free feeding weight. Rats remained on food restriction for the duration of the experiment. Water was available *ad libitum* in the home cage for the duration of the experiment. Animals were housed in groups of four in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All experimental procedures were carried out during the light phase of the cycle. Each rat was handled individually by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg (*n*= 8) or 2 mg/ml/kg (*n*= 8) depending on drug group allocation. The same concentration of saline was used for control (*n*= 8) injections and administered at an equivalent volume to that of the methamphetamine. In this experiment, drug was a between subjects factor, therefore methamphetamine or control injections were administered on two occasions only immediately prior to the test sessions.

Apparatus

The apparatus used in Experiment 7 was identical to that used in Experiment 6.

Procedure

Table 9 provides a summary of the key stages of the experimental procedure.

Table 9. Key stages of Experiment 7.

1	2	3	4
Magazine training	Lever press acquisition	Biconditional training	Test (following Meth or Sal treatment)

Magazine training.

In order to train rats to collect food pellets from the magazine, each animal underwent two, thirty-min sessions of magazine training, one in each context (Peppermint and Rose; C1 and C2). Rats were taken from the holding room, transferred to the laboratory, and each was placed inside an operant chamber. During magazine

training the house-light was illuminated to signal the start of the session and reward pellets (either grain or sucrose-grain, O1 or O2) were delivered to the magazine in the operant chamber on a random-time (RT) sixty-second schedule, whereby a reward was dispensed on average, every sixty seconds. Magazine entries were recorded during this training session. After 30 minutes the house light turned off to signal the end of the session, rats were taken out of the chambers and returned to the holding room.

Lever-press acquisition.

Following magazine training, all rats underwent lever-press acquisition. Initially, each rat received two sessions (one in each context) of lever press training on a continuous reinforcement schedule, whereby each lever-press was rewarded with a pellet. Each session began with the house-light being illuminated. During the session the left- and right-hand levers were presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials. Rats received O1 in one context, and O2 in the second context, counterbalanced. On the following day, rats then underwent one further session of training in each context. In these sessions, rewards were delivered on a random-interval (RI) fifteen second schedule, whereby pellets became available, on average, every fifteen seconds and delivered following the next lever press. During these sessions the left- and right-hand levers were once again presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials.

Biconditional discrimination training.

A summary of the experimental design for this phase of the experiment is shown in Table 8. During this phase of the experiment rats learned different biconditional discriminations in each context, an auditory discrimination in one context (C1) and a visual discrimination in the other context (C2). For the auditory discrimination, for

example, rats were required to learn to press the left-lever (LP1) in the presence of the tone (A1) stimulus and to press the right-lever (LP2) in the presence of the click (A2). For the visual discrimination, rats were required to learn to press the left-lever (LP1) in the presence of the flash (V1) stimulus and to press the right-lever (LP2) in the presence of the steady stimulus (V2). All cue and lever allocations were counterbalanced across animals. When the correct response was made rats were rewarded with the pellet type (O1 or O2) available in that particular context (C1 or C2) on a RI15 second schedule of reinforcement, such that rewards were available on average every fifteen seconds and delivered following the next correct lever press.

Each biconditional training session consisted of 24 trials (12, 60-s presentations of each discrimination stimulus). During each trial the stimulus was presented and simultaneously the left- and right-hand levers would be inserted into the chamber. During the first 10 s of the stimulus presentation rewards were unavailable. However, during the final 50 s of the stimulus presentation rewards were available on the RI 15 s schedule of reinforcement. Following the 60 s presentation of the stimulus, both levers retracted and remained in that state for the duration of the inter-trial interval (Halkitis et al.) which has an average duration of 60 s. Therefore, the duration of each session was 48 mins. The house-light remained of during all sessions and was only illuminated to provide the cue in the "Flash" condition. Correct and incorrect lever press responses were recorded during each trial. Biconditional training took place over 24 sessions

Test.

All rats completed two test sessions, one in each context. Rats underwent one test session per day, half of the rats were tested in the auditory context first and visual context second, and the order was reversed for the remaining half of the rats, counterbalanced across groups. Each test consisted of two probe types: single element

cues and incongruent compound cues. The duration of each trial was 60 s with an average 60 s ITI duration. Therefore each test session was 32 minutes long. During these trials stimulus presentations occurred in the presence of both levers but outcomes were not available during the first 10 seconds of trials. Correct lever pressing was rewarded only in the final 50 seconds of trials in order to prevent extinction. Each test session included 16 trials, presented in random order, which consisted of 12 single element probes and 4 incongruent compound probes. Again, correct and incorrect lever press responses were recorded during the first 10 seconds of each trial.

Prior to each test, rats received a single methamphetamine (1mg/kg or 2mg/kg) or saline control injection. On test day, rats were taken from the holding room to the laboratory where they were individually weighed. After weights were recorded, syringes were loaded with the appropriate volume of methamphetamine or saline, and each rat was injected according to their group allocation. Following a 5 minute delay to capture peak methamphetamine-onset, rats were placed into the operant chamber to commence the test session.

Results

A mixed between- and within-subjects ANOVA (via GLM using SPSS) was used to investigate the main effects and any interactions of the between subject, Drug at test (1mg/kg, 2mg/kg or saline), and within-subject variables Lever (Correct, Incorrect), and Probe (Single, Incongruent) on the dependent variable, lever response rate.

Magazine training and lever press acquisition

During these pre-training phases, all rats were successful in learning to collect both types of the food rewards from the magazines and press both left- and right-hand levers in each of the two contexts.

Biconditional discrimination training

All rats successfully acquired the biconditional discriminations in each context over 24 sessions. During the first 10s of each trial for the to-be 1mg/kg group, rats made more correct responses = 4.70 (*SD* = 1.54) compared to incorrect responses = 2.76 (*SD* = 0.87). The to-be 2mg/kg group also made more correct responses = 6.45 (*SD* = 3.71) compared to incorrect responses = 3.76 (*SD* = 1.98) during the first 10s of each trial. This trend was the same for the to-be saline group where more correct = 5.00 (*SD* = 2.14) than incorrect responses = 3.15 (*SD* = 1.82) were made during the first 10s of each trial. Successful acquisition was confirmed by a mixed between- and within-subjects ANOVA with factors of Drug (to be 1mg/kg, to be 2mg/kg, or to be saline) x Lever (correct, incorrect) revealing a significant main effect of Lever ($F_{(1,21)} = 16.84$, $p < 0.01$), but no main effect of Drug ($F_{(2,21)} = 1.32$, $p = .29$) and no Drug x Lever interaction ($F < 1$).

Test

Figure 18 shows the effect of acute methamphetamine administered at 1mg/kg or 2mg/kg, or saline control injections, administered immediately prior to test on correct and incorrect responding during single element and incongruent stimulus compound presentations at test. A 3x2x2 mixed between- and within-subjects ANOVA with a between-subjects factor of Drug (1mg/kg, 2mg/kg or saline), and within-subjects factors of Lever (correct, incorrect) and Probe (single element or incongruent compound) revealed a significant main effect of Lever ($F_{(1,21)} = 12.83$, $p = 0.01$), but no main effect of Drug ($F < 1$) and no main effect of Probe ($F < 1$). No significant interactions were observed; Lever x Drug ($F < 1$), Probe x Drug ($F < 1$), Lever x Probe ($F_{(1,21)} = 2.41$ $p = .14$), Lever x Probe x Drug ($F < 1$).

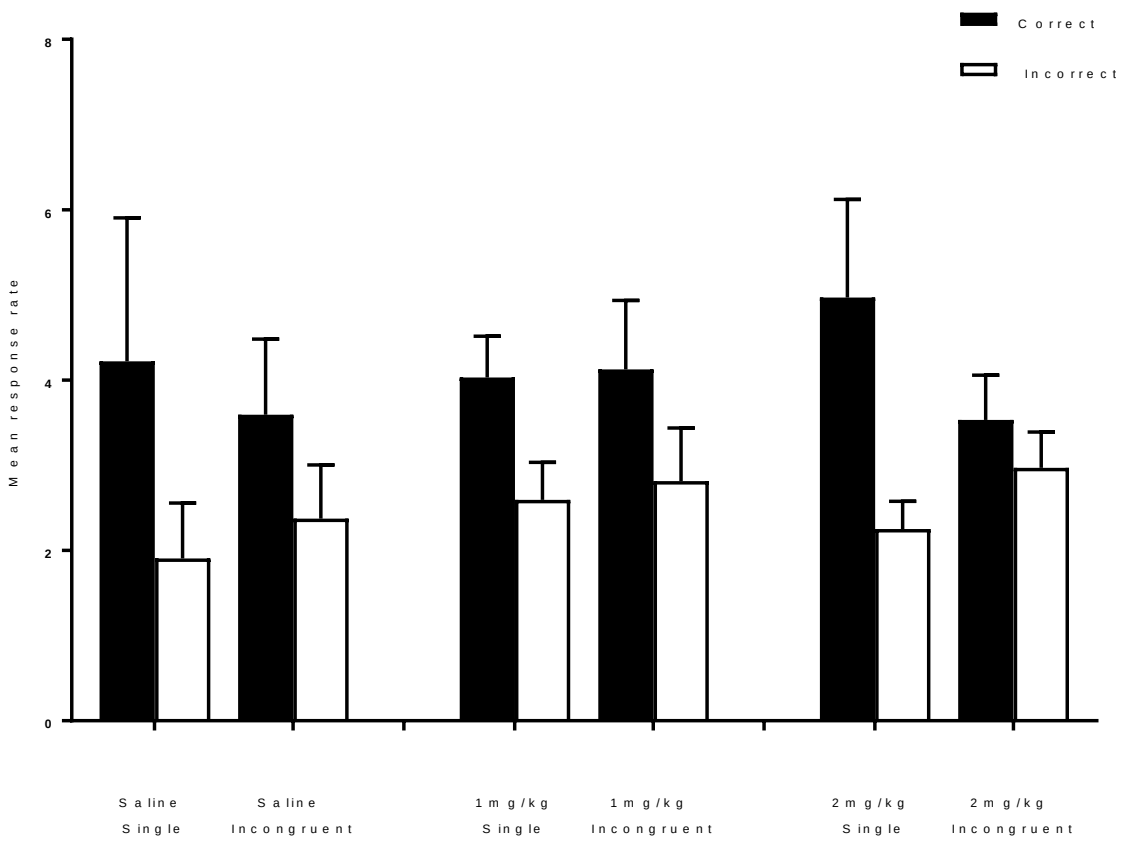

Figure 18. Effect of acute methamphetamine or saline control injections on responding to single element or audiovisual compound stimuli during the first 10s of trials. Correct responses (black bars) and Incorrect responses (white bars). Error bars represent +SEM.

However, as can be seen in Figure 18, the pattern of results for the 2mg/kg group does appear to differ from those of the 1mg/kg and saline groups. Although not appropriate and with a good deal of caution about interpretation, a separate mixed between- and within-subjects ANOVA was conducted to compare the 2mg/kg group to the 1mg/kg group. Again, this analysis yielded a significant main effect of Lever ($F_{(1,14)}$ = 13.54, $p < .01$), but no main effect of Drug ($F < 1$) and no main effect of Probe ($F < 1$). There were also no significant interactions; Lever x Drug ($F < 1$), Probe x Drug ($F < 1$),

Lever x Probe ($F_{(1,14)}$ = 2.58, p = .13), Lever x Probe x Drug ($F_{(1,14)}$ = 4.13, p = .18). Although again not strictly appropriate, another follow-up analysis was conducted in order to compare the 2mg/kg group to the saline control group. Once again, this analysis yielded a significant main effect of Lever ($F_{(1,14)}$ = 7.23, p = .02), but no main effect of Drug (F < 1) and no main effect of Probe (F < 1). There were also no significant interactions; Lever x Drug (F < 1), Probe x Drug (F < 1), Lever x Probe ($F_{(1,14)}$ = 2.68, p = .12) , Lever x Probe x Drug (F < 1). These analyses were only conducted with great caution to see if there was any potential effect of the dose of 2mg/kg methamphetamine on incongruent trial performance, but no significant differences were revealed.

Therefore, the results from this experiment indicate that acute administration of methamphetamine at 1mg/kg or 2mg/kg immediately prior to test does not have any significant effect on context appropriate responding during incongruent compound trials (and nor on performance of the underlying conditional discrimination). In this experiment methamphetamine treated animals responded in a manner that is not significantly different to saline control animals. In Experiment 8 we aimed to investigate whether methamphetamine administered immediately prior to test was able to have any influence on performance in this task. Therefore, we adopted a procedure used by Haddon and Killcross whereby the degree of training of the two biconditional discriminations is asymmetric, whereby one discrimination is "overtrained" in comparison to the other which is "undertrained". Therefore, during test, incongruent compound cues consist of one "undertrained" element and one "overtrained" element.

The typical finding replicates the Human Stroop where word reading is more familiar than colour naming which creates response conflict when the task is to name the colour. In the Haddon and Killcross paradigm, rats are able to perform in a context

appropriate manner when presented with incongruent compound cues at test in the "overtrained" context, however, performance in the undertrained context is impaired by presentation of the overtrained cue, that is, the overtrained cue disrupts context appropriate responding to the undertrained cue.

Method Experiment 8

Design

This study employed a 2x2x2 within-subjects factorial design. Independent variables were: Drug at test, (methamphetamine, saline), Lever (correct, incorrect) Probe (single element, incongruent compound). The dependent variable was lever press response rate.

Subjects

Twelve, male, Wistar rats (Laboratory Animal Services, University of Adelaide, Victoria, Australia) were used in the experiment. The rats weighed between 319g and 430g at the start of the experiment. Animals were housed in groups of four in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). All experimental procedures were carried out during the light phase of the cycle. Prior to behavioural training rats were placed on food restriction. During food restriction, each rat received 17.5g lab chow per day, and were kept at 85% of their free feeding weight. Rats remained on food restriction for the duration of the experiment. Each rat was handled individually by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Drugs

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered at a dose of 1 mg/ml/kg. The same concentration of saline was used for control injections and administered at an equivalent volume to that of the methamphetamine. Drug was a within subjects factor for this experiment so all rats were administered methamphetamine on two occasions and saline on two occasions, immediately prior to each of the four test sessions (see below for details).

Apparatus

The apparatus used in Experiment 8 was identical to those of previous experiments.

Procedure

Table 10 summarises the key stages of the experimental procedure.

Table 10. Key stages of Experiment 8.

1	2	3	4
Magazine training	Lever press acquisition	Biconditional training	Test

Magazine training.

In order to train rats to collect food pellets from the magazine, each animal underwent two thirty-minute sessions of magazine training, one in each context (Peppermint and Rose; C1 and C2). Rats were taken from the holding room, transferred to the laboratory, and each was placed inside an operant chamber. During magazine training the house-light was illuminated to signal the start of the session and reward

pellets (O1 and O2) were delivered to the magazine in the operant chamber on a random-time (RT) sixty second schedule, whereby a reward was dispensed on average, every sixty seconds. Magazine entries were recorded during this training session. After 30 minutes the house light turned off to signal the end of the session, rats were taken out of the chambers and returned to the holding room.

Lever-press acquisition.

Following magazine training, all rats underwent lever-press acquisition. Initially, each rat received two sessions (one in each context) of lever press training on a continuous reinforcement schedule, whereby each lever-press was rewarded with a pellet. Each session began with the house-light being illuminated. During the session the left- and right-hand levers were presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials. Rats received O1 in one context, and O2 in the second context, counterbalanced. On the following day, rats then underwent one further session of training in each context. In these sessions, rewards were delivered on a random-interval (RI) fifteen second schedule, whereby pellets became available, on average, every fifteen seconds and delivered following the next lever press. During these sessions the left- and right-hand levers were once again presented in an alternating fashion with each lever being presented 12 times, producing a total of 24, 60-s trials.

Biconditional discrimination training.

A summary of the experimental design for this phase of the experiment is shown in Table 8. During this phase of the experiment rats learned different biconditional discriminations in each context, an auditory discrimination in one context (C1) and a visual discrimination in the other context (C2). For the auditory discrimination, for example, rats were required to learn to press the left-lever (LP1) in the presence of the

tone (A1) stimulus and to press the right-lever (LP2) in the presence of the click (A2). For the visual discrimination, rats were required to learn to press the left-lever (LP1) in the presence of the flash (V1) stimulus and to press the right-lever (LP2) in the presence of the steady stimulus (V2). All cue and lever allocations were counterbalanced across animals. When the correct response was made rats were rewarded with the pellet type (O1 or O2) available in that particular context (C1 or C2) on a RI15 second schedule of reinforcement, such that rewards were available on average every fifteen seconds and delivered following the next correct lever press.

As with previous experiments rats underwent concurrent training in the two contexts. However for this experiment, half of the rats received fewer training trials ("undertrained; UT) for the visual context compared to the auditory context (overtrained; "OT") and this level of training was reversed for the remaining half of the rats. Each undertrained biconditional training session consisted of 8 trials (4, 60-s presentations of each discrimination stimulus) whereas the overtrained sessions consisted of 24 trials (12, 60-s presentations of each discrimination stimulus). During all trials the stimulus would be presented and simultaneously the left- and right-hand levers would be inserted into the chamber. During the first 10 s of the stimulus presentation rewards were unavailable. However, during the final 50 s of the stimulus presentation rewards were available on the RI 15 s schedule of reinforcement. Following the 60 s presentation of the stimulus, both levers retracted and remained in that state for the duration of the inter-trial interval (Halkitis et al.) which occurred on average every 60 s. Therefore, the duration of each undertrained session was 16 mins and each overtrained session was 48 mins. Correct and incorrect lever press responses were recorded for each trial. Training continued for 24 sessions in each context.

Test.

All rats completed four test sessions, two in each context, one in each context following an acute dose of methamphetamine, and one in each context following a control injection of saline (order counterbalanced across animals). Rats underwent one test session per day, half of the rats were tested in the auditory context first and visual context second and the order was reversed for the remaining half of the rats, counterbalanced across groups. Rats underwent one additional biconditional training session in each context between each test day. Thus, there was a washout period of 48 hours between methamphetamine- and saline-test sessions so that any residual methamphetamine from previous tests had been cleared (Cho, Melega, Kuczenski, & Segal, 2001). For all rats, the first two tests were conducted in the undertrained context (as this was of more direct experimental interest) and the final two tests were conducted in the overtrained context. Each test consisted of two probe types: single element and incongruent compound. The duration of each trial was 60 s with an average 60 s ITI duration. Therefore each test session was 32 minutes long. During these trials stimulus presentations occurred in the presence of both levers but outcomes were not available during the first 10 seconds of trials. Correct lever pressing was rewarded only in the final 50 seconds of trials in order to prevent extinction. Each test session included 16 trials, presented in random order, which consisted of 12 single element probes and 4 incongruent compound probes. Again, correct and incorrect lever press responses were recorded during the first 10 seconds of each trial.

Prior to each test, rats received a single methamphetamine injection or control saline injection. On each test day, rats were taken from the holding room to the laboratory where they were individually weighed. After weights were recorded, syringes were loaded with the appropriate volume of methamphetamine or saline, and

each rat was injected according to their drug allocation for that particular test. Following a 5 minute delay to capture peak methamphetamine-onset, rats were placed into the operant chamber to commence the test session.

Results

A within-subjects ANOVA (via GLM using SPSS) was used to investigate the main effects and any interactions of the three independent variables, Drug (methamphetamine, saline), Lever (Correct, Incorrect), and Probe (Single, Incongruent) on the dependent variable, lever response rate. Separate analyses were performed for the undertrained and overtrained contexts.

Magazine training and lever press acquisition

During these pre-training phases, all rats were successful in learning to collect both types of the food rewards from the magazines and press both left- and right-hand levers in each of the two contexts.

Biconditional discrimination training

All rats acquired the biconditional discriminations in both the overtrained and undertrained context over 24 sessions. This observation was confirmed by paired-samples t-tests. During the first 10s of each trial for the overtrained discrimination, rats made significantly more correct responses = 5.46 (SD = 2.63) compared to incorrect responses = 1.498 (SD = .81) ($t_{(11)}$= 5.28, p < .01). For the undertrained discrimination, rats made significantly more correct responses = 1.21 (SD = 0.75) compared to incorrect responses = 0.75 (SD = 0.28) ($t_{(11)}$= 3.64, p < .01) during the first 10s of each trial. Thus, despite the different degrees of training rats were able to accurately perform the discriminations in each context.

Test in Overtrained context

Figure 19 shows the effect of acute methamphetamine and saline control injections on correct and incorrect responding during single element and incongruent stimulus compound presentations at test in the overtrained. A 2x2x2 within-subjects ANOVA with factors of Drug at test (methamphetamine or saline), Lever (correct, incorrect), and Probe (single element or incongruent compound) revealed a significant main effect of Drug ($F_{(1,11)} = 4.81$, $p = .05$) and a main effect of Lever ($F_{(1,11)} = 13.03$, $p < .01$). However, there was no main effect of Probe ($F_{(1,11)} = 2.50$, $p = .14$) and no significant interactions Drug x Lever ($F_{(1,11)} = 1.20$, $p = .30$), Drug x Probe ($F < 1$), Lever x Probe ($F < 1$), and Drug x Lever x Probe ($F < 1$). Therefore, although rats performed more responses when they were administered methamphetamine prior to test compared to when they were administered saline, they did not show any specific task-related deficits. That is, all animals made significantly more correct than incorrect responses and thus were able to respond in a context appropriate manner regardless of the probe type and if methamphetamine was administered prior to the test.

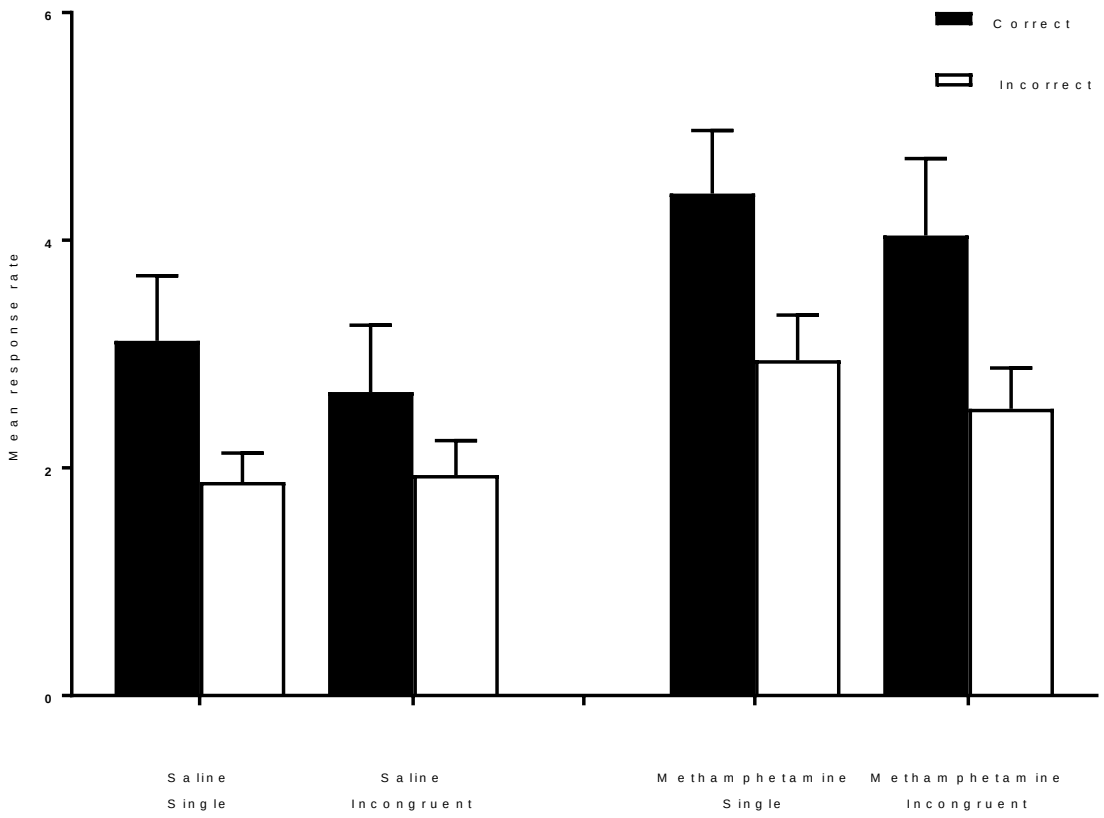

Figure 19. Effect of acute methamphetamine and saline control injections on correct and incorrect responding to single element or audiovisual incongruent compound stimuli during the first 10s of trials in the overtrained context. Correct responses (white bars) and Incorrect responses (black bars). Error bars depict +SEM.

Test in Undertrained context

Figure 20 shows the effect of acute methamphetamine and saline control injections on correct and incorrect responding during single element and incongruent stimulus compound stimuli presentations at test in the undertrained context. Notably, the incongruent compounds in this test comprise one context-appropriate but undertrained cue and one context-inappropriate overtrained cue. A 2x2x2 within-

subjects ANOVA with factors of Drug at test (methamphetamine or saline), Lever (correct, incorrect), and Probe (single element or incongruent compound) revealed no significant main effects of Drug ($F_{(1,9)} = 4.35$, $p = .07$), Lever ($F < 1$), or Probe ($F_{(1,9)} = 2.47$, $p = .15$), There were also no significant interactions Drug x Lever ($F_{(1,9)} = 1.16$, $p = .31$), Drug x Probe ($F_{(1,9)} = 1.62$, $p = .23$), and Drug x Lever x Probe ($F_{(1,9)} = 1.18$, $p = .21$) . However, there was a significant interaction between Lever x Probe ($F_{(1,9)} = 12.38$, $p < .01$). Simple effects analysis of the interaction revealed that rats performed significantly more correct compared to incorrect responses on the single element trials ($F_{(1,9)} = 16.50$, $p < .01$). However, there were no significant differences between correct and incorrect responses on the incongruent probe trials ($F_{(1,9)} = 3.18$, $p = .11$). Thus, regardless of drug treatment prior to test, presentation of the overtrained cue together with the context-appropriate yet undertrained cue, abolished the ability to respond in a context appropriate manner.

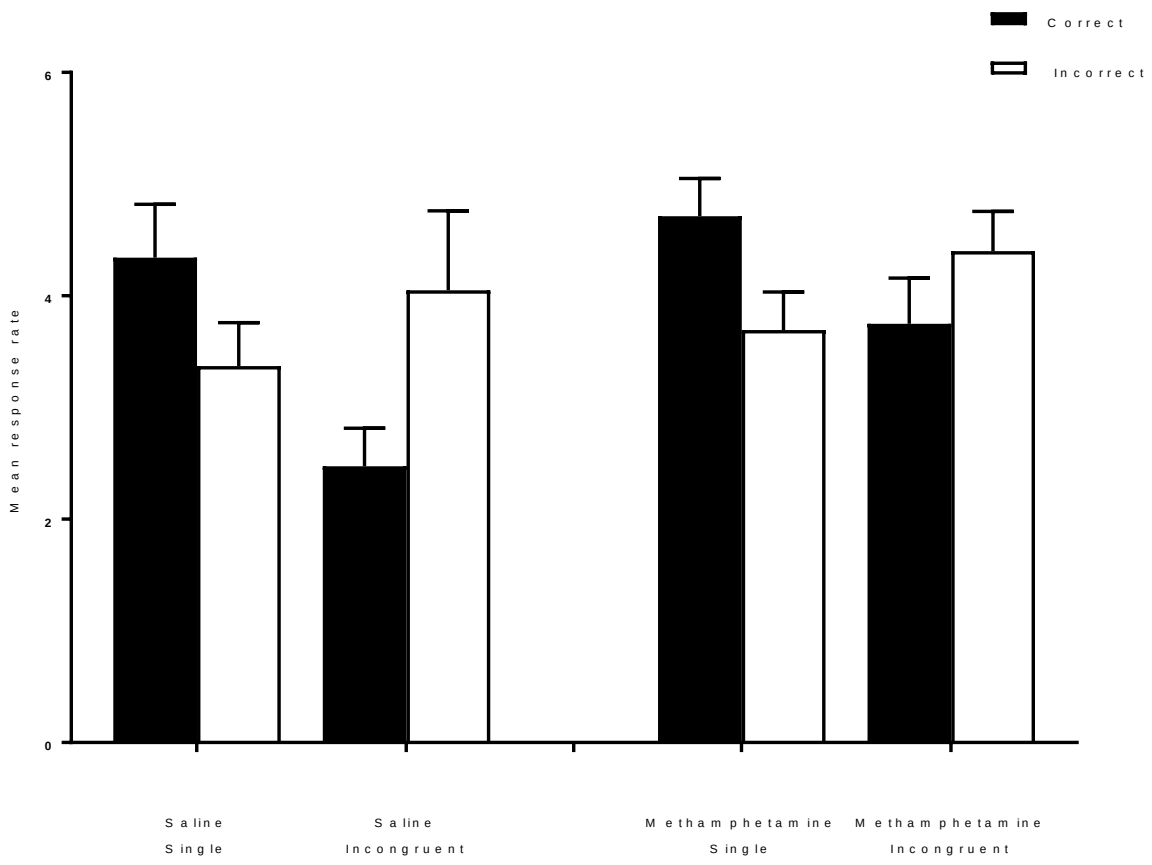

Figure 20. Effect of acute methamphetamine and saline control injections on correct and incorrect responding to single element or audiovisual incongruent compound stimuli during the first 10s of trials in the undertrained context. Correct responses (Black bars) and Incorrect responses (White bars). Error bars depict +SEM.

Discussion Experiment 8

Experiment 8 is the first empirical investigation of the influence of acute systemic methamphetamine on the ability to resolve response conflict in a context appropriate manner given differing degrees of training on the two discrimination tasks. In the context where the discrimination was "overtrained" no difference was found on correct responding to incongruent compound cues when rats were tested under

methamphetamine or saline. Somewhat surprisingly, acute methamphetamine prior to test seemed to have no detrimental effect on performance when the animals were tested in the undertrained context; regardless of having methamphetamine or saline control treatment prior to test, no differences in performance were found

Chapter 3 Discussion

These experiments examined the effect of both acute methamphetamine administered prior to test and chronic exposure to methamphetamine for several days prior to training, on the ability to use contextual information to resolve response conflict. Overall, the findings from the studies here provide mixed support for the existing literature. Experiment 6 provided the first evidence of chronic methamphetamine disrupting animals' ability to disambiguate conflicting information by using context to guide their responding. In this experiment, animals treated with methamphetamine acquired the discriminations as well as saline control animals during training and could perform accurately on simple probe trials during the test. However, these same animals were unable to perform in a context appropriate manner when faced with response conflict arising from the incongruent compound cues at test. This deficit in performance is in line with the deficits in executive function observed in current methamphetamine users or those in early sobriety (Farhadian et al., 2017; Han et al., 2008; Henry et al., 2011; Hosak et al., 2012; Monterosso et al., 2005; Nestor et al., 2011; Salo et al., 2013; Salo, Nordahl, et al., 2009; Salo et al., 2005; Simon et al., 2000b; Simon, Domier, et al., 2001; Tolliver et al., 2012).

However, the findings from Experiment 7 are in contrast to the earlier study employing this task conducted by Reichelt et al. (2013). In Experiment 7, when methamphetamine was administered acutely and prior to test, the performance of

animals treated with methamphetamine at both 1mg/kg and 2mg/kg doses was no different to the control group. Whereas, Reichelt found that 1.5mg/kg amphetamine administered acutely impaired animals' performance on incongruent compound probes and was able to restore context appropriate responding when amphetamine was co-administered with clozapine. Thus, Experiment 7 provides another example of differences in the effects of methamphetamine and amphetamine (Shoblock, Maisonneuve, et al., 2003; Shoblock, Sullivan, et al., 2003).

Experiment 8 supported the findings of Experiment 7 whereby acute methamphetamine administered acutely and immediately prior to test had no effect on performance in this task. In Experiment 8, rats underwent asymmetric training on the two biconditional discriminations, whereby one discrimination was overtrained relative to the other. Again, when animals were administered methamphetamine immediately prior to test, their performance did not differ to when they were tested under the same conditions but whilst under saline. Here, animals were able to perform as accurately on incongruent probe trials in the overtrained context whilst under methamphetamine or saline. Likewise, for the undertrained discrimination, regardless of whether methamphetamine or saline was administered immediately prior to test, accurate performance on incongruent probe trials was hindered by presentation of an overtrained cue in compound with a context-appropriate but undertrained cue.

Haddon and Killcross (2011) demonstrated that accurate performance in this task is sensitive to disruption in dopaminergic tone. Specifically, poor performers improved accuracy in the task following infusion of a dopamine agonist into the PFC. However, when performance was already optimal and animals were able to use the context to resolve the conflict arising from incongruent compounds, infusion of

dopamine agonist into PFC impaired performance. Thus, it is likely that the chronic methamphetamine regimen here disrupted dopaminergic tone in the PFC resulting in the animals' inability to use the contextual information to respond appropriately. However, it appears from Experiments 7 and 8 that acute methamphetamine does not disrupt dopaminergic tone sufficiently to observe short-term deficits.

Chapter 4

Effect of repeated methamphetamine on dendritic spine density in the dorsal striatum, and prelimbic and infralimbic cortices.

For several decades, the topic of drug-dependent neural plasticity has dominated addiction neuroscience. Advances in histological assays and imaging techniques have provided addiction researchers with new opportunities to examine this phenomenon in greater detail than ever before. Drug-dependent neural plasticity refers to structural modifications that occur in the brain as a result of drug use. Just like other experiences which cause plastic changes to the brain, such as: language development, environmental enrichment, or recovery of function following brain trauma, experience with drugs of abuse also changes the structure of the brain. It is now generally well accepted that at least some of the characteristic defining features of addiction (i.e. propensity for relapse, general behavioural changes, cognitive decline, etc.) are behavioural manifestations of drug-dependent neurological changes.

Psychostimulants are one class of drugs that have been the focus of numerous empirical investigations into drug-dependent neural plasticity in recent decades (Crombag, Gorny, Li, Kolb, & Robinson, 2005; Ferrario et al., 2005; Jedynak et al., 2007; Li et al., 2004; Li et al., 2003; Robinson et al., 2001; Robinson & Kolb, 1997, 1999; Singer et al., 2009). As discussed in Chapter 1, notable early work by Robinson and Kolb (Robinson & Kolb, 1997), used classic Golgi-Cox staining techniques combined with camera lucida drawings of the Golgi stained cells to examine structural morphology following a chronic regimen of amphetamine compared to drug-naïve animals. Robinson and Kolb chose to focus on the NAcc and PFC as regions of interest because of

their involvement in drug reward and behavioural sensitization. Of particular interest was the examination of changes in dendritic spine density on the major output neurons in the NAcc and PFC, medium spiny neurons (MSNs) and layer III pyramidal neurons, respectively. Dendritic spines are the locus of approximately 90% of all excitatory synapses in the CNS, so the number (or density) of spines on dendrites are considered a good index of synaptic connectivity. Thus, increases or decreases in dendritic spine density within these regions of interest would provide evidence of drug-dependent neural plasticity in key addiction brain regions.

Notably, Robinson and Kolb found good evidence of changes to synaptic connectivity following repeated amphetamine administration. In both the core and shell of the NA, significantly more spines were found per 10 μms of dendrite in animals exposed to repeated amphetamine (10 spines per 10 microns) compared to saline-controls (8 spines per 10 microns), increases representing 19.6% in the core and 25.9% in the shell. Also apparent were drug-dependent structural changes to the PFC pyramidal cells. In this region, Robinson and Kolb found increases in spine density on the apical (those most distal from the soma) dendrites of amphetamine-treated animals compared to control groups, however no differences were found between amphetamine- and saline-treated animals' spine density on basilar dendrites (those most proximal to the soma). These findings are good evidence of drug-dependent plasticity and suggest particularly strong synaptic connectivity in key addiction pathways following chronic amphetamine exposure.

Follow-up work from the same lab extended these findings of drug-dependent plasticity following chronic amphetamine exposure (Li et al., 2003). In this study Li and colleagues examined spine density for different segments of MSN dendrites. The

rationale was that dendrites proximal to the soma primarily receive inputs from other cells within the striatum, whereas dendrites more distal to the soma receive inputs from regions outside the striatum. Li also sought to examine whether the changes observed previously in the NAcc (ventral striatum) are similarly observed in the dorsal striatum (caudate-putamen, CPU). Using Golgi-Cox staining together with Neurolucida tracing software, Li found that compared to saline-control animals, amphetamine-treated animals showed increased spine density on distal dendrites of cells in both the NAcc and DS, but no effect of amphetamine on proximal dendrites in both regions. The distinction between changes in spine density between proximal and distal dendritic segments in the striatum are important because the distal dendrites receive dopaminergic and glutamatergic inputs from areas outside the striatum. In the case of the NA, MSNs receive input from the hippocampus and PFC, whilst MSNs in the DS receive input from the sensory-motor cortex. Thus, Li's work provides evidence that repeated exposure to amphetamine causes increases in spine density on the distal dendrites of MSNs throughout the striatum and suggests that both Dopamine and Glutamate are involved in these adaptations.

Of particular interest to the current experiments are the methamphetamine-dependent changes to spine density found in the dorsal striatum outlined in Chapter 1 (Jedynak et al., 2007). In this work, Jedynak and colleagues used an escalating dose of methamphetamine over 28 days, with a starting dose of 0.5mg/kg increasing to 6mg/kg. A behavioural assay three days after the last injection confirmed sensitization and the animals were then left in their home cages for a period of 3 months. Subsequently, in order to visualise cell morphology, Jedynak employed Sindbis virus to mediate Green Fluorescent Protein (GFP) expression in MSNs within the DS. Using GFP together with laser confocal microscopy allows for high visual acuity of cell morphology.

Z-stack images were then acquired with a confocal microscope and Neurolucida tracing software which allowed for spine visualization and quantification. Focusing on the distal segments of MSN dendrites in the dorsal striatum, Jedynak found that methamphetamine treated animal had significantly more spines per 10 μms in the DLS compared to the control animals. In the DMS however, methamphetamine-treated animals had significantly fewer spines per 10 μms compared to control animals. Thus, like amphetamine, repeated methamphetamine exposure causes distinct changes to spine density of distal dendrites on MSNs in lateral and medial portions of the dorsal striatum.

Taken together, the drug-dependent changes in spine-density discussed here suggest that repeated exposure to methamphetamine may result in increased synaptic connectivity in brain areas involved in habit formation. It may be that the specificity of these changes causes the S-R habit system to come to dominate performance more rapidly than is typical relative to the goal-directed system and that it is this shift in relative dominance that causes the rapid expression of S-R habits. However, due to the absence of behavioural data in the studies reviewed here, more evidence is needed to strengthen this hypothesis Therefore, given the drug-dependent changes in spine density found in the DLS, DMS and mPFC, and given the involvement of these areas in S-R habits and goal-directed behaviour, the aim of Experiment 9 was to employ the same methamphetamine dosing regimens that were used in the Chapter 2 Experiments 4 and 5, in order to provide a histological assay of spine density of rats chronically exposed to methamphetamine. In Experiment 5 we found that rats learning an instrumental response following a one week washout period from chronic methamphetamine demonstrated a rapid acquisition of S-R habits. In contrast, the animals in Experiment 4 who learned an instrumental responses following a six week washout period from

chronic methamphetamine exhibited normal outcome devaluation sensitivity. Thus, an analysis of spine density in rats having undergone the same dosing regimens and withdrawal periods will enable us to draw analogies between the behaviours observed in Experiments 4 and 5, and any changes to spine density found in the DLS, DMS, PL and IL in Experiment 9.

Method Experiment 9

Design

This study employed a 2x2 between subjects factorial design. The independent variables were Drug (methamphetamine or saline) and Period (3 weeks or 11 weeks). The dependent variable was number of dendritic spines per 10 μms. The four regions of interest: DLS and DMS, PL and IL, were analysed in two separate ANOVAs.

Subjects

Thirty male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) were used in the experiment. The rats weighed between 326 and 425g at the start of the experiment. Animals were housed in groups of eight in a climate-controlled holding room on a 12 hour light/dark cycle (lights on at 7 A.M.). Each rat was handled individually by the researcher prior to commencement of the experiment. All care and experimental procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition) and were approved by the University of New South Wales Animal Care and Ethics Committee.

Procedure

In order to match the procedures used in Experiments 4 and 5, rats were placed on food restriction. During food restriction, each rat received 15g lab chow per day, and

kept within 85% of their free feeding weight for the duration of the experiment. Rats had *ad libitum* access to water at all times in the home cage. Table 11 provides a summary of the next key stages of the experimental procedure which are explained in more detail in the following sub-sections.

Table 11. Key stages of Experiment 9.

1	2		3	4	5
Drug exposure	Washout	3 weeks	Euthanise and Rapid Golgi impregnation	Section and stain	Microscopy and quantification
	Washout	11 weeks			

Drug exposure and dosing regimen.

Methamphetamine (Australian Government, National Measurement Institute) was dissolved in sterile 0.9% saline solution and was administered i.p. at a dose of 1 mg/ml/kg. Rats administered methamphetamine in this experiment were given the drug on seven occasions on every second day for a period of 14 days. Saline control animals were administered 0.9% saline at an equivalent volume as the methamphetamine animals and the regimen was identical. On injection days, animals were weighed, injected with the appropriate volume of methamphetamine or saline, placed back inside their home cage and returned to the holding room. This dosing regimen was used to mirror that of the dosing regimen used throughout the experiments in this thesis. The dose of 1mg/kg was used in order to produce sensitisation observed in Chapter 2 experiments whilst avoiding the neurotoxicity that comes with higher doses. The time periods of this experiment represent the lengths of time between the last day of drug administration and date of test in Experiments 4 and 5. In Experiment 5, there was a 3 week period between the final day of

methamphetamine administration and test for response 2 and methamphetamine animals showed S-R dominance. In Experiment 4, there was an 11 week period between the final day of methamphetamine and test and here we found that methamphetamine animals were goal-directed at test. Table 12 summarises these relationships.

Table 12. Summary of the relationships between washout periods in Experiment 9 to key stages of Experiments 4 and 5.

Period*	Relationship to Chapter 2 experiments
3	Commencement of training Experiment 4
	Test date Experiment 5
11	Test date Experiment 4

*Period refers to the number of weeks between the last day of drug administration and date of euthanasia.

Golgi-impregnation procedure.

Following the respective washout periods for each group, rats were given a lethal dose of sodium pentobarbital. Post-mortem, brains were removed, rinsed briefly in Milli-Q, and immersed in Rapid Golgi Stain impregnation solution in individual vials. The impregnation procedure followed instructions provided in the Rapid Golgi Stain user manual (NeuroTechnologies, 2012).

Sectioning and staining.

Following the Rapid Golgi impregnation procedure, brains were removed, rapidly frozen, and 80 μm coronal sections were made through the PFC and striatum with a cryostat. Tissue was mounted onto gelatinised slides and subsequently stained following the procedure outlined in the Rapid Golgi Stain user manual. After staining, slides were cover-slipped and left to dry in the dark.

Confocal microscopy and quantification.

The rat brain atlas of Paxinos and Watson (Paxinos & Watson, 2014) was used by a trained observer to determine the location of tissue sections under the microscope. Cells of interest were initially identified with an Olympus FV1200 confocal microscope and motorised stage using the transmitted light detection unit and a 20x air objective (NA 0.7). MSNs in the DS were recognised by their soma size, dendritic projections and spines. Pyramidal cells in the PL and IL were recognised by the shape of their soma, axonal length, dendritic projections and spines. To be included in analyses dendrites had to be well stained with primarily intact processes that were able to be tracked to the soma of MSNs or the axons of Pyramidal cells. Cells could not be masked by astrocytes, blood vessels or obstructions caused by the staining/cover-slipping process.

Selected cells were then imaged using a 60x oil immersion objective (NA 1.4). Using Fluoview software confocal Z stacks were acquired at 2048 x 2048 pixels with an automated step size to optimise Z stack image acquisition. Once acquired, Z stacks were then collapsed into single projection images and second order dendritic segments from the soma for MSNs or the most distal segment of apical dendrites of Pyramidal cells, and were measured at the straightest length available with a 10 μm line. The number of spines visible along that length were counted. Only spines with a clear connection from the head of the spine to the dendrite were considered. Spines with more than one head were counted as spines according to the number of heads (i.e. if the number of multiple heads was three, the count of spines was three). One 10 μm segment was counted per cell. Spines were counted by two trained observers blind to drug group designation. The counts obtained from each observer were highly correlated with each other (r = 0.89). Data were obtained from a total of 6 cells per region in 23 individual rats

(methamphetamine *n*= 10, saline *n*= 13). The number of cells imaged was equivalent for the left- and right-hemispheres.

Results

A mixed between- and within-subjects ANOVA (via GLM using SPSS) was used to investigate the main effects and any interactions of the three independent variables, Drug (methamphetamine or saline), Period (3 weeks or 11 weeks), and Region – two separate analyses, (DLS, DMS) and (PL, IL) on the dependent variable spines visible along 10 μm length of dendrite. Figure 21 provides examples of Z-stack projection images acquired for methamphetamine- and saline-treated rats with 10 μm measurements along dendritic segments for each region of interest.

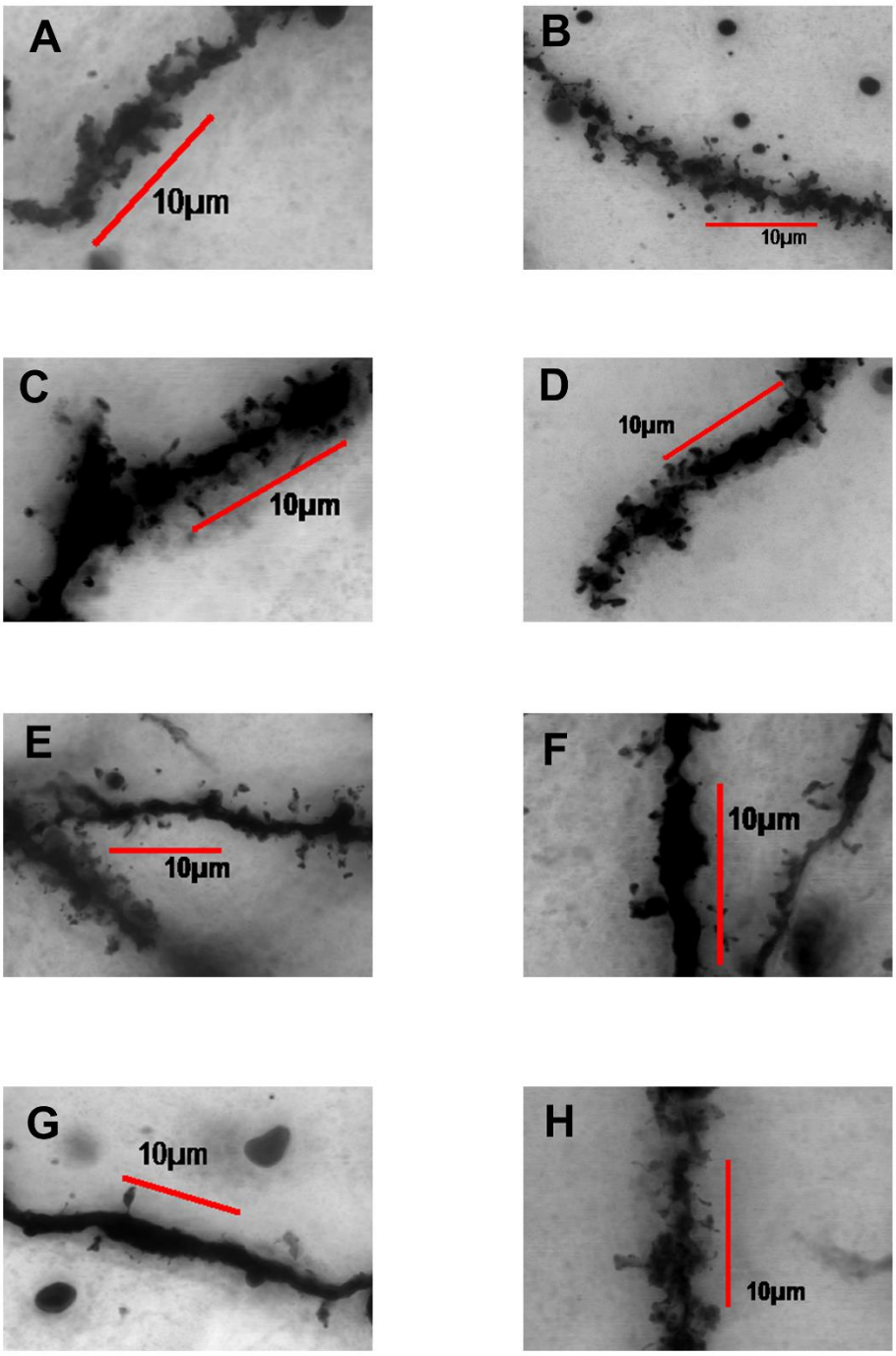

Figure 21. Effect of repeated methamphetamine (right column; B, D, F, H) or saline control (left column; A, C, E, G) injections on spine density of MSNs in the DLS (A, B) and DMS (C, D) and pyramidal cells in the PL (E, F) and IL (G, H).

Dorsal Striatum

A 2x2x2 mixed between- and within-subjects ANOVA with between-subjects factors of Drug (methamphetamine or saline) and Period (3 weeks or 11 weeks) and a within-subjects factor of Region (DLS, DMS) revealed no significant main effects of Drug ($F_{(1,20)} = 1.42$, $p = .25$), Period ($F_{(1,20)} = 1.42$, $p = .25$) or Region ($F < 1$). A significant interaction was observed between Drug x Region ($F_{(1,20)} = 4.22$, $p < .05$), but no other interactions were significant: Region x Period ($F < 1$), Drug x Period ($F_{(1,20)} = 1.42$, $p = .25$), Region x Drug x Period ($F < 1$). Simple effects analysis of the Drug x Region interaction revealed that although methamphetamine animals appeared to have greater spine density in the DLS compared to saline controls, the difference was not significant ($F_{(1,20)} = 4.02$, $p = .06$). No significant differences in spine density in the DMS were detected between methamphetamine and saline controls ($F < 1$). Therefore, as Figure 22 shows, although methamphetamine animals appeared to have greater spine density in the DLS compared to salines the difference was not significant, although this came very close.

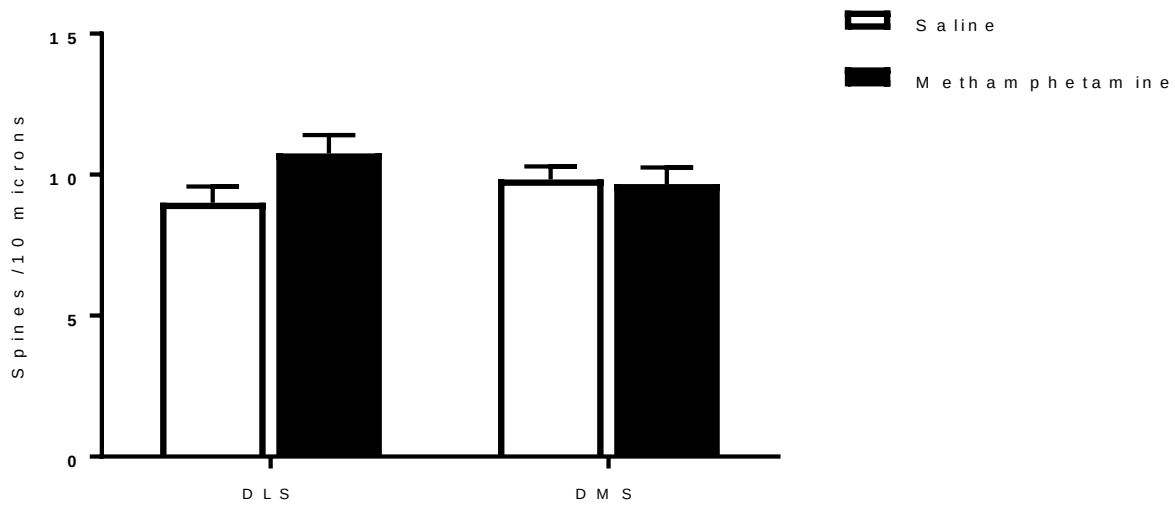

Figure 22. Effect of repeated methamphetamine or saline control injections on spine density of MSNs in the DLS and DMS. Chronic methamphetamine- (black bars) and saline-treated controls (white bars). Error bars represent S.E.M.

Medial Prefrontal Cortex

A 2x2x2 mixed between- and within-subjects ANOVA with between-subjects factors of Drug (methamphetamine or saline) and Period (3 weeks or 11 weeks) and a within-subjects factor of Region (PL, IL) revealed no significant main effects of Drug ($F_{(1,20)} = 1.00$, $p = .38$), Period ($F_{(1,20)} = 1.67$, $p = .21$) or Region ($F < 1$). A significant interaction was observed between Drug x Region ($F_{(1,20)} = 32.11$, $p < .01$), but no other interactions were significant: Region x Period ($F_{(1,20)} = 1.29$, $p = .27$), Drug x Period ($F_{(1,20)} = 1.00$, $p = .28$), Region x Drug x Period ($F < 1$). Simple effects of the significant interaction between Drug x Region revealed that methamphetamine animals had significantly less spine density in the PL compared to saline controls ($F_{(1,20)} = 24.64$, $p < .01$). This pattern was reversed for the IL region, whereby methamphetamine animals had significantly greater spine density compared to saline controls ($F_{(1,20)} = 17.78$, $p <$

.01). Therefore, as Figure 23 shows, methamphetamine animals have significantly less spines per 10 μms of dendrite in the PL, but significantly more in the IL compared to saline control animals.

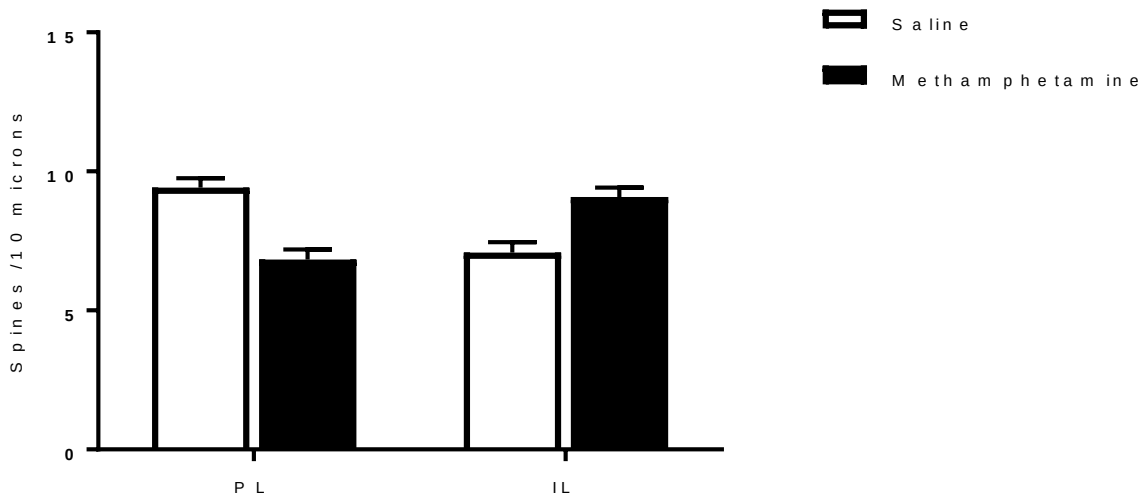

Figure 23. Effect of repeated methamphetamine or saline control injections on spine density of pyramidal cells in the PL and IL regions of the medial prefrontal cortex. Chronic methamphetamine- (black bars) and saline-treated controls (white bars). Error bars represent S.E.M.

Chapter 4 Discussion

Experiment 9 examined the effect of chronic methamphetamine exposure on dendritic spine density in the dorsal striatum and medial prefrontal cortex at two periods in time. Overall, our findings provide mixed support for the existing literature. Despite a trend of methamphetamine causing an increase in spine density on MSNs in the DLS and a significant Drug x Region interaction, simple effects of the interaction failed to find the difference between methamphetamine and saline animals significantly different. For the DMS, no significant differences in spine density of MSNs were found

between chronic methamphetamine animals and saline controls. Our findings are in contrast to those of Jedynak et al. (2007) whose work found significant differences in MSNs spine density between methamphetamine and controls. Specifically, Jedynak and colleagues found that methamphetamine animals had significantly more, and significantly fewer, spines in the DLS and DMS respectively, compared to controls. Our findings are also in contrast to those of Li and colleagues (2005) which found an increase in spine density of distal dendritic segments in the dorsal striatum of chronically exposed amphetamine animals. However, several methodological differences exist between Experiment 9 and the work of Jedynak (2007) and that of Li (2005) which may explain the differences observed (these will be addressed in following paragraphs).

Our findings in the medial prefrontal cortex were more consistent with previous research on drug-dependent neural plasticity. Notably, in the mPFC we specifically focussed on the PL and IL subregions. In line with Robinson and Kolb's (1997) research on amphetamine, we found evidence of chronic methamphetamine causing changes in spine density on the pyramidal cells in these regions. However, importantly, the findings of our work differs from Robinson and Kolb in that our chronic methamphetamine exposure caused a significant decrease in spine density in the PL and a significant increase in spine density in the IL, compared to drug naïve animals. In contrast, Robinson and Kolb detected increases in spine density only when the PFC was assessed as a whole region. This may be due to our distinction between the PL and IL cortices, or again this is an example of differences between methamphetamine and amphetamine, similar to our findings from Chapter 3 and those of Reichelt et al. (2013). To our knowledge, this is the first empirical finding of distinct changes in the PL and IL following chronic methamphetamine administration. Comparing the findings here to

those of experiments 4 and 5 allow us to draw analogies between the behaviours observed in Chapter 2 to the changes in synaptic connectivity observed in Experiment 9.

Notably, none of the analyses performed in Experiment 9 revealed a significant effect of Period, that is, the length of time between the last day of methamphetamine exposure and tests relative to Experiments 4 and 5. This is somewhat surprising given the behavioural differences observed in these experiments from Chapter 2. In experiment 5, animals who acquired an instrumental response following chronic exposure to methamphetamine showed a rapid acquisition of S-R habits. In contrast, animals in Experiment 4 who acquired an instrumental response 6 weeks after chronic exposure to methamphetamine showed sensitivity to reinforcer devaluation at test, indicating that overtime, the methamphetamine enhancing effect on rapid S-R habit dominance is diminished. However, mirroring the delay periods of Experiments 4 and 5 in Experiment 9, does not provide evidence that these structural modifications are likely to be the lone cause of these behaviours.

In light of the lack of difference in the spine density of both MSNs and Pyramidal cells in methamphetamine animals given different periods of drug washout, it is likely that neurochemical adaptations in these systems play a key role in the different behaviours observed in Experiments 4 and 5. One hypothesis is that despite enduring changes in neural architecture, over time these systems adapt at a neurochemical level in order to rebalance the system which then allows goal-directed behaviour to regain behavioural control over S-R habits. Or in other words, in time, rats are able to exert "top-down" control over their behaviour despite methamphetamine-induced changes in synaptic connectivity. Given that PL and IL pyramidal cells are glutamatergic, it is likely that the PL has reduced glutamate output, whereas the IL has

increased glutamate output in the early stages of methamphetamine withdrawal but balance is restored over time. Future work employing electrophysiological techniques would be needed to test this hypothesis.

Given the rapid acquisition of S-R habits following chronic methamphetamine observed in Chapter 2, it was surprising that changes in spine density on MSNs in the DLS or DMS were not observed, as was the case with Jedynak et al. (2007) and Li et al.'s (2005) work with amphetamine. It is worth noting however, that we did come very close to detecting a significant increase in spine density in the DLS (p = .06). It may be that the low dose used here (1mg/kg) was insufficient to cause the changes in spine density observed by Jedynak following a much higher dose and longer treatment regimen. It is less likely that differences in the techniques used to visualise cell morphology, Golgi-Cox in this case, GFP fluorescence microscopy in Jedynak's case, was any influence because, Li et al. (2005) also used Golgi-Cox staining and was able to detect changes in spine density on MSNs. It is also unlikely that differences in the time periods adopted in our study versus the 3 month delay employed by Jedynak influenced our lack of significant difference, because we failed to find any influence of Period overall. Nevertheless, our findings of changes to synaptic connectivity in the PL and IL regions following a low dose of methamphetamine (1mg/kg) and relatively brief treatment regimen (7 administrations over 14 days) is of note. As outlined in Chapter 1, methamphetamine is highly addictive and chronic abuse over long periods of time is common in this population.

Chapter 5

General Discussion

This thesis examined the influence of acute and chronic methamphetamine administration on S-R habits, contextual resolution of response conflict, and dendritic spine density on MSNs in the dorsal striatum and pyramidal cells in the mPFC. The experiments reported here had three broad aims. The first aim was to examine the influence of chronic methamphetamine and methamphetamine-paired contexts on the acquisition and performance of S-R habits using well-established behavioural paradigms. The second aim was to examine the influence of acute and chronic methamphetamine on animals' ability to use contextual information to resolve conflict and behave appropriately using a contemporary animal model of human executive function. The third aim was to examine whether chronic methamphetamine caused drug-dependent neural plasticity in key brain regions involved in S-R habits and executive function, and to ascertain whether different periods of abstinence have any effect on methamphetamine-dependent neural plasticity. This chapter will first summarise the empirical findings of this thesis and outline the theoretical implications of the findings with respect to Everitt and Robbins' model of addiction (Everitt & Robbins, 2005, 2016). The chapter will close with a discussion of the future directions and concluding remarks.

Summary of Experimental Results

The overall findings of the present thesis are that chronic exposure to methamphetamine speeds up the transition to S-R habit dominated behaviour in undertrained rats and impairs an animal's ability to use contextual information to

resolve response conflict. In contrast, acute methamphetamine administration did not impact resolution of response conflict, and limited (one drug-context pairing) exposure to methamphetamine prior to instrumental training did not impact early dominance of S-R habits This work also established that under some circumstances, that is, in "neutral" or saline-paired contexts or following a 6 week period of abstinence prior to commencing instrumental training, chronically exposed methamphetamine animals are capable of goal-directed behaviour. Finally, chronic methamphetamine causes changes in spine density on the dendrites of pyramidal cells in the PL and IL subregions of the mPFC. These findings will be discussed in more detail in the following sub-sections.

Chronic methamphetamine and methamphetamine-paired contexts lead to S-R habit dominance.

Experiment 1 investigated whether previous exposure to two distinct contexts, one paired with methamphetamine administration and another paired with saline control injections, leads to a context-dependent influence on an animal's ability to behave in a goal-directed manner. Here we found that exposure to a methamphetamine-paired context biases rats towards expression of S-R dominated behaviour at test in that context, but test in a matched saline-paired context permits expression of goal-directed behaviour in animals that received chronic methamphetamine exposure in the alternative context. In order to establish whether the S-R habits observed in Experiment 1 was purely caused by the methamphetamine-context, rather than an interplay between chronic methamphetamine exposure together with context, Experiment 2 assessed whether the same differential effects of methamphetamine- and saline-paired contexts was observed when animal's exposure to methamphetamine was limited to a single occasion. Experiment 2 found that limiting

methamphetamine exposure to a single occasion had no effect on the expression of goal-directed versus habitual performance at test in that context (relative to a control context). This would indicate either that the context-methamphetamine pairing alone was not responsible for the S-R habit bias observed in Experiment 1, or that this single treatment was insufficient to create strong contextual control, even though other studies have demonstrated this single exposure is sufficient to produce a robust conditioned place preference. Thus, the conservative conclusion is that an interaction between the methamphetamine-paired context (i.e. a psychological process) and chronic methamphetamine administration (i.e. a physical process) caused the promotion of the expression of habits found in Experiment 1.

Experiment 3 aimed to ascertain whether, like amphetamine, simple repeated methamphetamine exposure causes the behaviour of undertrained animals to come more rapidly under the control of S-R habits. The results from Experiment 3 revealed that following pre-training chronic exposure to methamphetamine, undertrained rats show rapid expression of S-R habits relative to control groups, mirroring the effect of rats exposed repeatedly to amphetamine (Nelson & Killcross, 2006, 2013). These findings support the notion of an interplay between context and repeated methamphetamine causing the effect found in Experiment 1. Also, findings from Experiment 3 suggest that chronic methamphetamine causes neurochemical or neurostructural changes that promote the rapid dominance of the expression of S-R habits. Experiment 4 aimed to examine whether the effect of chronic methamphetamine on the early development of S-R habits is observed long-term, by introducing a delay of 6 weeks between methamphetamine exposure and instrumental training. Experiment 4 showed that rats exposed chronically to methamphetamine 6 weeks prior to limited instrumental training remain goal-directed at test. This finding

stands in contrast to the results from Experiment 3, where animals repeatedly exposed to methamphetamine and trained one week later showed S-R habits at test. Thus, chronic methamphetamine causes rapid S-R habit dominance only when behaviours are acquired relatively soon after methamphetamine exposure. If behaviours are learned following a longer period of abstinence, goal-directed control dominates behaviour, as would be normal following the level of instrumental training given. Therefore, some recovery of normal function is observed over time. Experiment 4 also found that while there was a recovery of goal-directed behaviour following a 6-week period of abstinence, sensitisation (indexed by hyper-locomotion to a low-dose methamphetamine challenge) remained.

Experiment 5 aimed to demonstrate the dependence of this effect on pre-training treatment with methamphetamine more explicitly, This experiment investigated whether chronic methamphetamine causes instrumental responses that are learned before methamphetamine exposure are dominated by S-R habits when outcome devaluation and tests are conducted after drug exposure, and whether, in the same animals, a second response (leading to an alternative outcome) learned after chronic methamphetamine and tested in a second devaluation test, showed sensitivity to devaluation of the second outcome. The results from Experiment 5 provide strong evidence that chronic methamphetamine affects the relative acquisition of S-R habits and Action-Outcome associations, as opposed simply to affecting goal-directed performance. Therefore, methamphetamine-exposed animals are capable of goal-directed behaviour if instrumental training (but not test) occurred prior to methamphetamine exposure, but habits dominate when instrumental responses are acquired after chronic methamphetamine administration. Taken together, from the experiments in Chapter 1, we can conclude that chronic methamphetamine exposure

does not entirely abolish an animal's ability to be goal-directed. Chronic methamphetamine and exposure to methamphetamine-paired contexts facilitate a rapid shift in behavioural control that is biased towards the S-R habit system over the goal-directed system and this is likely due to a methamphetamine-induced imbalance in these systems. We can also conclude that chronically exposed methamphetamine-treated animals are capable of goal-directed behaviour following a longer period of abstinence. Therefore, goal-directed behaviour is not entirely abolished, S-R habits and goal-directed behaviours co-exist; it is the case that one system dominates the other under specific circumstances.

The experiments in Chapter 1 corroborates the findings of the existing literature. In line with the work of Furlong et al. (2015) we found that a methamphetamine-paired context disrupted goal-directed performance. However, in Experiment 2 where the methamphetamine-context pairing was limited to one occasion only, the behaviour of all animals was goal-directed, regardless of context. Thus, it seems most likely that an interplay between psychological (context) and physiological (drug-dependent neural changes) factors caused the rapid transition to S-R habit-dominance. In Experiment 3 we extended the findings of Nelson and Killcross' (2006; 2013) work with amphetamine. Here chronic methamphetamine exposure (without the influence of context) caused animals to demonstrate early dominance of S-R habits. This finding gave support to the hypothesis that methamphetamine-induced neural changes effect instrumental performance. Taking the findings of Experiments 1, 2 and 3 together with those of Furlong et al. (2015), it is likely that a context-modulated process comes into play when animals have previous experience with contexts that possess qualitatively different salient properties, and where animals are explicitly trained in a third, neutral

context, and the contextual discrimination is, in essence, an instrumental choice procedure.

Interestingly, Experiment 4 provided evidence of a dissociation between the classic sensitisation to psychostimulants and the rapid dominance of S-R habits. This experiment used exactly the same dosing regimen as our previous experiments with the exception of the period of abstinence between drug exposure and instrumental training. As discussed, this longer period of abstinence gave rise to the methamphetamine animals being goal-directed at test, in contrast to Experiment 1 and 3. However, despite being goal-directed at test, these animals still showed sensitisation to methamphetamine during the activity assay. Thus, the expression of goal-directed behaviour, appropriate given their low level of training, was not due to a failure of this dosing regimen to cause long-term sensitisation. This novel finding extends the current literature and suggests a dissociation between different drug-dependent effects and gives a strong indication that these processes are underpinned by separate neural systems.

Experiment 5 provides strong support to the existing evidence that drug-induced changes lead to a bias of the S-R habit system over the goal-directed system (Nelson & Killcross, 2006; 2013). Here, instrumental behaviours acquired before methamphetamine-induced disruption were goal-directed during the test which occurred after chronic methamphetamine administration had taken place. These same animals acquired a second instrumental response for a different outcome after methamphetamine-induced disruption. At test, the same animals showed rapid S-R habit dominance of this second instrumental response. Therefore, chronic methamphetamine animals are capable of goal-directed actions, as long as: the

instrumental response is learned prior to drug exposure, or learned after a long period of abstinence, or they occupy an environment that is familiar but not associated with methamphetamine.

The findings of Chapter 2 also align well with information garnered from human methamphetamine users. As discussed in Chapter 1, chronic methamphetamine users are known to become engrossed in bizarre stereotypies, referred to as "punding" or "tweaking". These behaviours take a variety of forms but are described as being involuntary, elicited by extraneous stimuli, and are difficult to disengage from, despite awareness of their useless, repetitive nature. Thus, these punding behaviours are akin to the automatic nature of habits which are also elicited by extraneous stimuli, rather than deliberate intention. Recall also from Chapter 1, the finding of Monterosso and colleagues (2005) who found that chronic methamphetamine users showed deficits in a response inhibition task. In Monterosso's work chronic methamphetamine users showed a specific impairment when they were required to inhibited a pre-potent response, which bares a qualitative resemblance to habits.

Chronic methamphetamine impairs performance in rodent model of executive function.

The rodent model of executive function developed by Haddon and Killcross (2005) provided an opportunity to empirically examine if methamphetamine negatively affects higher level executive function. Indeed, as discussed in Chapter 1, the answer to this question lacks consensus in the human literature. Haddon and Killcross' paradigm allowed us probe if animals chronically exposed to methamphetamine show deficits in resolving the conflict that arises in incongruent compound trials, similar to the classic human Stroop task, as outlined in Chapter 1. Thus, the aim of Experiment 6 was to

examine the effect of pre-training chronic methamphetamine administration on rat's ability to use contextual information to resolve response conflict arising from ambiguous audiovisual compound cues. The results of Experiment 6 provide the first evidence of chronic methamphetamine disrupting an animal's ability to behave appropriately within specific contexts when faced with conflicting information using the paradigm developed by Haddon and Killcross (2005).

The aim of Experiment 7 was to examine whether methamphetamine, like amphetamine (Reichelt et. al., 2013), administered at the doses of 1mg/kg and 2mg/kg but acutely, prior to test, had any impact on performance of this task of executive function. However, Experiment 7 did not find any significant effect of methamphetamine administration prior to test (at any dose) on context appropriate responding during incongruent compound trials (and nor on performance of the underlying conditional discrimination). Thus, based on the deficit Reichelt and colleagues found with acute amphetamine, the results of Experiment 7 provide another example of the differences between these two psychostimulants, as suggested by the work of Shoblock et al. (Shoblock, Maisonneuve, et al., 2003; Shoblock, Sullivan, et al., 2003).

Subsequently, in Experiment 8 we aimed to examine whether methamphetamine administered immediately prior to test was able to produce any disruption on performance in this task. Therefore, we adopted the asymmetric biconditional training procedure used by Haddon and Killcross (2006) in which one discrimination is "overtrained" in comparison to the other which is "undertrained". Differing the degrees of training in this manner leads to a test situation where the incongruent compound cues consist of one "undertrained" element and one "overtrained" element. This

manipulation mirrors the Human Stroop where word reading is more familiar than colour naming, which creates the particular pattern of response conflict when the task is to name the colour. As was the case with Experiment 7, we did not find any indication that methamphetamine administered prior to test caused deficits in performance on this version of Haddon and Killcross' task. In the context where the discrimination was "overtrained" no differences in performance were found when rats were tested under methamphetamine or saline. In the context where the discrimination was "undertrained" acute methamphetamine prior to test, again the drug did not appear to have any effect; regardless of having methamphetamine or saline prior to test, no differences in performance were found.

The findings of our Chapter 3 experiments provide strong evidence that there is a clear difference between the effects of acute and chronic methamphetamine administration on executive function. In Experiments 7 and 8 we failed to find any significant influence of acute methamphetamine on animals' ability to use contextual information as a guiding principle for appropriate responding when they were presented with ambiguous compound cues. However, our findings in Experiment 6 were remarkably different to those of Experiment 7 and 8. Here we found that rats chronically exposed to methamphetamine, under an identical dosing regimen to those in Chapter 2 experiments, demonstrated significantly impaired performance in this task. Animals under acute methamphetamine at test in Experiments 7 and 8 showed no deficits in executive function but chronically exposed animals in Experiment 6 were unable to resolve the conflict brought on by the incongruent stimulus compounds and behave in a context appropriate manner, despite acquiring the biconditional discrimination as well as saline controls and accurate responding to single element test stimuli. This the first evidence of chronic methamphetamine disrupting animals'

performance in a task that depends on intact executive functioning. Our Chapter 3 findings provide empirical support to existing human literature. Several investigations of chronic methamphetamine users performance in the classic Stroop task have found deficits in those who are current users, or those in early recovery (Farhadian et al., 2017; Han et al., 2008; Henry et al., 2011; Hosak et al., 2012; Monterosso et al., 2005; Nestor et al., 2011; Salo et al., 2013; Salo, Nordahl, et al., 2009; Salo et al., 2005; Simon et al., 2000b; Simon, Domier, et al., 2001; Tolliver et al., 2012).

Chronic methamphetamine causes long term changes to dendritic spine density in mPFC.

As outlined in Chapter 4, there is strong evidence of drug-dependent changes in spine density in the DLS, DMS and mPFC (Jedynak et al., 2007; Li et al., 2003; Robinson & Kolb, 1997, 1999). Given the involvement of these areas in S-R habits and executive function, the aim of Experiment 9 was to employ the same methamphetamine dosing regimens that were used in the Chapter 2 Experiments 4 and 5 and Chapter 3 Experiment 6, to provide a histological assay of spine density of rats chronically exposed to methamphetamine. Examining the spine density of rats that had undergone the same dosing regimen and withdrawal periods as the behavioural experiments 4, 5 and 6, enabled us to draw analogies between the observed behaviours to changes to spine density found in the DLS, DMS, PL and IL in Experiment 9.

The results for Experiment 9 were somewhat surprising for the dorsal striatum. For the DLS, despite a trend of methamphetamine causing an increase in spine density on MSNs together with a significant Drug x Region interaction, simple effects of the interaction failed to find a significant difference between methamphetamine- and saline-treated animals. For the DMS, no significant differences in spine density of MSNs

were found between methamphetamine-treated and saline, control animals. However, we did find significant differences between chronic methamphetamine- and saline-treated animal's spine densities on pyramidal cells of mPFC. Here we found that chronic methamphetamine exposure caused a significant decrease in spine density on PL pyramidal cells and a significant increase in spine density in the IL cells. To our knowledge, this is the first empirical finding of distinct changes in cell morphology in the PL and IL following chronic methamphetamine administration.

The findings of Experiment 9 provide mixed support for the existing literature on drug-dependent changes to spine density in the dorsal striatum and mPFC. In contrast to Jedynak's (2007) work where increases in spine density were found on MSNs in the DLS and decrease in spine density on MSNs in the DMS were found, we did not find any significant differences between chronic methamphetamine and saline controls animals. There are two possible explanations for the different findings. First, Jedynak and colleagues administered a much higher dose compared to our dosing regimen. Second, the visualisation techniques used to examine cell morphology differed. Nevertheless, we came close to replicating the significant increase in spine density on MSNs that Jedynak found and this could well be due to the small sample size acquired for each region (N= 6).

However, it is unlikely that the lack of detecting significant changes in cell morphology on MSNs in the DLS and DMS is only due to analysing six cells per region. Work by Robinson and Kolb (1999) and others (Crombag et. al., 2005) who have used the same Golgi-Cox staining procedure also analyse 5-6 cells per region of interest. It is more likely that our low dose of 1mg/kg compared to 3mg/kg of amphetamine and 15mg/kg of cocaine was not high enough to observe such changes to cell morphology in

the striatum. Another possibility is differences in the effects of methamphetamine compared to amphetamine and cocaine. Lastly, regional specificity may explain the lack of changes detected. Robinson and Kolb analysed cells in the ventral striatum whereas our focus was on cells in the dorsal striatum because of this regions involvement in S-R habits and the morphological changes to cells in this region found by Jedynak following chronic methamphetamine administration. Therefore, the most likely explanation for the lack of significant differences being found on MSNs in the DMS and DLS is likely due to a combination of differences in doses and visualisation techniques used in these experiments compared to those used by Jedynak and colleagues.

However, more in line with previous work on drug-dependent changes to spine density (Crombag et al., 2005; Li et al., 2003; Robinson & Kolb, 1997, 1999), we found evidence of drug-dependent changes in spine density of pyramidal cells in the mPFC. As discussed in Chapter 4, Robinson and Kolb's (1997) work with chronically exposed amphetamine animals revealed that, compared to saline controls, these animals showed increased spine density in the PFC. Notably, in our work, we divided the PFC into subregions; PL and IL because of strong empirical evidence supporting the functional heterogeneity of these subregions. Examining the PFC as distinct subregions in this manner revealed results that, although provide basic support for Robinson and Kolb's (1997) finding of drug-induced changes cell morphology, extend the findings of this work by providing a dissociation between recognised subregions of the PFC. Like Robinson and Kolb we detected significant increases in spine density of pyramidal cells in the PFC but this effect was restricted to cells in the IL. For pyramidal cells in the PL, we found the opposite pattern. In the PL, methamphetamine-treated animals had significantly lower spine density on pyramidal cells compared to saline-treated controls.

Although no behavioural assays were performed with these animals, the dosing regimen and periods of abstinence in this experiment mirrored that of Experiments 4, 5 and 6. Thus, the animals examined in Experiment 9 provide an example of the morphological state of those who had undergone behavioural assays in Chapter 2 and Chapter 3 following chronic methamphetamine treatment. Notably, none of the analyses performed in Experiment 9 revealed a significant effect of Period, that is, the length of time between the last day of methamphetamine exposure and tests relative to Experiments 4 and 5, despite the behaviour of Experiment 4 animals being strikingly different to Experiment 5 animals. Experiment 4 animals did not show an early transition to S-R habits despite findings of Experiment 9 indicating enduring changes to neural morphology. Experiment 5. This implies that perhaps neurochemical adaptations occur over time in order to restore balance to the system, despite enduring changes to structure of the systems. Such restoration of balance may allow for a return to normal function.

Theoretical implications

As discussed in Chapter 1, Everitt and Robbins' (Everitt & Robbins, 2005, 2016) model of addiction posits that drug addiction is an aberrant form of S-R habit learning caused by drug-induced neural plasticity on neural pathways involved in habits and goal-directed actions. Previous research in behavioural neuroscience has demonstrated that goal-directed actions are mapped onto distinct brain areas, namely, the DMS and PL. There is also a strong body of literature in the field that maps S-R habits onto the DLS and IL. Everitt and Robbins' model has good face validity, whereby it accounts for the characteristic of addiction that begins with the initial voluntary goal-directed pursuit of drugs which over time becomes increasingly more difficult to control and

eventually drug use dominates an individual's life, despite their goal to get, or to stay, abstinent. Evidently, the pursuit of drugs is habitual and executive control over the behaviour is diminished. The model also accounts for the ability of Pavlovian drug-paired CSs, such as drug-paired contexts, to exert influence on instrumental behaviour such as the pursuit of drugs.

The empirical findings of this thesis provide strong support for Everitt and Robbins' model of addiction and show that the paradigms used can provide the potential for translation to human populations. Firstly, in Chapter 1 we found that chronic exposure to methamphetamine caused a rapid shift in behavioural control to S-R habits. Second, we demonstrated that this shift to S-R dominance occurs only for behaviours learned after exposure to methamphetamine; behaviour acquired before exposure to the drug remained goal-directed (as would be expected following limited instrumental training); behaviours acquired after methamphetamine-exposure showed a rapid transition to control by habitual systems. Third, we demonstrated that a methamphetamine-paired context (a Pavlovian CS) was able to influence instrumental behaviour compared to behaviour in a saline-paired or "neutral" context. This effect was likely to be due to a combination of psychological and physiological mechanisms. Fourth, we found direct empirical support for diminished executive function, whereby animals chronically exposed to methamphetamine were impaired in a rodent model of the human Stroop task. Fifth, no effect of acute methamphetamine administration was found on any of the experiments in this thesis which supports the tenet of progressive change in Everitt and Robbins' model as well as data from individuals currently using methamphetamine (Simon et al., 2000b; van der Plas et al., 2009). Sixth, the results from Experiment 9 support the idea that drug-dependent neural plasticity may be an underlying mechanism of addiction as an aberrant type of habit learning, although there

was also evidence of dissociations between persistent neural changes and functional recovery (Experiment 4).

We found evidence that may extend Everitt and Robbins' model. Specifically, in Experiment 4, we found that instrumental behaviour acquired following a 6-week period of abstinence from methamphetamine was not controlled by S-R habits. Indeed, the methamphetamine-treated animals in this experiment showed a recovery of normal goal-directed control. However, in Experiment 9 we failed to find a significant effect of period of drug abstinence. That is, regardless of the length of time between the last drug administration and euthanasia, the same persistent drug-dependent changes to spine density on pyramidal cells in the PL and IL were found. This is particularly interesting because of the different behaviours observed in animals who had one week of methamphetamine abstinence (S-R habits dominate) and those who had 6 weeks of abstinence (goal-directed control). Thus, it seems likely that drug-dependent structural changes do occur and remain in the long-term, but behaviour nevertheless can return to normal. Therefore, despite these structural changes occurring on key neural pathways, functional adaptations which restore balance are likely to take place in these systems that allow behaviour to return to normal overtime. This hypothesis provides an exciting opportunity for future empirical investigations.

Limitations

For a number of reasons, it is difficult to compare methamphetamine administered in the laboratory to rodents to methamphetamine administered to humans in the real world with absolute certainty. Firstly, in order to increase financial gain to street dealers, methamphetamine that is sold on the black market is often adulterated (Irvine & Chin, 2009), whereas the methamphetamine we use in the

laboratory is in its pure, unadulterated form. Thus, a dose of 1mg/kg of adulterated street methamphetamine vs. 1mg/kg of unadulterated laboratory methamphetamine would differ in potency. Secondly, the route of methamphetamine administration can impact bioavailability. As discussed in Chapter 1, in humans, methamphetamine is most commonly smoked or intravenously injected (Cunningham et al., 2008). Both routes of administration produce rapid and intense effects. In our experiments we used intraperitoneal injections, as is commonly used in rodent models and in particular, the key background literature to this thesis (Nelson & Killcross, 2006; Furlong et. al., 2015). Third, the typical human hit varies considerably, particularly once drug-use is well-established and tolerance develops (Melega, Cho, Harvey, & Lacan, 2007). One of the main aims of the thesis was to model early-stage methamphetamine use, hence we used the same low dose for a brief period of time as Furlong and colleagues. It is important to note that a single dose of 40-60mg is sufficient to cause a significant rush and a 30mg dose is associated with peak plasma levels of 50ng/ml (Perez-Reyes et al., 1991). Considering these factors it is likely that 1mg/kg is a common starting dose for humans in the early stages of their drug use and therefore the dose used in our experiments is likely a valid model.

Another limitation inherent in animal models is matching the way a substance is used in the real world by humans (Ahmed, 2010) and the way that the pharmacokinetic properties of drugs are differ across species (Cho et al., 2001). In our experiments methamphetamine was administered to rodents by the experimenter. This is in contrast to human use where methamphetamine is self-administered. Thus, whether or not methamphetamine self-administration results in more profound executive deficits is an interesting line of enquiry for future research. Corbit et al (2012) found that extended access to alcohol lead to S-R habit dominated performance and this was also

the case when rats were self-administering nicotine (Clemens et al., 2014). In the human literature, Sjoerds and colleagues found that alcoholics were biased toward S-R habit learning (Sjoerds et al., 2013). However, in terms of neural morphology, Crombag and colleagues did not find any difference between self-administered vs. experimenter administered amphetamine on spine density of pyramidal cells in the rodent PFC (Crombag, 2005). Thus, one would expect deficits in executive function to be observed if animals were self-administering methamphetamine as they were with the experimenter administration procedure used here. However, whether greater deficits would be observed following self-administration compared to the deficits observed in this thesis is yet to be determined.

Future Directions

Future empirical investigations can build on and extend these findings in several ways. Firstly, given the results of Experiment 4 where methamphetamine-treated animals showed a return to goal-directed behaviour following a 6-week abstinence from methamphetamine prior to instrumental learning and the finding from Experiment 1 where sensitised animals were S-R habit biased in the methamphetamine-paired context but goal-directed in the saline-paired context, it would be interesting to use the same context-pairing procedure but introduce a 6-week delay between drug exposure and instrumental training. This would allow one to determine whether the context influence still exists when we know that animals should have recovered goal-directed performance following this period of time. Second, it would be interesting to examine whether introducing a 6-week withdrawal period following chronic methamphetamine exposure would improve an animal's ability to perform in a context-appropriate manner on incongruent compound trials in Haddon and Killcross' conflict resolution

task. Third, although not significant, given the trend toward a significant deficit in correct responding on incongruent compound trials at test following the 2mg/kg acute methamphetamine dose, warrants further examination. Future research could re-investigate this acute dose using the standard and undertrained/overtrained procedure of the rodent Stroop task. It may be the case that acute administration at the higher dose would negatively impact executive function as probed in this task. Forth, from the perspective of behavioural neuroscience further empirical investigations could also build on the existing findings. Investigations could assess how boosting PL function or turning off activity within the IL (for example, using optogenetics) would impact S-R habits and conflict resolution using the same behavioural paradigms, and following chronic methamphetamine treatment. Fifth, given that we were unable to detect changes to cell morphology on MSNs in the DLS and DMS in Experiment 9 yet experiments in Chapter 2 provided strong evidence of S-R habit dominance following chronic methamphetamine administration, future research could use microdialysis and single unit recordings to examine whether neurochemical or patterns of activity can be detected in the DLS and DMS in the absence of changes to cell morphology. Finally, in order to directly probe the neurochemical mechanisms underlying the cognitive control deficits observed following chronic methamphetamine, future research could employ microdialysis or single unit recording of pyramidal cells within the PL and IL.

Concluding remarks

At present, chronic methamphetamine use is a significant global health concern. Widespread use of methamphetamine continues to rise, placing an ever-increasing burden on the economy, as well as public health, social, and judicial systems. Considering methamphetamine's high addictive potential, it is vitally important to gain

a thorough understanding of this drug's acute and chronic neuropsychological effects, including the mechanisms that promote its addictive potential, as well as independent neuropsychological effects that impact everyday functioning. Currently, critical consensus on whether or not chronic methamphetamine use has detrimental effects on cognition and executive function is lacking. The aim of this thesis was to address this contention and provide clarity on the issue. Using well-established contemporary behavioural paradigms to model human cognitive function in laboratory rodents has allowed direct causal determinations to be made, without the confounds inherent in research on human methamphetamine users. Consequently, we have been able to translate our findings to existing literature on the human methamphetamine using population.

The empirical findings of this thesis make a significant novel contribution to our understanding of the neuropsychological effects of methamphetamine. We have shown that when methamphetamine is administered acutely, it has no significant effect on S-R habits, or executive function. However, we consistently found that animals chronically exposed to methamphetamine demonstrate a range of behavioural abnormities: a rapid transition to S-R habits for behaviours acquired following drug exposure; a persistent capacity for goal-directed behaviour whilst in the presence of non-drug paired contexts; diminished executive function indexed by a deficit in the ability to use contextual information to resolve response conflict; and, plastic changes to cell morphology in brain areas involved in S-R habits and executive function, implying changes in the dominance of different neural systems supporting goal-directed and stimulus-driven behaviours. Promisingly, we have also provided good evidence that following periods of abstinence, we see a return to normal, expected patterns of behaviour, despite the persistence of structural changes to the brain. These findings underscore the

importance of the acute phase of methamphetamine abstinence. The more we can support individuals through the fragile stage of early abstinence, and encourage them to avoid contexts associated with methamphetamine use, the better chance they have of reducing the negative impact their chronic use of methamphetamine will have on their lives in the future.

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