

**CONDITIONED COGNITIVE AND MOOD EFFECTS OF CAFFEINE
IN HUMANS**

By

ANGELA SUZANNE ATTWOOD

A thesis submitted to
The University of Birmingham
for the degree of
DOCTOR OF PHILOSOPHY

School of Psychology
The University of Birmingham
February 2007

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

ABSTRACT

Findings from animal studies suggest that stimuli present during the administration of psychostimulant drugs can acquire the ability to elicit drug-like conditioned increases in locomotor activity. Human pharmacological drug-like conditioned responses are less well researched. There is evidence suggesting that stimuli paired with psychostimulant administration can elicit drug-like physiological and subjective changes, as well as increases in drug craving. However, to date, no study has explicitly examined whether drug-induced facilitation of cognitive performance can be conditioned to drug-associated stimuli. The studies in the present thesis set out to test this and examine the extent to which the pattern of results conformed to the principles of Pavlovian classical conditioning. Caffeine was used as a model psychostimulant drug, due to its well-reported ability to facilitate various aspects of cognitive performance. However, due to difficulties obtaining reliable effects of caffeine, the factors that may influence the effects of caffeine in a caffeine consumer sample were also investigated and reviewed. These factors included dose, expectancy, absorption interval, type of task, withdrawal and level of habitual consumption. It was concluded that caffeine can enhance cognitive performance, however these effects are inconsistent and may be influenced by individual differences. In addition, findings from a screening procedure indicated that responses to caffeine differ even within an overnight-deprived caffeine consumer population, an effect that appears to be dependent on the level of habitual caffeine intake. Due to such individual variation in the responses to caffeine, the conditioned effects were examined using a differential (i.e. within-subjects) conditioning procedure in which one set of environmental stimuli were paired with caffeine, and another set were

paired with placebo. When subsequently tested free of drug, there were no differences in performance or mood responses at the conditioning test. However, there was evidence of caffeine facilitation on performance and mood during early conditioning trials that was lost on later conditioning trials due to a systematic improvement in the placebo condition. It was argued that this may be due to a conditioned response being acquired in the caffeine paired context which generalised to the placebo paired context. To test this hypothesis a second differential conditioning paradigm was conducted with fewer trials to establish whether evidence of a conditioned response could be observed in the caffeine-paired context during a placebo challenge. Evidence of a conditioned facilitation of reaction time was found, suggesting that the environment in which caffeine is ingested can acquire drug-like facilitations of cognitive performance.

Acknowledgements

Firstly, I would like to thank my supervisors Dr Philip Terry and Dr Suzanne Higgs. Not only are you so good at what you do, but you are also two of the nicest people that I could have wished to learn from. I really cannot thank you enough for your constant support, advice and good humour. It has been a pleasure working with you.

Thanks also to Dave Barber for help with the research and capsules! To the “guys in the office” – Maria Michail, Becki Jenks, Jon Catling, Claire Tweed, Steph Walker, Claire Goodchild, Jon Grey and Kulbir Birak - for making coming in to work everyday all the more enjoyable.

I would also like to thank my friends and family for their support - with special thanks to: my nan; my godchildren and co. – Jean-Paul, James, Christopher, Ellie, Tommy and Millie who never held it against me that they received their Christmas presents at Easter and their birthday presents at Halloween because Angie was “at school a long way away in Birmingham”; my best friends Jackie, Dee, Shell and Maria who remained my best friends despite not seeing me for three years, and to Gaz for being there for three years. Finally, I would like to thank my mum, without whose endless support, time and love I would not have been able to get here. This is as much her hard work as it is mine. I love you so very much mum – thank you... for everything.

TABLE OF CONTENTS

CHAPTER 1: General Introduction.....	1
CHAPTER 2: Caffeine: Pharmacology, Physiological and Behavioural Effects.....	6
2.1 Introduction.....	6
2.2 Pharmacology of caffeine	7
2.2.1 Pharmacokinetics	7
2.2.2 Mechanisms of action	7
2.3 Physiological effects of caffeine.....	11
2.3.1 Physiological arousal	11
2.3.2 Effects on sleep	12
2.3.3 Cardiovascular effects.....	12
2.4 Performance and subjective effects	13
2.4.1 Psychomotor performance	14
2.4.2 Cognitive performance.....	17
2.4.3 Performance and arousal.....	20
2.4.4 Subjective effects	21
2.5 Chronic effects of caffeine	22
2.6 Caffeine withdrawal and the withdrawal-reversal hypothesis	25
2.7 Caffeine dependence and reinforcement.....	28
2.8 Summary	30
CHAPTER 3: Classical Conditioning and Conditioned Drug Effects.....	33
3.1 Introduction.....	33
3.2 Associative learning and conditioning theory.....	34
3.2.1 Classical conditioning.....	35
3.2.2 Factors affecting conditioning	36
3.3 Conditioned drug effects.....	45
3.3.1 Drug-like conditioned responses.....	46
3.3.2 Drug-opposite conditioned responses	48
3.3.3 Predicting the direction of the CR	51
3.4 Establishing a role of classical conditioning.....	54
3.4.1 Methodological considerations of drug-conditioning studies.....	54
3.4.2 Evaluating the role of conditioning in supposed conditioned drug effects	57
3.5 Conditioned effects of caffeine	61
3.6 Aims and objectives	62
CHAPTER 4: General Methods.....	64
4.1 Participants.....	64
4.2 Inclusion and exclusion criteria	65
4.3 Caffeine administration.....	66
4.4 Measures	66
4.5 Post-study questionnaires.....	67
4.6 Data analysis	68
CHAPTER 5: Experiment 1: Conditioned Performance, Subjective and Cardiovascular Effects of Caffeine	70
5.1 Introduction.....	70
5.2 Method	75
5.3 Results.....	80
5.4 Discussion	86

CHAPTER 6: Experiment 2: Effects of Caffeine Information/Expectancy on the Effects of Caffeine	91
6.1 Introduction.....	91
6.2 Method	94
6.3 Results.....	97
6.4 Discussion	100
CHAPTER 7: Experiment 3: Effects of Two Doses of Caffeine on Selected Tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB)	103
7.1 Introduction.....	103
7.2 Method	106
7.3 Results.....	113
7.4 Discussion	117
CHAPTER 8: Experiment 4: Conditioned Effects of 400 mg Caffeine on CANTAB Task Performance, and Subjective and Cardiovascular Measures	121
8.1 Introduction.....	121
8.2 Method	123
8.3 Results.....	126
8.4 Discussion	133
CHAPTER 9: Experiment 5: Conditioned Performance and Subjective Effects of Caffeine Using a Differential Conditioning Paradigm	136
9.1 Introduction.....	136
9.2 Method	139
9.3 Results.....	144
9.4 Discussion	156
CHAPTER 10: Experiment 6: Conditioned Performance and Subjective Effects of Caffeine with Two Conditioning Trials.....	163
10.1 Introduction.....	163
10.2 Method	164
10.3 Results.....	166
10.4 Discussion	175
CHAPTER 11: Experiment 7: Differential Responsiveness to Caffeine and Perceived Effects of Caffeine in Moderate and High Caffeine Consumers	182
11.1 Introduction.....	182
11.2 Method	184
11.3 Results.....	186
11.4 Discussion	192
CHAPTER 12: General Discussion.....	197
12.1 Overview of conditioning findings.....	197
12.2 Overview of caffeine findings	200
12.3 Limitations and future research	209
12.4 Summary	214
REFERENCES.....	217
APPENDIX – Publications and Presentations	279

List of Tables

Table 5.1 Test schedule.....	77
Table 5.2 Participant characteristics	80
Table 5.3 Mean group changes on mood factors at the conditioning trials and test...	84
Table 5.4 Substances the unpaired group believed that they had received during the conditioning trials	85
Table 5.5 Substances the paired group believed that they had received during the conditioning trials	85
Table 6.1 Participant characteristics	97
Table 6.2 Mean blocked times on simple reaction and semantic verification tasks ...	98
Table 6.3 Substances the groups believed that they had received during the placebo and caffeine sessions.....	99
Table 7.1 Test schedule.....	111
Table 7.2 Mean block reaction times on RVIP after 3 doses of caffeine	116
Table 7.3 Mean changes on mood factors after 3 doses of caffeine	117
Table 8.1 Test schedule.....	124
Table 8.2 Participant characteristics	127
Table 8.3 Mean reaction and movement durations for simple reaction time task	127
Table 8.4 Mean group changes on mood factors at the conditioning trials and test.	131
Table 8.5 Substances the unpaired group believed that they had received during the conditioning trials	132
Table 8.6 Substances the paired group believed that they had received during the conditioning trials	132
Table 8.7 Number of participants who experienced caffeine withdrawal	133
Table 9.1 Test schedule.....	142
Table 9.2 Mean group changes in sleepiness and positive mood at the conditioning trials and test	153
Table 9.3 Substances participants believed that they had received in CS- context ..	155
Table 9.4 Substances participants believed that they had received in CS+ context .	155
Table 9.5 Number of participants who experienced caffeine withdrawal	156
Table 10.1 Mean changes on mood factors during the conditioning trials and test..	173
Table 10.2 Substances participants believed that they had received in CS- context	174
Table 10.3 Substances participants believed that they had received in CS+ context	174
Table 10.4 Number of participants who experienced caffeine withdrawal	175
Table 11.1 Participant characteristics	187
Table 11.2 Subjective effects of caffeine in moderate and high consumers.....	192

List of Figures

Figure 5.1 Simple reaction time in caffeine-paired and -unpaired groups 81

Figure 5.2 Choice reaction time in caffeine-paired and -unpaired groups 82

Figure 7.1 Simple reaction time performance after 3 doses of caffeine 113

Figure 7.2 Choice reaction time performance after 3 doses of caffeine 114

Figure 7.3 Match-to-sample visual search performance after 3 doses of caffeine ... 115

Figure 8.1 Blocked choice reaction time reaction durations in caffeine-paired and –
unpaired groups..... 128

Figure 9.1 Simple reaction time reaction durations in the CS+ and CS- contexts in the
conditioning trials and conditioning tests 144

Figure 9.2 Simple reaction time movement durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 145

Figure 9.3 Choice reaction time reaction durations in the CS+ and CS- contexts in the
conditioning trials and conditioning tests 147

Figure 9.4 Choice reaction time movement durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 148

Figure 9.5 Match-to-sample visual search reaction durations in the CS+ and CS-
contexts in the conditioning trials and conditioning tests..... 149

Figure 9.6 Number of RVIP errors in the CS+ and CS- contexts in the conditioning
trials and conditioning tests 151

Figure 9.7 Changes in ratings of sleepiness, positive mood, mental clarity and mental
repose in the CS+ and CS- contexts..... 153

Figure 9.8 Changes in ratings of mental clarity and headache in the CS+ and CS-
contexts on the test of conditioning 154

Figure 10.1 Simple reaction time reaction durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 167

Figure 10.2 Simple reaction time movement durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 168

Figure 10.3 Choice reaction time reaction durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 169

Figure 10.4 Choice reaction time movement durations in the CS+ and CS- contexts in
the conditioning trials and conditioning tests 170

Figure 11.1 Simple reaction time performance in moderate and high caffeine
consumers after caffeine and placebo 188

Figure 11.2 Choice reaction time performance in moderate and high caffeine
consumers after caffeine and placebo 189

Figure 11.3 Changes in ratings of tense mood, anxiety, mental repose and sleepiness
in moderate and high consumers after caffeine and placebo 191

CHAPTER 1:

GENERAL INTRODUCTION

Psychopharmacological research and anecdotal evidence suggest that stimuli associated with drug-taking can elicit drug-related reactions when presented in the absence of the drug. These drug-related responses can be similar to those of the original drug effect (Tilson & Rech 1973; Childress, McLellan & O'Brien 1986; Adams, Careri, Efferen & Rotrosen 2000) or opposite to it (Poulos, Hinson & Siegel 1981; Poulos & Cappell 1991; Ehrman, Ternes, O'Brien & McLellan 1992; Cepeda-Benito & Tiffany 1995; Siegel 1999). For example, stimuli associated with amphetamine administration produce amphetamine-like increases in locomotor behaviour in rats (Tirelli & Terry 1998); and morphine-paired stimuli can produce drug-opposite hypothermia when presented alone (Siegel 1975a). It is commonly assumed that drug-associated stimuli acquire the ability to elicit drug-related responses through the processes of Pavlovian conditioning, and that these responses may contribute to the maintenance of drug use and to relapse after drug abstinence (e.g. Carter & Tiffany 1999; Siegel 1999; See 2002; Franken 2003).

Many commonly-used drugs produce benefits on mood and cognitive performance which are likely to contribute to their rewarding potential (Stolerman 1993) and thus play a role in their continued use. Despite some evidence of conditioned compensatory effects of alcohol-induced cognitive impairment (Shapiro & Nathan 1986), no study to date has explicitly examined whether drug-related performance benefits can be conditioned to stimuli present at the time of drug ingestion. Previous

research has focused predominantly on the conditioning of physiological responses (Muntaner et al. 1989) and subjective reports of craving (Foltin & Haney 2000). Cue reactivity studies, for example, have shown increased reports of drug craving in the presence of drug-associated stimuli, which presumably would increase the likelihood of drug-use in the presence of such cues (Rohsenow, Niaura, Childress, Abrams & Monti 1990; Childress et al. 1993)

In addition, the majority of previous studies have measured the responses to cues already associated with drug administration prior to test, such as videos of drugs being prepared and drug paraphernalia (Teasdale 1973; Sideroff & Jarvik 1980; Ehrman, Robbins, Childress & O'Brien 1992). It is assumed that prior exposure to these cues in the presence of drug administration will have led to acquisition of CS-US association. However, using such an approach means that it is impossible to control for prior drug-related cue exposure. For example, there may be differences in the number of drug-stimuli pairings; the number of stimuli-alone presentations (e.g. a beer drinker may sometimes opt for alcohol-free lager); drug dose; and post-ingestive experiences of drug use (i.e. some drinkers may consume excessive amounts of alcohol so that consumption is associated with post-ingestive hangovers); all of which could affect the degree and/or nature of a conditioned response (see section 3.2).

In addition, specific drug-vehicle stimuli such as a cigarette or a beverage have often been the cues of choice in cue reactivity studies (Newlin 1986; Ehrman, Robbins et al. 1992; Glautier & Drummond 1994; Mucha, Pauli & Angrilli 1998; Mahler & de Wit 2005). However, the results from animal studies (Tirelli & Terry 1998; Tirelli &

Heidebreder 1999; Crombag et al. 2001) and more recent studies of human participants (Conklin 2006) imply that the ability of drug-related stimuli to gain control over drug-taking behaviour extends beyond the proximal cues traditionally associated with drug administration. Specifically, evidence suggests that the environmental context in which the drug is administered can enter into a direct association with the drug, and acquire the ability to elicit drug-related responses (Shapiro & Nathan 1986; Conklin 2006). The role of compound contextual cues in mediating drug effects is particularly important as they are more representative of real-life situations, in which a multitude of cues are present during drug exposure.

Therefore, the first aim of the present thesis was to examine whether drug-induced facilitation of cognitive performance and mood could be conditioned to a previously neutral environment paired with a stimulant drug. A pilot study was conducted prior to this Ph.D. project (Attwood, Terry & Higgs 2004) that paired a caffeinated, novel-tasting beverage with a particular environment to test whether the environment could later elicit caffeine-like improvements in cognitive and psychomotor performance when presented alone. In this study, two groups of participants attended four conditioning trials, at which one group received a caffeinated juice beverage in a novel context (paired group), and the other group received a similar beverage containing placebo (unpaired group) in the same context. To test for a conditioned response, both groups returned for a final session and received the placebo beverage in the test cubicle. A significant effect of caffeine was obtained on the conditioning trials, whereby the paired group demonstrated faster reaction times across all four conditioning trials in comparison to the unpaired group. Importantly, this significant

group difference was also evident at the conditioning test when both groups received placebo, indicating that the paired group showed caffeine-like conditioned performance effects. However, this study was conducted single-blind and baseline measures were not taken. Therefore, experimenter effects, or the possibility that the groups differed significantly in performance prior to the experimental sessions, could not be ruled out. Therefore, the initial experiment of this thesis replicated this pilot study using an improved methodology.

The second aim of the current thesis was to investigate the role of classical conditioning in the effects observed in the pilot study. Some studies have demonstrated effects that do not support a classical conditioning explanation for the responses produced by drug-paired stimuli. Therefore, alternative explanations have been offered to account for the findings such as pseudoconditioning effects (Izquierdo 1974; Di Giusto & Bond 1977; Tomie, Burger, Di Poce & Pohorecky 2004), sensitisation effects (Tirelli, Michel & Brabant 2005) or drug-induced anti-habituation effects in the drug-paired environment (Gold, Swedlow & Koob 1988; Damianopoulos & Carey 1992). Classical conditioning theory posits testable predictions about how the conditioned response should be affected by specific methodological manipulations. For example, a conditioned response is expected to: extinguish following repeated presentation of the CS without the US; be disrupted by pre-exposure to the CS; and generalise to similar CSs (Pavlov 1927). A series of studies is planned to explore the extent to which the conditioned response conforms to the principles of classical conditioning. Establishing whether these effects are due to conditioning will have important implications for the understanding of drug-taking

behaviour and the mechanisms by which the environment can come to control drug-related behaviour, and for conditioning-based addiction therapies, whose efficacy relies on the validity of classical conditioning principles such as extinction.

The drug chosen was caffeine, as it is widely regarded as having stimulant properties that can facilitate various psychomotor and cognitive functions (see Smith 2002; Lieberman 2003 and Lorist & Tops 2003 for reviews), particularly in acutely-deprived caffeine consumers (Zwyghuizen-Doorenbos et al. 1990; Frewer & Lader 1991; Hasenfratz & Bättig 1994; Smith, Maben & Brockman 1994; Kenemans & Lorist 1995; Warburton 1995; Durlach 1998; Warburton, Bersellini & Sweeney 2001; Yeomans, Ripley, Davies, Rusted & Rogers 2002). In addition, there is evidence that caffeine-associated cues can act as conditioned stimuli. For example, taste-preference learning studies have shown that novel flavours repeatedly paired with caffeine can acquire reinforcing properties, so that when tested free of caffeine, participants rate these flavours as more pleasant than novel flavours paired with placebo (Yeomans, Jackson, Lee, Nesic & Durlach 2000). However, to date, there has been no direct investigation of whether the performance enhancing effects of caffeine can be conditioned to the context in which the drug is taken. The next chapter describes the behavioural effects of caffeine, with particular emphasis on its cognitive, psychomotor, and mood effects in order to highlight its suitability as a drug US for the current series of experiments. In addition, aspects of caffeine's pharmacology and its dependence profile are reviewed, as they have important implications for experimental design.

CHAPTER 2:

CAFFEINE: PHARMACOLOGY, PHYSIOLOGICAL AND BEHAVIOURAL EFFECTS

2.1 INTRODUCTION

Caffeine is a constituent of many frequently consumed foodstuffs including coffee, tea, soft drinks and cocoa products. It is also an ingredient in prescription and over-the-counter medicines, including cold remedies, analgesics and slimming medications (James 1991). Although long-term caffeine use has been associated with negative health consequences such as osteoporosis (Hernandez-Avila 1991), spontaneous abortion (Cnattinguis et al. 2000) and cardiovascular disease, particularly in hypertensive individuals (James 1997), caffeine is generally believed to have no serious health consequences when used in moderation by healthy individuals. Moreover, moderate doses of caffeine are associated with mildly stimulant effects, in particular increased arousal and reduced feelings of fatigue (Herz 1999; Kamimori et al. 2000; Smith 2002). These psychostimulant properties, along with the perceived absence of serious side effects, have made caffeine the world's most popular psychoactive drug (see Fisone, Borgkvist & Usiello 2004; Thompson & Keene 2005 for reviews). Such widespread use and low toxicity provides major methodological benefits for psychopharmacological experiments as caffeine can be administered easily and safely to a large proportion of the general population.

The following sections discuss the pharmacological and physiological effects of caffeine. An understanding of factors such as the rate of absorption of caffeine, and

possible disruption of caffeine's effects by the use of other drugs is important for the design of effective experiments to test the effects of caffeine, and provides information on potential mechanisms that may underlie its behavioural effects. Furthermore, it is important to review the specific effects of caffeine on behaviour to be able to select measures that will be suitable for testing conditioned effects of the drug.

2.2 PHARMACOLOGY OF CAFFEINE

2.2.1 Pharmacokinetics

Caffeine is absorbed rapidly from the gastrointestinal tract (Blanchard & Sawyers 1983), and peak plasma levels of caffeine are reached within 15-120 minutes of oral ingestion (Arnaud 1987). The half-life of caffeine is approximately 3-5 hours (Lorist & Tops 2003), although this is subject to high levels of inter-individual variability (Magkos & Kavouras 2005), and can be affected by concomitant drug use, such as nicotine (Parsons & Neims 1978) and/or the contraceptive pill (Abernethy & Todd 1985). Caffeine is primarily metabolised by the liver (Grant, Campbell, Tang & Kalow 1987) and has several active metabolites including theophylline and paraxathine.

2.2.2 Mechanisms of Action

Caffeine affects the activity of CNS neurotransmitters such as dopamine, acetylcholine, serotonin, GABA, glutamate and noradrenaline, and via this widespread action influences cardiovascular, renal and respiratory functions in the

PNS (Dunwiddie & Masino 2001; Smith, Brice, Nash, Rich & Nutt 2003). However, extensive evidence indicates that at doses consistent with normal human consumption, caffeine acts primarily as an antagonist at adenosine receptors (Snyder, Katims, Annau, Bruns & Daly 1981; Boulenger, Patel, Post, Parma & Marangos 1983; Smits, Boekema, De Abreu, Thien & van't Laar 1987; Fastbom & Fredholm 1990). Therefore, caffeine is commonly categorised among a class of drugs known as the methylxanthines; a group of mild stimulants that block adenosine receptors.

Adenosine is a neuromodulator that regulates many transmitter systems within the CNS as well as peripheral biological systems such as the cardiovascular system (Smits et al. 1987; Riberio, Sebastião & de Mendonca 2003). Four types of G-protein coupled adenosine receptors have been identified: A₁, A_{2A}, A_{2B} and A₃ receptors. Caffeine is only a weak antagonist at the A_{2B} and A₃ receptor sites and exerts its primary actions at the adenosine A₁ and A_{2A} receptors (see Fredholm, Bättig, Holmén, Nehlig & Zvartau 1999 for review).

Adenosine A₁ receptors are widespread throughout the brain, with highest concentrations in the hippocampus, cerebral and cerebellar cortices and some thalamic nuclei (Rivkees, Price & Zhou 1995). They regulate CNS activity via an inhibitory action on central neurotransmitters (Dunwiddie & Masino 2001). It has been proposed that caffeine and other methylxanthines increase dopamine and glutamate in the shell of the nucleus accumbens by antagonising A₁ receptor activity, suggesting that caffeine's action at this receptor may play a role in its rewarding properties (Solinas et al. 2002).

Unlike the near-ubiquitous A₁ adenosine receptor, the A_{2A} receptor is highly localised in dopamine-rich regions of the brain. Particularly high densities have been identified within the striatum of both rodent and human brains (Jarvis & Williams 1989; Martinez-Mir, Probst & Palacios 1991). The striatum is the main projection site of the basal ganglia, and is heavily involved with planning and executing movement (DeLong 1990; Graybiel 1990; Freund & Hefter 1993). Therefore, antagonism of inhibitory striatal A_{2A} receptors is a potential mechanism by which caffeine stimulates motor activity (Ferré, von Euler, Johansson, Fredholm & Fuxe 1991; Ferré, O'Connor, Fuxe & Ungerstedt 1993; Ferré, Fredholm, Morelli, Popoli & Fuxe 1997).

Thus, the stimulant actions of caffeine are thought to be due to a disinhibition of transmitter systems that are normally under tonic inhibitory control of adenosine. For example, release of inhibition of dopamine by adenosine antagonism (Ferre, Fuxe, von Euler, Johansson & Fredholm 1992) is a likely mechanism by which caffeine increases dopamine (DA) release and turnover (Fredholm et al. 1999). Studies show that caffeine enhances DA-related locomotor behaviour (Ferre et al. 1992), and that DA receptor antagonists can block the motor activating effects of caffeine (Garrett & Holtzman 1994).

Caffeine also increases the synthesis and turnover of noradrenaline, as demonstrated by its ability to reverse the performance deficits induced by administration of a noradrenergic antagonist (Smith et al. 2003). Noradrenaline is well known to be important for arousal (Robbins 1984) and therefore caffeine-induced increases in

noradrenaline may contribute to its reported alerting effects (see Nelig, Daval & Debry 1992, for review).

Additionally, caffeine may increase arousal via indirect stimulation of cholinergic activity. Rainnie, Grunze, McCarley and Green (1994) demonstrated that infusion of adenosine decreases firing of cholinergic neurons in the laterodorsal tegmental nucleus and the pontine tegmental nucleus. During periods of wakefulness, when both metabolic and cholinergic activity are high, adenosine may be co-released, inhibiting the cholinergic neurons. In support, caffeine attenuates performance deterioration induced by cholinergic antagonists (Riedel et al. 1995).

Finally, caffeine has been shown to induce various regional changes in the levels of other neurotransmitters in rodents. For example, caffeine decreases the levels of GABA in the pons and medulla (Wajda, Banay-Schwarz & Lajtha 1989). However, the neurochemical effects of caffeine do not occur in isolation, but interact with and modulate one another, and factors such as dose, tolerance and the regional function of the host receptor will influence the behavioural outcome of acute caffeine intake.

The above section demonstrates that there are many neurochemical effects of caffeine that may contribute to its potential to increase arousal and offset fatigue. These alerting effects of caffeine have been supported by measures of physiological arousal including galvanic skin response and EEG studies, which have shown increased activation following acute caffeine ingestion.

2.3 PHYSIOLOGICAL EFFECTS OF CAFFEINE

2.3.1 Physiological Arousal

Physiological data support an arousing effect of caffeine. For example, caffeine intake increases skin conductance (SC) levels (Flaten 1998; Flaten, Aasli & Blumenthal 2003). However, some caution must be applied to the interpretation of such data as caffeine is frequently administered in hot beverages, which themselves can increase SC regardless of caffeine content (Quinlan et al. 2000). Nevertheless, Quinlan et al. (2000) demonstrated an additional effect of caffeine on SC compared to the effects of hot water, and Flaten and Blumenthal (1999) reported that 2 mg/kg of caffeine significantly increased SC in abstinent (at least 12 hours) consumers regardless of whether it was administered in coffee or orange juice. In addition, Mikalsen, Bertelsen and Flaten (2001) reported that caffeine-related increases in skin conductance were correlated with self-reported increases in alertness.

In addition, caffeine has been associated with increased cortical activation in EEG studies (Bruce, Scott, Lader & Marks 1986; Kenemans & Lorist 1995). Hasenfratz and Bättig (1994) reported dose-dependent effects of caffeine on dominant EEG frequencies in α and β bands. In addition, Patat et al. (2000) found that caffeine reversed the negative effects of sleep deprivation on EEG.

Finally, caffeine's stimulant effects are also associated with increases in glucose utilization in numerous brain regions including those involved in the control of locomotion and the regulation of the sleep/wake cycles. For example, Nehlig, Duval,

Boyet and Vert (1986) reported widespread increases in cerebral glucose utilization in rats given 10 mg/kg caffeine. Similar effects, albeit reduced, were also reported in chronically caffeine-maintained compared to caffeine-naïve rats, indicating limited tolerance to these effects.

2.3.2 Effects on Sleep

Caffeine reverses some of the negative effects of sleep deprivation (Penetar et al. 1993). Caffeine also interferes with normal sleep behaviour, increasing the time taken to fall asleep (Zwyghuizen-Doorenbos et al. 1990), and reducing total sleep duration, particularly at high doses (>3 mg/kg) (Hicks, Hicks, Reyes & Cheers 1983). There is evidence of tolerance to these effects, with reduced sleep interference in higher consumers (Zwyghuizen-Doorenbos et al. 1990). Caffeine consumers may avoid problems with sleep interference by limiting intake to times when increased arousal is beneficial, for example early in the day or during the post-lunch dip (see Smith 2002 for review).

2.3.3 Cardiovascular Effects

In addition to its arousing effects, caffeine also increases systolic blood pressure and, to a lesser extent, diastolic blood pressure, due to its action as a vasodilator (Benowitz, Jacob, Mayan & Denaro 1995; Smith, Cranford & Green 2001). These changes are small in magnitude (in the region of 5-10 mm/Hg), but may be of clinical significance, particularly in hypertensive individuals for whom an increase in blood pressure of just 5-9 mm/Hg has been associated with increased risk of death (Lane &

Manus 1989). Although tolerance to these pressor effects has been reported (Kourtidou-Papadeli, Papadelis, Louizos & Guiba-Tziampiri 2002), it appears to be at best incomplete (Lane, Adcock, Williams & Kuhn 1990).

Despite claims that caffeine has direct tachycardic effects (Passmore, Kondowe & Johnston 1987), caffeine ingestion is often associated with a decrease in heart rate (Whitsett, Manion & Dix Christensen 1984; Tiffin, Ashton, Marsh & Kamali 1995). This effect has been attributed to baroreceptor-mediated actions in response to the increases in blood pressure. However, Kourtidou-Papadeli et al. (2002) reported a dose-dependent decrease in heart rate that was not correlated with blood pressure changes, suggesting that another mechanism, such as direct vagal stimulation, may underlie the bradycardic effects of caffeine.

The cardiovascular effects of caffeine are complex, with caffeine acting differentially on cardiac muscle, blood vessels, vagal nerves and vasomotor centres of the brain stem (Hasenfratz & Bättig 1994). The effects also vary with factors such as dose and population sample. In sum, although some of the effects of caffeine on cardiovascular functioning may be small, there is clear evidence that caffeine ingestion elicits increases in blood pressure and is most often associated with decreases in heart rate.

2.4 PERFORMANCE AND SUBJECTIVE EFFECTS

This section reviews research examining the psychomotor, cognitive and subjective mood effects of caffeine. These studies provide information on the nature of

caffeine's behavioural effects, and were used to guide the choice of behavioural measures adopted in this thesis.

2.4.1 Psychomotor Performance

2.4.1.1 Simple (SRT) and Choice (CRT) Reaction Time

Reaction time tasks test the ability to attend and respond to a target stimulus, with response speed as the dependent variable. The SRT task typically comprises one target and one response (e.g. button press in response to a flash of light). The CRT task comprises multiple targets and/or responses (e.g. different button presses in response to different targets), although the nature of the stimuli, responses and other features of the tasks can differ widely.

Caffeine decreases reaction time in SRT and CRT tasks (Lieberman, Wurtman, Emde, Roberts & Coviella 1987; Roache & Griffiths 1987; Smith, Maben & Brockman 1994; Smith, Kendrick & Maben 1992; Smit & Rogers 2000; Brice & Smith 2002a). Benefits of caffeine on reaction time have been reported at low to moderate doses of caffeine ranging from 12.5 mg (Smit & Rogers 2000) to 400 mg (Jacobson & Edgley 1987), with less consistent effects at higher doses of around 600 mg (Jacobson & Edgley 1987; Roache & Griffiths 1987). Although some studies have shown net benefits of caffeine on performance (Haskell, O'Kennedy, Wesnes & Scholey 2005), other studies have reported effects only in consumers withdrawn from caffeine. For example, Rogers, Martin, Smith, Heatherley & Smit (2003) reported significant effects of caffeine on reaction time in a group of overnight-deprived caffeine consumers, but not in a group of non-consumers, suggesting that the benefits of

caffeine may be in part mediated by the alleviation of negative effects of caffeine withdrawal.

Reaction time responses involve a series of cognitive processes including attentional processes that identify the target stimulus, and psychomotor processes that underlie the motor response. However, it is often not possible to elucidate the extent to which caffeine affects the individual processes that underlie the response. The effects of caffeine on motor speed have been directly tested using tasks that isolate the motor response from decision-making. For example, it has been reported that caffeine improves performance of a tapping rate task that requires a continuous motor action that is not made in response to an external stimulus (Kaplan et al. 1997).

2.4.1.2 Additional measures of psychomotor performance

Caffeine can increase performance on tasks that require participants to continually tap a keyboard key or a specially devised stylus for a period of around 1-2 minutes (Kaplan et al. 1997). However, null effects have also been reported across a wide range of doses (Bruce et al. 1986; Lieberman et al. 1987; Richardson, Rogers, Elliman & O'Dell 1995; Lane 1997; Rogers & DERNONCOURT 1998). These inconsistencies may in part be due to variation in task length with some tasks lasting just 20 seconds (Lane 1997), and others incorporating breaks (Richardson et al. 1995). Under these conditions, participants are more likely to maintain a ceiling level of responding in the placebo condition, leaving little scope for caffeine-induced improvement. In support, Kaplan et al. (1997) conducted multiple tests that could have promoted task fatigue or

boredom in the placebo condition and which caffeine was able to offset via its well-documented ability to increase arousal (see sections 2.4.3 and 2.4.4).

Caffeine-induced increases in tapping rate suggest that caffeine can be beneficial if increased motor action is required. However, the deterioration of caffeine on hand-steadiness (Gilliand & Bullock 1984) implies that caffeine can be detrimental in situations where reduced movement is necessary. For example, Arnold, Springer, Engel and Helveston (1993) demonstrated decreased hand-steadiness in a group of ophthalmologist trainees after 200 mg of caffeine, a potentially dangerous consequence for individuals required to perform delicate microsurgical techniques. However, as research on the cognitive effects of caffeine has focussed on whether caffeine has performance benefits, measures of hand-steadiness have been relatively seldom-used experimentally.

Kuznick & Turner (1986) demonstrated that tolerance may occur to the psychomotor agitating effect of caffeine. Performance on a rotary pursuit task, in which contact was maintained between a metal disk and a metal stylus mounted on a rotating turntable, improved after caffeine in a caffeine consumer group but deteriorated in a group of non-users (Kuznicki & Turner 1986). Findings from tracking tasks are also mixed, with reports of both caffeine-induced performance improvements (Kerr, Sherwood and Hindmarch 1991) and impairments (Patat et al. 2000). However, methodological differences, including task difficulty, caffeine dose, participant age and level of withdrawal, could explain the contradictory findings. These findings suggest that caffeine's behavioural effects are mediated by several factors and that

tasks that have been shown to be sensitive to the effects of caffeine in some circumstances may nevertheless be insensitive in other situations or consumer samples. Therefore, in addition to selecting a suitable task for studying the effects of caffeine, consideration should also be given to factors such as task length, task difficulty, consumer-status of participants, duration of caffeine abstinence and caffeine dose, all of which may influence whether an effect of caffeine is observed.

2.4.2 Cognitive Performance

2.4.2.1 Information Processing

Significant performance benefits of caffeine have been reported on a wide range of information processing tasks including visual scanning (Lieberman, Tharion, Shukitt-Hale, Speckman & Tulley 2002), selective visual processing (Kenemans & Lorist 1995), rapid visual information processing (RVIP) (Frewer & Lader 1991; Hasenfratz & Bättig 1994; Warburton 1995; Warburton, Bersellini & Sweeney 2001; Yeomans, et al. 2002), delayed and immediate pattern recognition (Durlach 1998), digit symbol substitution (Kaplan et al. 1997), semantic verification (Smith et al. 1992; Warburton 1995) and Stroop tests (Kenemans, Wieleman, Zeegers & Verbaten 1999).

As mentioned previously, caffeine can enhance psychomotor responding, and many cognitive tasks involve a motor response. Therefore, in many cases, caffeine may simply enhance performance of cognitive tasks by decreasing motor response speed. However, Lieberman et al. (1987) showed significant improvements on an auditory vigilance task with a low dose of 32 mg of caffeine, yet no significant effects on six other cognitive tasks, all of which included a motor response, suggesting that

vigilance may be particularly sensitive to the effects of caffeine. Caffeine has been associated with improvements in sustained attention, offsetting the performance deterioration that otherwise commonly occurs over time in long and/or fatiguing tasks (Frewer & Lader 1991; Robelin & Rogers 1998). This is in keeping with the contention that caffeine attenuates symptoms of fatigue, and suggests that caffeine may exert its facilitatory action on performance via its enhancement of arousal. In general, the effects of caffeine have been more readily detected on simple tests of psychomotor performance and cognition, than on tests of higher cognitive functions such as memory (Smith 2002; James & Rogers 2005).

2.4.2.2 Caffeine and Memory

Effects of caffeine on memory have not been reported consistently. Warburton (1995) found a significant effect of caffeine on delayed word recall after auditory presentation in a group of non-abstinent caffeine consumers. However, in the same study no effects of caffeine were observed on immediate recall or working memory (spatial recognition and visuo-spatial memory tasks), and Rogers and Dernoncourt (1998) also found no effect of caffeine on immediate recall when word lists were presented visually (delayed recall was not tested). Further null or weak effects of caffeine have been reported on: immediate and delayed recall (Loke, Hinrichs & Ghoneim 1985; Gupta 1991; Keleman & Creeley 2001; Warburton et al. 2001; Smit, Cotton, Hughes & Rogers 2004); recognition memory (Gupta 1993); and spatial working memory (Warburton et al. 2001).

In contrast, there is evidence that long-term caffeine consumption may be beneficial for memory (Jarvis 1993; Hameleers et al. 2000). Hameleers et al. (2000) tested a large sample of consumers who differed in their levels of habitual caffeine consumption, and found that higher levels of consumption were associated with better long-term memory. In addition Smith et al. (1994) reported a facilitation of immediate recall following administration of 4 mg/kg caffeine but only 2 hours after ingestion, suggesting that a shorter ingestion-test interval may explain reports of null findings.

Reports of no significant effects of caffeine on memory have been accompanied by reports of enhancement of other cognitive processes using the same procedure (see Smith 2002 for review), which undermines suggestions that the weak or null effects on memory are due to methodological shortcomings. However, differences in task characteristics (e.g. task duration, number of items to be remembered, sensory modality of stimuli presentation) may explain some of the variability in the findings.

Nehlig and Debry (1994) argue that caffeine does not directly facilitate memory, rather it enhances other aspects of performance such as reaction time or information processing speed, which, in turn, influence overall task performance. As with other aspects of performance, caffeine's ability to enhance arousal has been suggested to mediate its effects on memory, suggesting that significant effects of caffeine will only be obtained on tasks in which increases in arousal are beneficial.

2.4.3 Performance and Arousal

It has been argued that the arousing effect of caffeine mediates the performance enhancing effects observed following its administration (Kenemans & Lorist 1995). The Yerkes-Dodson principle (1908) states that there is an “inverted-U” relationship between cortical arousal and performance, whereby moderate levels of arousal are associated with performance facilitation, and low and high levels of arousal are associated with performance impairment. Furthermore, tasks with a high memory load benefit less from increases in arousal than simple tasks (e.g. letter cancellation), due to a negative relationship between arousal and short-term memory processes (Humphreys & Revelle 1984). Anderson and Revelle (1983) reported caffeine-induced enhancement on a cognitive task with low information processing demands, but impairment on the same task when there was a higher information processing demand, suggesting that caffeine’s performance enhancing effects may be dependent on the extent to which task performance is benefited by an increase in arousal.

In support, caffeine is particularly effective in improving performance in situations associated with low arousal (Smith 2002), such as sleep deprivation (Kamimori et al. 2000), early morning performance (Smith et al. 1992), benzodiazepine-induced fatigue (Johnson, Spinweber & Gomez 1990), caffeine withdrawal (Rogers et al. 2003), and task fatigue (Rogers & DERNONCOURT 1998). Brice and Smith (2002b) note that caffeine consumption varies with time of day indicating that consumers may use caffeine to offset fatigue associated with certain times of the day (e.g. the post-lunch dip – Smith, Rusted, Eaton-Williams, Savory & Leathwood 1990).

In sum, there is good evidence that caffeine can increase arousal, which, in turn, can facilitate some aspects of cognitive performance. However, because increased subjective and cortical arousal after caffeine has been reported with no corresponding facilitation of performance (Herz 1999; Ruijter, Lorist, Snel & Ruiter 2000), and performance facilitation has been reported without any subjective increases in arousal, caffeine may also improve performance by mechanisms unrelated to arousal.

2.4.4 Subjective Effects

Caffeine-induced alterations in mood have been reported extensively. For example, caffeine has been reported to increase tension (Kaplan et al. 1997; Smit & Rogers 2000), excitement (Kaplan et al. 1997), irritation (Kaplan et al. 1997), nervousness (Charney, Galloway & Heninger 1984), vigour (Evans & Griffiths 1992; Penetar et al. 1993) friendliness, happiness and clear-headedness (Warburton 1995). It has also been reported to decrease calmness (Charney et al. 1984), tension (Warburton 1995) and alleviate symptoms of caffeine withdrawal (Schuh & Griffiths 1997). Reports of both decreased (Warburton 1995) and increased (Kaplan et al. 1997) tension after caffeine, may be partly due to methodological issues. In a comprehensive review, Smit and Rogers (2002) describe several common methodological pitfalls of mood measurement, including “insufficient or ambiguous instructions” and the use of irrelevant or ambiguous mood adjectives.

As with performance, non-linear dose-response relationships have been observed between caffeine and mood. Low to moderate doses are associated with positive subjective reports, while higher doses tend to produce negative reports of anxiety,

tension and jitteriness. Negative effects can manifest at lower doses in individuals whose normal intake of caffeine is low, suggesting some tolerance to these effects (Evans & Griffiths 1992). A clinically significant syndrome, “caffeinism”, has been associated with excessive caffeine use upward of 1000 mg/day. The symptoms include anxiety, mood fluctuation, cardiac arrhythmias and hypertension; and it has been described as being “virtually indistinguishable” from general anxiety disorder (Greden 1974).

In summary, the biphasic effect of caffeine on subjective reports of arousal is well documented. At low doses, caffeine can generate positive feelings of alertness but as the dose increases, reports of anxiety and tension are more common, indicating a state of over-arousal (Kaplan et al. 1997). High doses may be associated with anxiogenic effects but these are not likely within the limits of normal consumption.

2.5 CHRONIC EFFECTS OF CAFFEINE

Long-term habitual use of a drug can induce neural and/or metabolic adaptations that attenuate the perturbations caused by drug ingestion. For example, receptor density/or efficacy can change in response to repeated drug use and this can result in the drug-user requiring progressively larger amounts of drug to experience an optimal or desired effect (i.e. tolerance). Tolerance occurs to the effects of both recreational and medical drugs (Schenk & Partridge 1997; Bailey & Connor 2005; Gonzalez, Cebeira & Fernandez-Ruiz 2005; Zapantis & Leung 2005). Due to tolerance, the acute effects of a drug often vary with the level of an individual’s habitual intake of the drug. Therefore, prior to using a drug in an experimental situation, it is important to

understand its tolerance profile, as this will have implications for the dose required to achieve optimal effects.

Tolerance develops to some, but not all, effects of caffeine. Although the underlying mechanisms of caffeine tolerance have not been established, there is evidence of adenosine receptor changes after chronic use of caffeine in both rodent and humans models (Fredholm 1982; Chou, Khan, Forde & Hirsh 1985; Svenningsson, Nomikos & Fredholm 1999; Varani et al. 1999; Varani et al. 2005). The development of tolerance may be influenced by dose (Mukhopadhyay & Podder 1998), dosing regime (Meliska, Landrum & Landrum 1990) and route of administration (Rozin, Reff, Mark & Schull 1984).

Numerous studies report tolerance to the locomotor effects of caffeine in rodents (Rozin et al. 1984; Chou et al. 1985; Ahlijanian & Takemori 1986; Finn & Holtzman 1986; Finn & Holtzman 1987). However, the evidence is less convincing in humans, which, in part, could be due to the lack of control over previous exposure to the drug. Zahn and Rapoport (1987) reported significantly less autonomic nervous system responsivity in a group of high versus low caffeine consumers that may be indicative of tolerance. However, this effect may have also been due to underlying traits predisposing individuals to varying levels of daily intake.

Because caffeine consumption has been linked with stress, hypertension and cardiovascular disease, there has been much interest in whether tolerance develops to the pressor effects of caffeine. The general consensus is that tolerance, if it occurs at

all, is incomplete and short-lived (Lane & Manus 1989; Pieper, Phillips-Bute, Bryant & Kuhn 2002; Watson, Deary & Kerr 2002; Farag et al. 2005a, b). Overnight abstinence is sufficient to reverse tolerance, suggesting that habitual consumers are continually affected by caffeine-induced increases in blood pressure, which are implicated in increased risk of cardiovascular disease.

It has been reported that habitual caffeine consumers experience fewer negative mood effects after caffeine than do non-consumers (Evans & Griffiths 1992). This effect could be explained by tolerance to the aversive effects of caffeine in the habitual consumers, but equally could be due to heightened sensitivity to these effects in the non-consumers. In addition, Warburton (1995) reported that caffeine increased ratings of calmness, happiness and clear-headedness in minimally deprived caffeine consumers, implying only modest tolerance to the mood enhancing effects of caffeine. Evidence of tolerance to caffeine's cognitive effects is also limited. Watson et al. (2002) found that caffeine decreased choice reaction times similarly in caffeine consumers who consumed either caffeine or placebo for a week, indicating an absence of tolerance.

In sum, there is evidence of cellular changes in response to long-term caffeine use. There is also evidence of tolerance to the effects of caffeine, however when tolerance occurs, it is often incomplete. Furthermore, caffeine consumption does not generally follow the characteristic pattern associated with tolerance, that is, increasingly larger doses being administered to achieve the same level of response (Strain & Griffiths

1995; Daly & Fredholm 1998). Instead, the number of doses (e.g. cups of coffee) and strength of dose administered by individuals tends to be relatively stable over time.

2.6 CAFFEINE WITHDRAWAL AND THE WITHDRAWAL-REVERSAL HYPOTHESIS

It has been reported that 35-100% of participants in studies of the effects of caffeine abstinence experience withdrawal effects after abrupt cessation of caffeine use (Juliano & Griffiths 2001). However, the prevalence of reported withdrawal symptoms varies between studies and is higher in prospective (e.g. laboratory-based experiments) compared with retrospective studies (e.g. surveys), possibly due to recall inaccuracies. In addition, studies vary in the duration of monitoring and the scope of symptoms assessed.

Withdrawal onset begins approximately 12 to 24 hours after caffeine cessation, and peaks at 24 to 48 hours (Fredholm et al. 1999). Symptoms generally cease after approximately 1 week of abstinence (Griffiths et al. 1990), although residual symptoms have been reported as late as 21 days after caffeine cessation (Richardson et al. 1995). Griffiths et al. (1990) suggested that withdrawal symptoms are even experienced following termination of relatively low dietary doses caffeine (100 mg/day) and may also occur after as little as 3 days of caffeine exposure.

The most commonly cited effects of caffeine deprivation are self-reported increases in headache and fatigue, and decreases in arousal (see Smit and Rogers 2002 for review). Physiological studies show that caffeine deprivation is associated with increases in

EEG theta power as well as increases in mean systolic and diastolic blood flow velocity in the cerebral arteries (Jones, Herning, Cadet & Griffiths 2000). These changes in cerebral blood flow may underlie the headaches reported during caffeine withdrawal (Mathew & Wilson 1985). Other, less prevalent, symptoms include sleepiness, lethargy, flu-like symptoms, insomnia, anxiety, psychomotor impairment, hand/limb tremor, caffeine craving and apathy; and in extreme cases, confusion, weakness, dysphoria, delirium, nausea, vomiting, rhinorrhea, and muscle tension and pain (see Griffiths & Woodson 1988; Fredholm et al. 1999; Feinstein et al. 2000; Dews, O'Brien & Bergman 2002; Smit & Rogers 2002; Juliano & Griffiths 2004 for reviews). The caffeine withdrawal syndrome may be exacerbated by expectation (Dews et al. 2002) and may correlate with habitual consumption levels (Juliano and Griffiths 2004).

There is less evidence that impaired cognitive performance is associated with caffeine withdrawal (Smith 2002). Detrimental effects of caffeine deprivation have been reported on tapping performance (Silverman, Evans, Strain & Griffiths 1992) and on complex functioning in a series of "real-world" managerial simulations (Streufert et al. 1995). In addition, Lane and Phillips-Bute (1998) reported slower and less accurate vigilance performance following overnight caffeine abstinence compared to *ad libitum* caffeine consumption. However, as the *ad libitum* group were not free of drug, it was not possible to ascertain whether the group differences were due to impaired performance after deprivation or enhanced performance after caffeine. Similarly, Rizzo, Stamps and Fehr (1988) reported slower reaction times in caffeine-deprived consumers compared to non-users, but this was due to faster performance in

the non-user group relative to their baseline, indicating that the effect was not mediated by withdrawal in the consumer group. Furthermore, some studies have reported negative mood, but no evidence of impaired performance during caffeine withdrawal (Rogers, Richardson & Démoncourt 1995; Lane 1997; Watson et al. 2000).

Nevertheless there is evidence that the effects of caffeine on cognition and mood are more robust in acutely-deprived caffeine consumers compared to non-consumers (James 1994; Rogers et al. 2003; James 2005). Furthermore, the effects of caffeine are reduced in situations where caffeine has been consumed *ad-libitum* prior to test (Lane 1997) and in long-term withdrawn consumers (Richardson et al. 1995). These data suggest that caffeine has little net benefit on mood or performance, and instead merely acts to alleviate caffeine-withdrawal. However, in contrast to this suggestion, beneficial effects of caffeine have been reported in non-caffeine users (Rogers, Richardson & Elliman 1995; Haskell et al. 2005) and following minimal abstinence (Warburton 1995; Christopher, Sutherland & Smith 2005; Smith, Sutherland and Christopher 2005) suggesting that caffeine withdrawal is not necessary to experience positive effects of caffeine. One explanation for these contradictory findings may be that arousal levels are low in caffeine-withdrawal meaning that it may be a sufficient, but not necessary state for caffeine facilitation of performance and mood. This suggests that requiring overnight abstinence in caffeine consumers may be a useful experimental tool for eliciting robust effects of caffeine, and may be easier to achieve and confirm than other fatigued states such as sleep deprivation.

Thus, there is persuasive evidence of a withdrawal syndrome upon acute caffeine cessation, which is characterised chiefly by increased headache and fatigue. The evidence of withdrawal effects on cognitive function is somewhat mixed but in some circumstances performance may be adversely affected by withdrawal-induced decrements in arousal. Furthermore, there is evidence that caffeine effects are particularly robust in caffeine-deprived consumers.

2.7 CAFFEINE DEPENDENCE AND REINFORCEMENT

Whether or not consumers can become dependent on caffeine has been much debated (Hughes, Oliveto, Helzer, Higgins & Bickel 1992; Strain & Griffiths 1995; Daly & Fredholm 1998; Feinstein et al. 2000) and the answer largely depends on how caffeine dependence is defined. Although caffeine does not generally fit the criteria for a drug of abuse, as it does not usually result in lifestyle problems (Hughes et al. 1992; Nehlig 1999), evidence of withdrawal and tolerance to the effects of caffeine suggests that it may be considered a dependence-producing drug. However, the compulsion to use a drug may occur in the absence of a robust withdrawal syndrome (Gawin & Kleber 1988; Withers, Pulvirenti, Koob & Gillin 1995), suggesting that negative reinforcement explanations of drug dependence (e.g. alleviation of withdrawal) do not fully account for continued drug use. Instead, positive reinforcement theories of drug dependence have emphasised a role for the positive effects of drugs (e.g. euphoria, arousal) in maintaining drug use and supporting dependence (Schuster & Thompson 1969; Stolerman 1993).

The reinforcing potential of a drug can be inferred by the extent to which it is self-administered when there is free access to it (Hartnoll 1991; Withers et al. 1995).

However, self-administration of caffeine in non-human animals is weak and levels of administration usually fall below those observed with other psychostimulant drugs such as amphetamine and cocaine (Deneau, Yanagita & Seevers 1969; Atkinson & Enslen 1976; Collins, Weeks, Cooper, Good & Russell 1984; Griffiths & Woodson 1988).

Hughes, Oliveto, Bickel, Higgins and Badger (1993) reported that 31% of human participants demonstrated preferential self-administration of caffeinated coffee compared to placebo. In addition, participants were twice as likely to self-administer caffeine if they suffered from withdrawal headaches, implying that caffeine is negatively reinforcing. However, Griffiths, Bigelow and Liebson (1989) reported that participants self-administered caffeine even after a substantial period of caffeine abstinence, suggesting that withdrawal is not prerequisite for caffeine to act as a reinforcer. Similarly, Mitchell, de Wit and Zacny (1995) found that caffeine was self-administered in coffee regardless of deprivation state.

In a choice procedure, Evans, Critchfield and Griffiths (1994) reported that consumers chose caffeine capsules over placebo on 80% of occasions. In a similar study, Griffiths and Woodson (1988) found that the percentage of participants choosing caffeine over placebo was inversely related to dose, supporting an inverted U-shaped dose-response function with adverse effects at highest dose of caffeine (600 mg).

More convincing evidence for the reinforcing properties of caffeine comes from conditioned place preference (CPP) studies in animals, in which one context is paired with drug administration and another context is paired with placebo. The findings from these studies depend chiefly on the dose tested. Lower doses (approximately 3 mg/kg) induce CPP, while higher doses (up to 30 mg/kg) result in a conditioned place aversion (Brockwell, Eikelboom & Beninger 1991). This suggests that a dose window exists for the reinforcing potential of caffeine.

Moreover, caffeine's rewarding potential may be mediated by its post-ingestive consequences. Mobini, Elliman and Yeomans (2005) showed that flavour preference for a caffeinated beverage was acquired if the drink was administered at breakfast, but a decrease in liking was reported if the same beverage was administered at night. Presumably, caffeine-induced increases in arousal were not beneficial or rewarding before sleep.

2.8 SUMMARY

The stimulant action of caffeine is likely to be due to disinhibition of transmitter systems that are normally under tonic inhibitory control of adenosine. Caffeine's physiological effects include changes in cardiovascular function and cerebral blood flow, and increases in indices of physiological arousal. Caffeine can also increase the latency to fall asleep and alter sleep patterns.

Caffeine can enhance cognitive performance, with particularly reliable effects on psychomotor performance and tasks requiring sustained attention, although it can

have detrimental effects on hand steadiness. Caffeine may exert its facilitatory effects via its capacity to increase arousal, which may explain why less reliable effects are often observed on higher cognitive functions such as memory, which are thought to benefit less from high levels of arousal. The positive effects of caffeine are particularly robust in states of low arousal, in particular during caffeine withdrawal in regular caffeine consumers. Clear detrimental effects of withdrawal from caffeine have been reported on mood, but detrimental effects of withdrawal on cognitive performance are less consistent, suggesting the enhancements of caffeine may be secondary to increases in arousal.

Evidence of a caffeine withdrawal syndrome suggests some degree of caffeine dependence, although the profile of dependence is unlike that for other drugs of abuse. There is no evidence of disruption of everyday life or work obligations due to caffeine use. However, physiological alterations following chronic caffeine have been reported, although tolerance, when reported, is often incomplete. Finally, caffeine has reinforcing properties, although, for the most part, these appear to be related to withdrawal reversal.

The research reviewed above supports the use of caffeine in the current series of studies. The low toxicity, widespread use and relatively low dependence potential of caffeine mean that it can be administered safely to the general population.

Furthermore, the use of caffeine can be disguised using capsule administration, thereby reducing the expectancy or prior conditioned effects that may be associated with coffee or tea ingestion. Of particular relevance to the study of conditioned

cognitive effects, the evidence suggests that caffeine is a robust psychostimulant, capable of improving cognitive performance and mood. These facilitatory effects of caffeine appear to be particularly reliable for measures of reaction time and sustained attention among overnight-deprived caffeine consumers. Therefore, it is planned to employ these measures and recruit relatively high caffeine consumers with a stipulation of overnight caffeine abstinence. Cardiovascular and mood effects of caffeine will provide additional measures of caffeine reactivity.

CHAPTER 3:

CLASSICAL CONDITIONING AND CONDITIONED DRUG EFFECTS

3.1 INTRODUCTION

Stimuli that are present during drug ingestion can acquire the ability to elicit drug-related responses when presented without the drug. These responses are important because they are likely to play a role in motivating and maintaining self-administration of drugs (O'Brien, Childress, McLellan & Ehrman 1992; Siegel 1999; See 2005). The following review will introduce classical conditioning as a type of associative learning and provide a brief overview of various factors that affect conditioning. The literature on conditioned drug effects will be discussed with particular reference to the role of classical conditioning in these effects and the nature of the conditioned response. Discussion of the theoretical background of classical conditioning provides a framework for the general aims of the present thesis, and highlights testable predictions regarding the direction and nature of the conditioned response. Furthermore, findings from pharmacological conditioning studies demonstrate the important role of conditioned effects in the behavioural responses to drugs, and emphasises the paucity of research examining the conditioned cognitive effects of drugs. These findings address important issues related to the design of the experiments in the present thesis such as the intra- and inter-trial timeframe and the nature of the drug-paired stimuli.

3.2 ASSOCIATIVE LEARNING AND CONDITIONING THEORY

Associative learning concerns the learning of the predictive and/or causal relationship between two or more events. Such learning is fundamental for survival in an ever-fluctuating environment. For example, in order to seek out foods that are nutrient-rich, and avoid those that are potentially toxic, an animal must learn to associate the stimulus properties of a food (i.e. sight, taste, smell) with its post-ingestive consequences. From a practical viewpoint, the study of associative learning has provided a framework that has furthered the understanding of human learning, which, in turn, has led to practical applications, including the treatment of addictions and phobias (Eysenck & Rachman 1965; Rohsenow et al. 1990).

Two types of associative learning have been identified: operant and classical conditioning. Operant (or instrumental) conditioning (Thorndike 1911; Hull 1943) is thought to involve the learning of an association between a stimulus and a response. In contrast, classical conditioning (which is most relevant to this thesis) refers to the learning that occurs when two stimuli are presented together.

Unlike cognitive theories of learning that discuss complex and dynamic neural networks (Gallistel 1990), associative learning involves relatively simple associations that occur automatically. For some years, the study of conditioning decreased in popularity, with greater emphasis on cognitive approaches to learning (Wasserman 1993). However, more recently there has been a return to examining the role of cognition (e.g. awareness, expectation, imagery) in associative learning (Martin and Levey 1988, Shanks 1990; Kirsch, Lynn, Vigorito and Miller 2004).

3.2.1 Classical Conditioning

Pavlov (1927) observed that repeated pairing of a previously neutral stimulus, which elicited no inherent response (the conditioned stimulus – CS) with a second stimulus (the unconditioned stimulus – US) that elicited a physiological response (the unconditioned response – UR), led to the CS acquiring the ability to elicit a response similar to the UR (the conditioned response – CR). Stimulus-substitution theory (SST), attributed to Pavlov (1927), claimed that this learning was due to a direct link forming between the “brain centres” responsible for processing the CS and US, so that activation of the CS “brain centre” resulted in an automatic activation of the US centre which in turn elicited the unconditioned response (UR). Therefore, CR was expected to be quantitatively and qualitatively similar to the UR.

An alternative explanation for classical conditioning suggests that one stimulus does not substitute another, but rather the CS signals the arrival of the US (Tolman 1932). In this case, the two stimuli would be expected to elicit similar, but not necessarily identical, responses. This model allows for preparatory responses that are dissimilar to the UR. However, it should be pointed out that although Pavlov (1927) described the CR and UR as “equal”, he did not adhere to stimulus substitution in its strictest sense. He explicitly discussed a signalling role of the CS, and reported orientating responses towards the door (i.e. source of US) when a light (CS) was presented (i.e. preparatory response).

Since the time of Pavlov, conditioned responding has been consistently demonstrated in the laboratory in many different species and across many response systems. Early

work focussed on the autonomic nervous system, reporting conditioning of: salivation (Kintsch & Witte 1962; Rozin, Reff, Mark & Schull 1984); hormone release (Bykov 1957; Stockhorst et al. 1999; Pacheco-Lopez et al. 2004); galvanic skin responses (Hunt & Daily 1974); cardiovascular function (Cohen & Randell 1984); blood glucose responses (Siegel 1975b; Stockhorst, Steinburger & Scherbaum 2000); eyeblink responses (Steinmetz, Tracy & Green 2001; Christian & Thompson 2003); and nausea (Matteson, Roscoe, Hickok & Morrow 2002; Stockhorst, Steingruber, Enck & Klosterhalfen 2006). More recently conditioning of emotional and cognitive responding (Shapiro & Nathan 1986; Davis 1990; Zwuyghuizen-Doorenboos et al. 1990; Buchel & Dolan 2000; Foltin & Haney 2000) and feeding-related behaviours (Weingarten 1983; Arwas, Rolnick & Lubow 1989; Yeomans, Durlach & Tinley 2005) has also been documented. Despite the variability in the response systems examined, the factors affecting conditioned responding are strikingly similar.

3.2.2 Factors affecting conditioning

3.2.2.1 Contiguity and Contingency

Pavlov's (1927) early findings suggested when two stimuli occur together (i.e. are contiguous), a learned association will form between them. In support, the strength of conditioning generally increases as a function of the temporal and/or spatial proximity of the CS and US (Pavlov 1927). However, Rescorla (1966; 1968) reported that conditioning only occurred if the CS signalled an increased probability of the US, i.e. if the CS was present on more US presentations than it was absent. In contrast, no conditioning occurred when a CS was present on 50% of US presentations. These findings suggested that contiguity alone was not sufficient to explain conditioning, as

conditioning would be expected in any situation where the CS and US occur together. In support, the strength of the CR varies as a function of the level of contingency between the CS and US; being strongest when the CS occurs on 100% of US presentations (Rescorla 1966).

This raises an important consideration regarding the temporal arrangement of the CS and US. For the CS to signal the US, it should precede the US. In support, stronger conditioning effects have been observed with this arrangement of stimuli (forward conditioning), compared with paradigms where the CS follows the US (backward conditioning), or occurs simultaneously with the US (Durkovic and Damianopoulos 1986; Murdock 1951; Pavlov 1927). The optimal inter-stimulus interval (ISI) varies depending on the nature of the US and CS, but can range from several hundred milliseconds (Frey & Ross 1968) to several hours (Garcia, Ervin & Koelling 1966). The CS-US interval during simultaneous conditioning is 0 ms, after which conditioning strengthens to a point as the CS-US interval is increased and then begins to weaken (Ost & Lauer 1965).

Furthermore, while Pavlov (1927) argued that for conditioning to occur there should be temporal overlap between the CS and US, acquisition of CRs in trace conditioning procedures, in which the CS onset and offset precedes US presentation, argues against this (Yeo, Hardiman, Moore & Russell 1984; Graves & Solomon 1985; Finkbiner & Woodruff-Pak 1991; Akins & Domjan 1996). However, trace procedures generally produce weaker or less consistent conditioning than procedures in which CS presentation overlaps the US onset (Akins and Domjan 1996; Graves and Solomon

1985; Pavlov 1927), and the strength of trace CRs may be negatively correlated with the CS-offset/US-onset interval (Marlin 1981).

3.2.2.2 Partial Reinforcement

Partial reinforcement schedules pair the CS with some, but not all, of the US presentations. As a general rule of thumb, conditioning should occur when the CS is present on more US trials than it is absent, as the probability of the US occurring is greater if the CS is presented; and the strength of the CR positively correlates with the level of reinforcement (Rescorla 1966). In general, in comparison to 100% reinforcement schedules, partial reinforcement schedules produce a slower rate of learning and show a marked resistance to extinction (Jenkins & Stanley 1950)

3.2.2.3 Latent Inhibition

Latent inhibition refers to the retardation of conditioning that occurs due to pre-conditioning exposure to the CS, and has been demonstrated across several species and using a variety of measures (Mackintosh 1983; Kiernan & Westbrook 1993; Crombag et al. 2001). The general consensus is that that pre-conditioning exposure reduces attention paid to the CS, as it is not linked to any significant event (Lubow & Moore 1959; Mackintosh 1975; Pearce & Hall 1980; Bouton 1993).

Latent inhibition has been shown to vary as a function of: CS intensity (Crowell & Anderson 1972; Rodríguez & Alonso 2002); the number of CS pre-exposures (Domjan & Siegel 1971); the conditioning to test interval (Crowell & Anderson 1972; Kraemer & Roberts 1984); and the presence of other stimuli during pre-exposure

(Lubow, Schnur & Rifkin 1976). Furthermore, there is evidence that latent inhibition can be affected by context: it is attenuated if the CS is presented in a different context to that in which it was pre-exposed (Lovibond, Preston & Mackintosh 1984). Finally, when the CS is a complex compound cue, pre-exposure to the CS can facilitate, rather than retard, conditioning (Fanselow 1990; Bennett, Tremain & Mackintosh 1996). It has been proposed that this may be due to the additional time required to develop a coherent configural representation of the compound stimulus (Fanselow 1990).

3.2.2.4 Effects of Interference

External Inhibition: Conditioning to one CS (CS1) can be impeded by the presence of a second CS (CS2) if the CS2 is more salient or better predicts the US (Pavlov 1927; Wasserman & Miller 1997). These inhibitory effects on conditioning have been attributed to less attention being paid to CS1 due to it being considered irrelevant in comparison to either a pre-conditioned CS2 (blocking) or a more salient CS2 (overshadowing) (Jones, Gray & Hemsley 1990).

Blocking occurs when a pre-trained CS blocks the development of conditioning to a second CS when they are subsequently paired together with the US (Kamin 1969). The effects of blocking were of particular theoretical interest as they demonstrated that classical conditioning does not occur due to mere presentation of the CS and US together; rather the CS must provide some information regarding the likelihood of US occurrence. Blocking has been well-established in non-human models (Garrud et al. 1984; Schactmann, Kaspro, Chee & Miller 1985; Giannaris, Cleland & Linster

2002), and has also been reported in humans although the results are usually somewhat weaker (Kimmel & Bevill 1996; Martin & Levey 1991).

Overshadowing occurs when two (or more) CSs are simultaneously paired with the US, and one is more salient than the other (Pavlov 1927; Kehoe 1982; Blaisdell, Denniston & Miller 1998). Research has used overshadowing techniques to reduce the nausea associated with cytotoxic drug treatment in cancer patients. There is evidence that when a distinctive beverage is paired with drug treatment, it acquires the aversive properties of the treatment, and overshadows the development of aversion to the treatment context. Thus, later exposure to the context alone should result in reduced anticipatory- and post-treatment nausea (Stockhorst et al. 1998).

Conditioned Inhibition: In the above examples of blocking and overshadowing, conditioning to one CS is disrupted due to a preferential excitatory association between the US and an alternative CS. However, a CS can also acquire conditioned inhibitory properties when the CS predicts the absence of the US (Pavlov 1927). For example, in a standard conditioning trial, a well-established CS is paired with a US, and reliably comes to elicit a CR. However, if on some trials a second CS is introduced (CS2), and on these trials the US does not occur, the CS2 acquires the properties of a “conditioned inhibitor” (Pavlov 1927). This results in CR inhibition when the CS is presented in parallel with CS2 (Wagner, Mazur, Donegan & Pfautz 1980; Yeo, Hardiman, Moore & Russell 1984; Neumann, Lipp & Siddle 1997; Lambert & Whitehouse 2002; Kearns, Weiss, Schindler & Panlilio 2005).

3.2.2.5 Occasion Setting

Occasion setters are stimuli that are present during conditioning that may modulate the CS-US association, particularly when there is ambiguity surrounding the CS-US association (Bouton & Swartzentruber 1986; Holland 1992). Unlike conditioned inhibition, however, occasion setters are not assumed to enter into a direct association with the US. Occasion setters are not restricted to discrete stimuli present during conditioning training, but can be the environmental context in which the training takes place (Bouton and Swartzentruber 1986). Some theorists have described occasion setters as retrieval cues, which serve to “select” the most appropriate representation or “meaning” of the CS (Bouton & Swartzentruber 1986; Maren & Holt 2000); a phenomenon akin to context-dependent memory, whereby the retrieval of a memory is largely influenced by the context in which retrieval takes place (Tulving & Thomson 1973). Thus, occasion setters appear to serve as “rules of thumb”, providing information about when a CS is likely to signal a US and when it is not. Occasion setters may also play an important role in post-conditioning changes in the signalling value of the CS, as occurs during extinction training (Bouton 2004).

3.2.2.6 Extinction

Extinction refers to the gradual attenuation, and eventual termination, of an established CR that reliably occurs after repeated presentation of the CS without the US (Pavlov 1927). Rescorla and Wagner (1972) attributed this to an “unlearning” of the original CS-US association. However, under certain conditions the CR can be re-established, suggesting that the original learning is not eradicated (Pavlov 1927; Bouton 2002, 2004; Delamater 2004). In addition, context has a profound effect on

responding following extinction. For example, if conditioning occurs in one context and extinction in a different context, conditioned responding can be reinstated on return to the original context (renewal effect) (Brooks & Bouton 1994; Bouton & King 1993). It has been argued that during extinction an additional CS-no US relationship is learnt and that the context may serve as retrieval cue (or occasion setter) for either the CS-US or CS-no US association (Bouton 2004; Delamater 2002). Furthermore, the re-expression of an extinguished conditioned response to a CS can occur after re-exposure to the US alone (reinstatement) (Rescorla & Heth 1975) or spontaneously due to the mere passage of time (spontaneous recovery) (Rescorla 2004). Taken together, renewal, reinstatement and spontaneous recovery provide persuasive evidence that the CS-US association is not “unlearned” during extinction.

3.2.2.7 CS Generalisation Effects

Stimuli that are sensorily similar to a CS, but not identical to it may come to elicit CRs in a similar way to the original CS. For example, Pavlov (1927) noted that when a tone was trained to elicit a CR, similar tones of lesser or greater pitch spontaneously acquired the ability to also elicit CRs, and the strength of the generalised CR was related to the level of similarity to the original CS (Pavlov 1927). Stimulus generalisation has been replicated across species and response systems (Linster & Smith 1999; Devriese et al. 2000; Baldi, Lorenzin & Bucherelli 2004; Chandra & Singh 2005; Ginane & Dumont 2006). Findings have also demonstrated that stimulus generalisation can be attenuated by pre-exposure to the would-be generalised CS (Bennett, Wills, Wells & Mackintosh 1994; Chotro & Alonso 2003; Sanjuan Mdel, Alonso & Nelson 2006).

3.2.2.8 Higher Order Conditioning and Sensory Preconditioning

In both higher order conditioning and sensory preconditioning, a CS comes to elicit a CR without any direct association with the US. Sensory preconditioning involves the pairing of two stimuli (CS1 and CS2), prior to CS1 being paired with a US. When subsequently presented alone, the CS2 evokes a CR via its previous association with CS1 (Archer, Mohammed, Danysz, Jarbe & Jonsson 1986; White & Davey 1989; Muller; Gerber, Hellstein, Hammer & Menzel 2000). In a similar vein, in higher order conditioning, a CS, which through previous pairings with a US reliably evokes a CR, is paired with a second CS (CS2) (Holland & Rescorla 1975; Amiro & Bitterman 1980; Green & Schweitzer 1980). This results in the CS2 eliciting a CR, despite not being directly paired with the US.

3.2.2.9 Contingency Awareness

While it is generally accepted that for conditioning to occur there should be some degree of contingency between the CS and US, what is less clear is the extent to which knowledge of the CS-US contingency is necessary for conditioning. For example, Dawson and Biferno (1973) manipulated levels of contingency awareness of a CS (tone)-US (shock) relationship by means of verbal instructions. Clear conditioned effects were only observed in participants who demonstrated contingency awareness, and only at the point at which this awareness was expressed. Much of the early research examining contingency awareness utilised autonomic conditioning procedures, in which the general consensus was that conditioning only occurred in participants who were contingency aware (Dawson & Schell 1985; Davey 1987).

Devriese, Winters, van Diest and van den Bergh (2004) reported conditioning of odours in a differential conditioning paradigm, but only in participants who displayed CS-US contingency awareness. However, not all contingency aware participants displayed conditioning, suggesting that awareness was necessary but not sufficient to support conditioning.

There are some methodological concerns regarding the reliability and validity of awareness measurements in a conditioning paradigm. For example, concurrent measures of awareness are considered as more reliable as they are closer in time to the event and, thus less susceptible to forgetting than retrospective measures. However, concurrent measures may interfere with the test procedure, be sensitive to demand characteristics or, if not sufficiently masked, may prime participants to the contingency itself. Furthermore, there is some question regarding the validity of the ability to verbalise contingency as a measure of underlying contingency knowledge (see Lovibond & Shanks 2002 for review).

In non-autonomic preparations, there is evidence of conditioning without explicit contingency knowledge. For example, it is relatively common to obtain conditioned evaluative responses in the absence of contingency awareness (Martin & Levey 1978; Baeyens, Eelen & van den Bergh 1990; Kirsch, Lynn, Vigorito & Miller 2004; Walther & Nagengast 2006), although Field (2000) argues that evaluative conditioning may be a distinct form of conditioning with regards to its insensitivity to contingency awareness. However, evidence of conditioned effects with subliminal

stimuli (Öhman & Soares 1993; Soares & Öhman 1993,) suggests that conditioning can occur without contingency knowledge.

Finally, the influence of contingency awareness may vary with the nature of the conditioning paradigm. Ross and Nelson (1973) suggested that cognitive factors including contingency awareness may only be important in differential conditioning procedures rather than in single cue conditioning procedures. In addition, Clark and Squire (1998) demonstrated successful delay conditioning, but disrupted trace condition, when participants were classified as contingency unaware.

In sum, although there is some evidence of conditioning in the absence of CS-US contingency knowledge, a large body of research suggests that in many situations such contingency knowledge is necessary for the development of conditioning. However, there are problems associated with both concurrent (e.g. contingency priming) and retrospective (e.g. recall inaccuracies) measures of contingency awareness. Furthermore, when participants demonstrate contingency awareness, it is often difficult to elucidate whether the effects elicited by a CS are due to conditioning or expectancy. The importance of contingency awareness may also be influenced by the response system being measured.

3.3 CONDITIONED DRUG EFFECTS

Conditioning studies typically involve training laboratory animals to learn an association between a US and an arbitrary CS such as a tone, light or odour. This approach is advantageous because the properties of the CS (e.g. intensity, duration)

can be strictly controlled. In addition, the CS is unlikely to have been encountered previously, particularly in connection with the US, thus the likelihood of interference effects due to CS pre-exposure or conditioning history are reduced. However, it is likely that people and other animals learn about relationships between natural stimuli that co-occur in their environment (Domjan 2005). For example, it is common for chemotherapy patients to experience nausea in the presence of cues that have been associated with their treatment (Bovbjerg 2006; Stockhorst et al. 2006). Similarly, there is substantial evidence showing that drug effects can act as a US and be conditioned to associated stimuli, however the nature of the CR may vary (Eikelboom & Stewart 1982). The following section reviews the drug conditioning literature, detailing studies that have found CRs that are drug-like and those that have reported CRs that are drug-opposite to the original drug effect. Factors that may influence the direction and nature of the CR are also discussed.

3.3.1 Drug-like conditioned responses

Pavlov (1927) was the first to condition drug effects by pairing the administration of morphine with a neutral stimulus (tone) in dogs. The morphine injection alone induced salivation, and after repeated pairings, the neutral tone began to elicit drug-like salivation when presented alone. In subsequent tests, it was found that the stimuli associated with the injection procedure, e.g. syringe, skin swab, experimenter, had also acquired the ability to elicit salivation.

More recently, drug-like CRs to the locomotor activating effects of psychostimulants such as cocaine (Carey & Gui 1998a; Adams, Careri, Efferen & Rotrosen 2000;

Tirelli, Michel & Brabant 2005), amphetamine (Pickens & Crowder 1967; Tirelli & Terry 1998; Ahmed, Oberling, Di Scala & Sandner 1996; Martin-Iverson & Fawcett 1996) and apomorphine (Godoy & Delius 1999; Tirelli & Heidebreder 1999) have been shown in rats. Iso-directional conditioned drug effects have also been obtained with drugs not generally considered to be stimulants, such as morphine, when these drugs produce stimulatory effects on behaviour measures including locomotor activity and salivation (Hayashi, Ohashi & Tadokoro 1980; Badiani, Oates & Robinson 2000). This seems to suggest that iso-directional CRs are expressed when the US has a stimulatory effect on behaviour.

Conditioned drug-like effects have also been reported in humans. For example, Muntaner et al. (1989) reported that an intravenous infusion procedure that had previously been used to administer cocaine acquired the ability to elicit conditioned cardiovascular responses similar, albeit of a lesser magnitude, to cocaine. More recently, Foltin and Haney (2000) reported cocaine-like increases in heart rate and blood pressure in experienced cocaine users who were presented with a set of cues that had been repeatedly paired with cocaine. Furthermore, these responses decreased across subsequent extinction trials, which is consistent with the suggestion that these effects were due to conditioning. Enhanced craving for cocaine was also observed in response to presentation of drug-paired stimuli compared with placebo-paired stimuli. This finding is consistent with the assertion that drug-paired stimuli can acquire incentive-motivational properties that can sustain drug use or initiate relapse (Robinson & Berridge 2003; See 2005).

Drug-like conditioned responding in humans may explain the findings of laboratory-based cue reactivity studies (Drummond 2000). Many studies have reported drug-like responding and craving in the presence of drug-related paraphernalia in drug users (Ehrman, Robbins et al. 1992; Avants, Margolin, Kosten & Cooney 1995; Drummond 2000). In addition, drug addicts often report craving in the presence of drug-related cues in real-world situations (Siegel 1999).

It has also been argued that summation of iso-directional CRs with the drug response results in a progressive potentiation of the drug effect (Tilson & Rech 1973; Hayashi et al. 1980), which may explain some instances of sensitisation to the effects of drugs. In support, it has been reported that gradual increases in the magnitude of the drug response occur with repeated drug exposure in the presence, but not the absence, of drug-related cues (Tilson & Rech 1973; Post, Lockfeld, Squillace & Contel 1981; Weiss, Post, Pert, Woodward & Murman 1989; Quandros, Souza-Formigoni, Nobrega & Oliveira 2003). Moreover, evidence of context specific sensitisation indicates that sensitisation is not solely due to neural adaptations as a result of repeated drug exposure. Finally, sensitisation may be attenuated by extinction trials, as would be predicted by conditioning theory (Battisti, Uretsky & Wallace 2000).

3.3.2 Drug-opposite conditioned responses

Siegel (1978) first demonstrated that stimuli repeatedly paired with morphine administration elicited hypothermia in rats in the absence of drug, a response that was opposite to the acute hyperthermic effect of morphine. Siegel (1975) referred to such responses as “conditioned compensatory responses” (CCR), and suggested that they

counteract a homeostatic disturbance caused by the drug or are made in anticipation of drug presentation (Macrae, Scholes & Siegel 1987). CCRs have been observed following drug-induced disturbance of several homeostatically regulated systems including temperature (Siegel 1978; Lê, Poulos & Cappell 1979; Mansfield & Cunningham 1980), heart rate (Newlin 1986; Macfarlane & White 1989; Schwartz-Stevens & Cunningham 1993) and blood glucose (Siegel 1975b), and in anticipation of analgesic (Siegel 1975a) and eyeblink reflex effects of drugs (Andrews, Blumenthal & Flaten 1998; Flaten, Simonsen, Waterloo & Olsen 1999).

CCRs have also been observed in spontaneous ongoing behaviours that are not homeostatically regulated, nor have an absolute baseline level of responding, such as locomotor activity and cognitive performance. For example, Duncan, Alici and Woodward (2000) administered alcohol or saline to rats in two distinctly different environments. After repeated pairings, the rats were given saline in the environment previously paired with alcohol, and significant increases in locomotor activity, indicative of a conditioned compensatory response, were observed. A similar finding has been shown to the detrimental effects of alcohol on cognitive performance in humans (Shapiro & Nathan 1986).

It has been proposed that the antagonistic CCR strengthens as a result of repeated CS-US pairings and increasingly attenuates the drug effect, which could explain some forms of drug tolerance. In support, Siegel (1975) found environmental-specific tolerance to the analgesic effects of morphine. Tolerance was only evident in rats that were tested for pain sensitivity in the same environment in which they had received

morphine (test environment). In contrast, rats that had received the same number of morphine infusions in the home cage, showed no evidence of tolerance when subsequently tested in the test environment.

Situational specific tolerance to the effects of drugs has also been demonstrated in humans. Social drinkers were found to show tolerance to the tachycardic effects of ethanol in an environment previously paired with ethanol administration, but not in an environment previously paired with placebo (Dafters & Anderson 1982). These findings have been replicated in rats and humans using various drugs including morphine (Mucha, Volkovskis & Kalant 1981; Shapiro, Dudek & Rosellini 1983), nicotine (Epstein, Caggiula & Stiller 1989), carisoprodol (Flaten, Simonsen, Waterloo & Olsen 1997), naloxone (Goodison & Siegel 1995), alcohol (Le et al. 1979) and haloperidol (Poulos & Hinson 1982).

A conditioning explanation of tolerance is further supported by studies showing that procedures that retard or potentiate the development of a CR similarly affect the development of tolerance. For example, tolerance has been shown to undergo extinction (Siegel 1975a), spontaneously recover following extinction trials (Millin & Riccio 2002), and be attenuated by CS pre-exposure (latent inhibition) (Tiffany & Baker 1981), and by the introduction of a novel stimulus (external inhibition) (Siegel 1999).

It has been proposed that CCRs may contribute to continued drug use and/or relapse (Poulos & Cappell 1991; Siegel 1999). Greater behavioural and physiological

symptoms of drug withdrawal in the presence of previously drug-related stimuli have been reported, and Poulos and Cappell (1991) argue that these withdrawal symptoms are CCRs that have not been offset by the effects of the drug. This is supported by the observation that withdrawal symptoms are often qualitatively opposite to the direct effects of the drug (Siegel 1999).

3.3.3 Predicting the direction of the CR

Understanding the factors that may affect whether a drug-like or drug-opposite CR is expressed is vital to be able to predict the direction of the CR. One factor that may have particular bearing on the direction of the CR is the nature of the unconditioned drug effect (Domjan, 2005). In general, depressant effects or effects that perturb well-regulated physiological systems tend to initiate compensatory responses that can be conditioned and expressed as CCRs (Siegel 1975a; 1978). In contrast, drug effects that do not disrupt homeostasis or have stimulant effects tend to condition drug-like responses (Ahmed et al. 1996; Tirelli and Terry 1998). For example, the conditioned effects of context on locomotor activity have been reported to be drug-like when the unconditioned drug effect is one of psychostimulant activation (Foltin & Haney 2000; Tirelli et al. 2005), but have been reported to be drug-opposite when the unconditioned effect is the depressant action of alcohol (Duncan et al. 2000). Furthermore, it has been reported that tolerance rapidly develops to the aversive, but not euphoric effects of drugs (Babbini & Davis 1972; McAuliffe & Gordon 1974; Esposito & Kornetsky 1977; Collier 1980), suggesting that physiological systems initiate mechanisms to counteract negative, but not positive, effects of drugs (Stewart & Badiani 1993). CCRs have also been observed in response to drug effects that



cause perturbations in well-regulated physiological systems, with no CCRs observed in response to positive subjective drug effects that are not explicitly regulated by a homeostatic mechanism (Rozin, Reff, Mark & Schull 1984; Ehrman, Ternes et al. 1992).

While consideration of the nature of the UR may enable predictions to be made concerning the direction of the CR, it should be noted that theoretically it has been argued that all CRs are similar to the UR (Pavlov 1927). Eikelboom and Stewart (1982) have argued that failure to correctly identify the US and the UR explains why some CCRs may be mistakenly referred to as drug-opposite. These authors suggest that the US and the UR are often identified as the drug and the drug effect respectively, when in fact the drug effect is the US and the compensatory adjustment is the UR (Eikelboom & Stewart 1982). For example, in the case of morphine conditioned hyperanalgesia, the analgesic effect of morphine acts at the US, which instigates a compensatory hypoanalgesic response (UR) that is conditioned to the stimuli present at the time of morphine administration. Therefore, the CR is qualitatively similar to the UR as would be predicted by Pavlov (1927) and SST.

However, although the Eikelboom and Stewart (1982) model provides a reasonable account of the data, it is often difficult to determine the locus of the drug action. Identification of the UR/CR in a study of autonomic responding is relatively straightforward, as the drug often induces a deviation from a homeostatic “optimum”. However, with behaviours such as locomotion, the drug-induced response is not a “novel” response that deviates from an absolute baseline; rather it involves a

modulation of an ongoing behaviour (Damianopoulos and Carey 1994). Furthermore, the model seems to have trouble accounting for the finding that different CRs may be elicited in the same response system depending on the type of CS used. For example, Staiger and White (1988) reported conditioned compensatory cardiac effects when participants were tested in a room that had been paired with alcohol, but drug-like cardiac effects following presentation of a drug-paired drink cue. Eikelboom and Stewart (1981) found similar effects of morphine in rats, with conditioned hyperthermia occurring in response to discrete environmental cues and conditioned hypothermia occurring in response to temporal cues.

These findings support the argument that two types of conditioned response exist (Konorski 1967): a “consummatory” response that substitutes the UR (i.e. elicits a response in the same direction as the UR); and a “preparatory” response that enables the organism to deal with the US in the most efficient way, which can lead to responses that minimise the impact of the US (i.e. CRs that are opposite to the UR). Furthermore, the data suggest that the nature of the CS may be a factor in determining whether a consummatory or preparatory conditioned response is observed.

It should also be noted that unconditioned drug effects can be biphasic and often affect multiple physiological systems. These effects may change over time, may vary with dose, and can interact with each other. Different conditioned responses may develop to different aspects of the unconditioned drug effect at different times, further complicating the predictability of the CR (Stewart, de Wit and Eikelboom 1984).

3.4 ESTABLISHING A ROLE OF CLASSICAL CONDITIONING

The previous section summarised the evidence suggesting that drug effects can be conditioned to drug-associated stimuli, and that conditioned drug responses can be in a similar or opposite direction to the drug effect. There are however, inconsistencies in the drug conditioning literature, with some studies finding little or no evidence of conditioning and others reporting findings that conditioning theory has difficulties accounting for. Collectively, these studies have raised questions as to whether the effects obtained in conditioning experiments can be attributed to classical conditioning processes or whether they can be explained by other associative and/or non-associative mechanisms (Kesner & Cook 1983; Macrae, Scoles & Siegel 1987; Damianopoulos & Carey 1992; Ahmed, Cador, LeMoal & Stinus 1995; Montgomery & Kirsch 1997; Niaura 2000; Ohmori, Abekawa, Ito & Koyama 2000). The following section discusses these issues, and addresses methodological factors that may have contributed to the lack of conditioned effects in some studies.

3.4.1 Methodological considerations of drug-conditioning studies

While there have been many reports of conditioned responding to cues associated with drug use (see sections 3.2.1 and 3.2.2) some studies have failed to demonstrate CRs in tests of conditioning (Shapiro, Dudek & Rosellini 1983; Hakan & Ksir 1988). The development of conditioning may, in part, depend on the response measured and drug dose used, as a single drug can elicit effects on multiple biological systems that can have different temporal profiles, which, in turn, may display different conditioning properties (Paletta & Wagner 1986).

The progression from the study of autonomic drug effects (Pavlov 1927; Eikelboom & Stewart 1982) to more complex behavioural effects such as locomotor activity in animals (Tirelli and Terry 1998; Tirelli et al. 2005) or subjective effects in humans (Foltin & Haney 2000) is also a potential source of increased variability in findings. Behaviours that display robust levels at baseline may obstruct the detection of small conditioned changes and thus increase the likelihood of a Type II error (Duncan et al. 2000). The analysis of such behaviours is further complicated by the fact that they are influenced by other factors associated with multiple testing including stress, novelty and habituation (Carey & Gui 1997). Discrepancies may also arise due to variance in the measure used. For example, many studies have examined drug effects on motor activity in rodents, however motor behaviour can take various forms including grooming, rearing, climbing, magazine entries etc, which are not necessarily equivalent behaviours and may be differentially affected by drugs and conditioning procedures. Damianopoulos and Carey (1994) found that cocaine decreased grooming behaviour but increased levels of rearing, and subsequent CRs to cocaine-paired stimuli mirrored these differential effects. In addition, Martin-Iverson and Fawcett (1996) found that of 26 behaviours affected by amphetamine, only eight of these were similarly expressed by drug-paired stimuli at a test of conditioning. Why some behaviours were more susceptible to the development of a CR than others is unclear, but emphasises the importance of considering the reliability of the behavioural measure in the interpretation of results.

The nature of the CS may also play a role in determining whether a CR is expressed. There is evidence that a naturalistic cue (e.g. sight or smell of food) will produce more

robust conditioning than an arbitrary stimulus (see Domjan 2005). This could be in part because a naturalistic CS is more salient or biologically relevant (Miller & Matute 1996). For example, stimuli that elicit a strong orientation response, such as the sight of a predator, are more likely to be attended to than a light or a tone. Moreover, the functional perspective on conditioning (Domjan 2005) contends that taste cues are a particularly effective CS for the post-ingestive effects of food or drugs. One implication of this is that drug vehicles that comprise strong sensory elements (e.g. a strong tasting beverage) may support stronger conditioning than, for example, a capsule, as they may form particularly strong associations with the post-ingestive effects of the drug. In addition, it has previously been reported that a CS of high intensity can block conditioning to another CS of lesser intensity, and therefore blocking may occur to an environmental CS when a salient taste cue is present (Feldman 1975).

A CS may also have different conditioning properties depending on whether it is a property of the US (e.g. taste of a drug-containing drink) or a separate entity (e.g. context cue) (Paletta and Wagner 1986). Meachum and Bernstein (1990; 1992) demonstrated qualitatively different CRs to a discrete CS that was a component of the US (taste) compared to a contextual CS, when associated with the toxin, lithium chloride in rats. The discrete cue elicited behaviour that was remarkably similar to the effects of the drug (lying on abdomen), whereas the contextual cue induced freezing behaviour, which was interpreted as an anticipatory avoidance response (Meachum & Bernstein 1992).

Other factors that have been argued to affect the likelihood of observing conditioning include: the relative novelty of environmental stimuli (Dafters & Bach 1985; Badiani, Browman & Robinson 1995); individual differences (Stockhorst et al. 1999); the inter-stimulus interval (Broadbent & Cunningham 1996); and the CS duration (Schwarz-Stevens & Cunningham 1993).

Finally, while many studies have examined the learning of associations between drug effects and exteroceptive cues, it is also the case that conditioned associations can occur between drug effects and other types of cues, including interoceptive cues induced by drug-elicited changes in the internal environment (Poulos, Hinson & Siegel 1981; Kim, Siegel & Patenall 1999) and temporal cues (Arvanitogiannis, Sullivan & Amir 2000). These conditioned associations may occur alongside, and thus interfere with, conditioning to external stimuli. Eikelboom and Stewart (1982) reported conditioned hyperthermia to environmental cues and conditioned hypothermia to temporal cues after morphine administration in rats. The authors argued that if they had not selectively examined the responses to each cue type, the net effect would have been no change in behaviour and the conclusion that no conditioning had occurred.

3.4.2 Evaluating the role of conditioning in supposed conditioned drug effects

Findings have been reported from drug conditioning studies that are discrepant with a conditioning interpretation. For example, CRs have been shown to differ qualitatively from the acute drug effect. Rotational CRs elicited in the presence of cocaine-paired contextual cues have been shown to be dissimilar to the motor responses elicited by

cocaine itself (Damianopoulos & Carey 1992), and rats have developed CRs that were in a similar direction to the effects elicited by lithium chloride (suppression of activity), but were qualitatively different (Damianopoulos & Carey 1992).

Many studies have reported CRs that are much weaker than the drug effect (see Ohmori, Abekawa, Ito & Koyama 2000), but similar in magnitude to the level of responding observed in naïve animals on first exposure to the test environment (Martin-Iverson & McManus 1990; Damianopoulos & Carey 1992). Consequently, it has been suggested that so called conditioned effects may be explained by differential habituation to test environment in the group that receives drug in the test context (paired group) versus the group that receives drug outside of the test context (unpaired group). Specifically, it is argued that the behaviour of the unpaired group habituates to the test context whereas the drug interferes with habituation in the drug-paired group such that on the test day when both groups receive placebo, the activity in the paired group is greater than the unpaired group and is wrongly attributed as CRs. Damianopoulos and Carey (1992) found greater locomotor activity in a group of rats given cocaine in an environment previously paired with the drug relative to an unpaired group that had not received cocaine in the test environment. However, this difference was not due to a reliable increase in responding in the paired group (i.e. sensitisation) from the initial response. Rather, it was due to a decline in locomotion in the unpaired group that accounted for the group differences at test.

In addition, the role of conditioning in drug sensitisation and tolerance has similarly been questioned. Procedures that weaken conditioning by reducing the importance of

the context (e.g. extinction, latent inhibition) often do not fully abolish the development of tolerance or sensitisation as the conditioning model would predict (Carey & Gui 1998a; Drew & Glick 1988; Reimer & Martin-Iverson 1994; Anagnostaras & Robinson 1996). As little as 1 hour of CS pre-exposure inhibited the development of a conditioned rotational response to amphetamine, but had no effect on the development of sensitisation in a distinct environment (Crombag et al. 2001). Alternatively, it has been argued that sensitisation occurs via a non-associative mechanism, whereby the environment acts as a stressor and potentiates the drug effect. In support, sensitisation is observed more robustly in novel environments, and neurobiological evidence indicates that a novel environment is stress-inducing (Badiani et al. 1998). An alternative associative account of sensitisation suggests that contextual cues act as “occasion setters” that modulate the expression of sensitisation independently of a direct association with the US (Anagnostaras & Robinson 1996).

Alternative explanations of conditioned tolerance include behavioural tolerance, which involves the learning of a strategic response that counteracts drug-induced behavioural disturbances. For example, the experienced drinker learns to adjust his/her stance to offset the loss of balance induced by alcohol. Here, the attenuated response is not due to a CR that compromises the pharmacological efficacy of the drug. Rather, an alternative behaviour is learned and improved through practice, which compensates for the drug effect. Hayes and Mayer (1978) claim that Siegel (1976) prematurely attributed his findings of environment-specific tolerance of morphine analgesia in rats to conditioning, when the findings could be explained by behavioural tolerance.

Furthermore, a number of studies have functionally dissociated conditioned responding from tolerance and/or sensitisation. There are reports of situational-specific tolerance and sensitisation without evidence of CRs in placebo challenge tests (Shapiro, Dudek & Rosellini 1983; Hakan & Ksir 1988; Cepeda-Benito, Tiffany & Cox, 1999). Although Tirelli, Michel and Brabant (2005) found both sensitisation and drug-like CRs to the locomotor activating effects of cocaine in rats, the CR persisted for longer than the sensitised effect. If the CR was important for the expression of sensitisation, then a sensitised effect should have occurred as long as a CR was evident. Moreover, the within-session time courses of sensitisation and the CR did not occur in parallel as the conditioning model would predict. Kosowski and Liljequist (2005) also observed sensitisation prior to the expression of a drug-like CR. Also, it has been reported that haloperidol attenuated sensitisation of cocaine-induced locomotor activity without affecting the CR, suggesting a pharmacological dissociation (Reimer & Martin-Iverson 1994).

In contrast, there have also been reports that the CR responds in a way that conforms to the principles governing classical conditioning. For example, the CR has been shown to: extinguish following CS presentation without the US (Macfarlane & White 1989; Tirelli & Heidebreder 1999); decay with time (Barr et al. 1983); be retarded by CS pre-exposure (latent inhibition) (Norman & Cassaday 2004); be contingent on the level of reinforcement (Macrae et al. 1987); be disrupted by the introduction of a novel stimulus (external inhibition) (Macrae et al. 1987); and to generalise to a similar stimulus (Weiss, Post, Woodward & Murman 1993). However, few studies have

explicitly examined whether a conditioned response can be modified according to a range of classical conditioning principles.

3.5 CONDITIONED EFFECTS OF CAFFEINE

Studies have demonstrated that the effects of caffeine can be conditioned to stimuli paired with its administration. For example, cues associated with caffeine ingestion (e.g. taste and smell of coffee) can increase physiological and subjective arousal (Flaten and Blumenthal 1999), and improve cognitive performance (Zwyguizen-Doorenbos et al. 1990) similar to caffeine. However, it is often difficult to elucidate whether these effects are conditioned effects or expectancy effects based on previous experience or the social perception of caffeine as a stimulant drug.

The use of arbitrary cues that have no prior association with caffeine however, provides more convincing evidence for a role of conditioning. In non-human animals, these effects have predominantly been studied using conditioned place preference procedures in which one context is paired with caffeine and another with placebo. The animals are subsequently allowed free access to both contexts. Significantly greater amounts of time spent in the caffeine-paired (relative to placebo-paired) context is taken as evidence of a conditioned place preference, indicating that the positive effects of caffeine have become associated with the context in which it was administered (Bedingfield, King and Holloway 1998; Brockwell, Eikelboom and Beninger 1991; Patkina and Zvartau 1998). Conditioning of the reinforcing effects of caffeine has also been shown in humans in studies which pair novel flavours with caffeine and placebo administration. Flavour preferences are acquired over time to

novel caffeine-paired flavours compared to novel placebo-paired flavours (Yeomans et al. 1998; Yeomans et al. 2000; Tinley Yeomans & Durlach 2003; Yeomans, Durlach & Tinley 2005). However, these conditioned effects in humans seem to be somewhat dependent on a state of caffeine withdrawal in regular caffeine consumers (Yeomans, Spetch & Rogers 1998; Tinley et al. 2003; Yeomans, Ripley, Lee and Durlach 2001).

In sum, the conditioned effects of caffeine on physiological and cognitive responses have predominantly examined cues already associated with caffeine (e.g. a cup of coffee) and studies employing previously-neutral cues have predominantly examined changes in evaluative responses. In contrast, little research has examined physiological, cognitive and mood effects of previously-neutral stimuli paired with caffeine. Finally, the results of taste preference studies suggesting that the reinforcing effects of caffeine can be conditioned to caffeine-paired drink cues, further supports the use of caffeine in a conditioning paradigm.

3.6 AIMS AND OBJECTIVES

In summary, there is a paucity of studies examining whether drug-induced facilitation of cognitive and psychomotor performance can be conditioned to drug-associated environmental stimuli. Furthermore, there remains some contention as to the extent that reports of conditioned drug effects are actual conditioned responses or may be explained by alternative mechanisms. The aim of this thesis was to investigate whether drug-associated environmental stimuli could elicit caffeine-like facilitation of performance and mood. To evaluate the role of conditioning in these effects, studies

were planned to assess whether the apparent conditioned response: extinguished following CS alone presentations; was retarded by CS-pre-exposure; generalised to similar CS and was blocked by a pre-trained CS; as would be predicted by conditioning theory.

CHAPTER 4:

GENERAL METHODS

This section describes methodological factors that were common to all of the experiments in this thesis.

4.1 PARTICIPANTS

Participants were staff or students of the University of Birmingham who took part in return for either cash or course credits. Several methods of recruitment were employed, including poster and campus newspaper advertisements. In addition, recruitment drives were held in the student union at which willing participants registered on a campus-wide database to be contacted when studies occurred. In order to disguise the nature of the study, the studies were advertised as “effects of over-the-counter drugs” and participants were told that they may receive one of several possible substances at each session: caffeine, paracetamol, aspirin or ibuprofen (with the exception of Experiment 2 – see Chapter 6). Participants were those who were first to meet the inclusion criteria.

For each session, participants were asked to refrain from consuming psychoactive substances (including caffeine and alcohol) after 11pm the previous night; sessions were rescheduled if participants reported non-compliance. On arrival at the laboratory for testing, participants gave verbal confirmation that they had adhered to this requirement. To promote compliance with abstinence, a saliva sample was taken at the start of each session, although this was not analysed (Richardson et al. 1995).

From Experiment 3 onwards, additional procedures were used to promote compliance: a reminder email was forwarded to each participant the day before an experimental session, and participants were asked to sign a slip confirming that they had abstained overnight from all psychoactive substances.

4.2 INCLUSION AND EXCLUSION CRITERIA

Participants were non-smoking, regular caffeine consumers. Consumption levels were estimated by means of a screening questionnaire emailed at the time of initial contact, and a further questionnaire was administered during the first experimental session. The data from that latter questionnaire are reported as the most recent estimate. The screening questionnaires asked about the use of over-the-counter drugs in line with the study advertisements. Participant's reports of caffeine use were converted into estimated mg/day consumption quantities. The caffeine contents of drinks (per serving) were estimated as: coffee - 80 mg; tea - 50 mg; cola - 30 mg (James 1991; Garattini 1993; Richardson, Rogers, Elliman & O'Dell 1995). Unless otherwise stipulated, participants were required to consume at least 200 mg/day of caffeine (Rogers et al. 1995).¹ Participants were required to be in good health and not taking any prescribed medication (except the contraceptive pill), with normal or corrected to normal vision. Participants were not excluded based of their body mass index (BMI), but BMI was considered in the data analyses, as differences in BMI may

¹ Although all participants met the inclusion criteria for caffeine consumption on the initial questionnaires, these ratings were estimates and occasionally differed from the second measure. Therefore, the range of consumption levels given in the method sections may fall below the stipulated mg/day criterion. 30 mg/day difference was allowed for participants to continue in the study, but if their estimates were more than 30 mg/day lower, they did not take part.

affect drug absorption and therefore drug potency at the time of test (Cohen, DiBiasio, Lisco & Hurford 1997; Miya et al. 2001).

4.3 CAFFEINE ADMINISTRATION

Caffeine (Sigma-Aldrich Co, Poole, UK) was administered in gelatine capsules (Shionogi Qualicaps, size-00, Madrid, Spain) with water (100 ml). The placebo capsule contained arrowroot matched for the weight of caffeine (Supercook, Leeds, UK). The dose of caffeine used in each study is noted in the relevant method section. All capsule administration was double-blind.

4.4 MEASURES

Psychomotor and cognitive performance: The performance tasks varied across the experiments, so they are described in detail in the relevant experimental sections.

Mood: Mood was assessed using a questionnaire adapted from Rogers et al. (1995) that was designed to assess the effects of caffeine. Sixteen adjectives (“Friendly”, “Alert”, “Cheerful”, “Drowsy”, “Anxious”, “Energetic”, “Angry”, “Muddled”, “Calm”, “Tired”, “Dejected”, “Tense”, “Clear-headed”, “Relaxed”, “Thirsty” and “Jittery”) were rated on a 100-mm visual analogue scale from 0 mm (*not at all*) to 100 mm (*extremely*). In addition, “Headache” was added as a seventeenth item (Experiments 2-6), as several studies have reported that increased headache is often associated with caffeine withdrawal (e.g. Smit and Rogers 2002). Participants were

told to consider each line as the extreme spectrum of that emotion and to bisect the line appropriately to show how they were feeling at that specific moment.

Heart rate and blood pressure: For Experiment 1, heart rate was measured by palpation of the radial pulse (0 and 30 minutes post-capsule ingestion). For experiments 2 to 4, a Dinamap Pro-series 100-400 cardiovascular monitor (Gemedical Systems, France) was used to measure heart rate and systolic and diastolic blood pressure. This entailed an inflatable cuff being placed round the upper arm of the participant. Recordings were taken twice before and twice after capsule ingestion. The first recording acted as a habituation trial for participants to become accustomed to the procedure, the second recording constituted the pre-drug baseline, the third and fourth recordings were taken at 30 and 45-50 minutes post ingestion respectively to track any temporal changes. These measures were dropped from Experiments 5 and 6 due to the absence of reliable effects in the previous experiments.

4.5 POST-STUDY QUESTIONNAIRES

Two additional questionnaires were completed by the participants: one at the end of each experimental session (post-session questionnaire – PSQ); and one after all experimental sessions had been completed (post-experiment questionnaire – PEQ).

4.5.1 Post-session questionnaires

The PSQ asked participants what substance they thought that they had received during that session. A list of options were presented and participants opted by placing a tick next to the relevant choice. In line with the study information, several over-the-counter drugs (aspirin, caffeine, ibuprofen and paracetamol) were given as options in

addition to placebo and “other/don’t know”. A second question asked participants whether they had felt any effects of the capsule (response: tick yes or no), and if so to describe these effects.

4.5.2 Post-experiment questionnaires

Using open-ended questions, participants were asked what they thought the study was testing and what aspects of the procedure made them think this. For the conditioning experiments (Experiments 1, 4, 5 and 6), participants were also asked if they had been aware of any changes in the procedure during the study. Participants were also asked to describe the effects that they believed each of the aforementioned over-the-counter drugs (aspirin, caffeine, ibuprofen and paracetamol) had on mood, cognition, heart rate and blood pressure. For Experiments 4, 5 and 6, questions were added asking participants if they experienced caffeine withdrawal upon abstinence, and if so had they experienced caffeine withdrawal during the experimental sessions. Finally, at the end of the PEQs used in Experiments 5 and 6, participants were informed that they had received caffeine in one cubicle and placebo in the other. They were then asked if they could identify the cubicle in which they had received caffeine.

4.6 DATA ANALYSIS

Statistical analyses were conducted using the SPSS statistical package (version 11.5) for windows.

A principle components analysis (PCA) was performed on the mood items to reduce the number of possible statistical tests. Analysis of the 17 mood items using a

varimax rotation revealed five factors with eigenvalues greater than 1 that accounted for 69% of the variance. Items were retained on a factor if the loading was greater than .5 and less than .4 on all other factors. The five factors were: “Tense negative mood” (items loaded onto this factor were: Tense, Jittery, Angry, Dejected), “Sleepiness/hydration” (items loaded onto this factor were: Drowsy, Tired, Thirsty), “General positive mood” (items loaded onto this factor were: Energetic, Cheerful, Friendly) and “Mental clarity” (items loaded onto this factor were: Alert, Clear-headed, Muddled) “Mental repose” (items loaded onto this factor were: Calm, Relaxed). Participant’s scores for each factor were calculated by adding the scores of the all items loading onto the factor. Two ratings did not load clearly onto any factor (anxiety and headache) and were analysed separately.

For mood and cardiovascular measures “change-from-baseline” scores were calculated by subtracting the post-session scores from the baseline scores.

Measurements were taken at 0, 30 and 50 minutes post-caffeine ingestion, resulting in three change scores (0-30, 0-50 and 30-50 minutes). On most occasions the 0-30 and 30-50 data either produced no significant effect or was similar to the 0-50 data. Thus, for brevity, the 0-50 data is reported. On occasions where there was an additional significant effect at the 0-30 or 30-50 time point, these data are reported with the relevant time point given in parenthesis.

CHAPTER 5:

EXPERIMENT 1: CONDITIONED PERFORMANCE, SUBJECTIVE AND CARDIOVASCULAR EFFECTS OF CAFFEINE

5.1 INTRODUCTION

Studies of the conditioning of drug effects have demonstrated that stimuli paired with drug ingestion can acquire the ability to elicit drug-related conditioned responses (Pavlov 1927). These conditioned responses can be opposite to the original drug effect, particularly in situations where the drug has negative effects or perturbs a homeostatically regulated physiological system (Siegel 1975a, 1978; Mansfield & Cunningham 1980; Newlin 1986; Macfarlane & White 1989). In contrast, drug-related stimuli have been reported to elicit drug-like physiological effects and craving (Pickens & Crowder 1967; Ehrman, Robbins et al. 1992; Carey & Gui 1998a; Foltin & Haney 1999). Although conditioned drug effects have been well-studied, there is a paucity of literature examining the conditioned effects of drugs on psychomotor and cognitive performance. Shapiro and Nathan (1986) demonstrated a conditioned compensatory response to the deleterious effects of alcohol on performance, and Zwyghuizen-Doorenboos et al. (1990) reported caffeine-like improvements on vigilance performance after decaffeinated coffee, although the latter was not a study of conditioning and furthermore the effects may have been influenced by expectancy or other constituents of coffee. Therefore, the present study was designed to examine whether caffeine-like improvements on performance could be conditioned to stimuli previously paired with caffeine ingestion.

A typical between-subjects conditioning paradigm was used (Robbins & Ehrman 1992) as has been employed frequently in both animal (Tilson & Rech 1973; Carey & Gui 1998b; Adams et al. 2000; Tirelli et al. 2005) and human (Newlin 1986; Shapiro & Nathan 1986; Macfarlane & White 1989; Yeomans et al. 2000) conditioning studies. A group of caffeine consumers attended four conditioning trials at which they received caffeine in a test cubicle (caffeine-paired group). This provided an opportunity for a learned association to be formed between the effects of caffeine and the environmental context. On the final session, participants were administered placebo in the test cubicle. These effects were compared against a control group who received placebo in the test cubicle on all sessions (caffeine unpaired group). A between-subjects paradigm is preferential over a within-subject paradigm (in which one CS is paired with drug and a matched CS is paired with placebo) as learning in the drug-paired context in a within-subjects paradigm cannot be isolated from learning in the placebo-paired context. Consequently, factors such as stimulus generalisation and/or inhibition may attenuate or obscure conditioning in a within-subjects design. Therefore, although within-subjects paradigms have been used successfully to demonstrate conditioned effects of drugs (Foltin & Haney 2000; Field & Duka 2001), a between-subjects design was considered preferential initially. To control for pseudo-conditioned effects (i.e. significant effects at the conditioning test due to mere exposure to the US and not to a CS-US association), the groups received the converse capsule at the end of each conditioning trial in a different location. This ensured that by the conditioning test all participants had received equal amounts of drug; the only group difference was the relationship between drug administration and CS exposure.

This procedure replicated that of a pilot experiment conducted by Attwood, Terry and Higgs (2004), in which two groups of overnight-withdrawn participants received either 250 mg of caffeine or placebo on four sessions in a constant test environment. The dose was selected because previous studies have reported facilitatory effects of caffeine on psychomotor and cognitive performance at doses ranging from 200 to 300 mg (Lieberman et al. 1987; Roache & Griffiths 1987; Zwyghuizen-Doorenbos et al. 1990; Kerr, Sherwood & Hindmarch 1991; Lieberman, Tharion, Shukitt-Hale, Speckman & Tulley 2002; van Duinen, Lorist & Zijdewind 2005). Overnight-withdrawn regular caffeine consumers were recruited as they are reportedly particularly sensitive to the effects of caffeine (James & Rogers 2005)

In the Attwood et al. (2004) study, the group that received caffeine paired with the test context showed faster reaction times, relative to the unpaired group, and this advantage persisted in a subsequent conditioning test, at which both groups received placebo in the test context. However, baseline measures were not taken, and although there is little reason to expect that group performances differed significantly at baseline, this cannot be ruled out. Therefore, the present study incorporated baseline measures, which were completed in a different test cubicle, thus reducing the possible influence of latent inhibition (see Section 3.2.2.3). In addition, the earlier study was conducted single-blind and therefore the effects may also have been influenced by experimenter bias. Thus the present study used a double-blind procedure.

The present study administered caffeine and placebo via capsules as opposed to a novel juice vehicle that was used in the pilot study (Attwood et al. 2004). The use of

capsules minimised the possibility that the participants would detect the absence of drug on the conditioning test in the paired group. Also, because the capsules could be made up in advance, there was no need for a second experimenter to be present on each test day to make up the drinks. Finally, because the beverage had a strong, unusual taste, and taste cues are reported to be particularly salient for the conditioning of post-ingestive effects (Domjan 2005), the juice drink CS may have overshadowed other stimuli associated with the test context (see section 3.2.2.4).

The present study comprised four caffeine-CS pairings (or placebo-CS pairings for unpaired group). This replicated the number of trials used in the pilot study, at which caffeine-like effects were obtained during the test of conditioning. Furthermore, previous drug studies have reported drug-related CRs after four pairings of drug and a neutral CS (Staiger & White 1988; Foltin & Haney 2000; Stockhorst et al. 1999). For example, Field and Duka (2002) demonstrated the acquisition of conditioned responses by neutral stimuli paired with alcohol over four conditioning trials.

Inter-trial intervals were no less than 24 hours and no more than 7 days. An upper limit of 7 days was stipulated in order to keep data collection within a suitable timeframe (i.e. within one University semester). A minimum inter-trial interval of 24 hours was adhered to for several theoretical reasons. First, there is evidence that massed rather than spaced trials may disrupt conditioning (Sunsay, Stetson and Bouton 2004; Yin, Barnet and Miller 1994). Also, due to an approximate 4 hour half-life, caffeine may still be exerting pharmacological actions at a second trial if two trials were to be conducted on the same day. This would mean that the unpaired

group (who received caffeine at the end of each session) may not be strictly “unpaired” at the time of the second trial. Finally, as has been frequently suggested here, caffeine is particularly effective when participants are in a state of overnight caffeine withdrawal. Robelin and Rogers (1998) found significant effects of an initial dose of caffeine, with no additional effects of a second dose (see also Heatherley, Hayward, Seers & Rogers 2005). Therefore, the paired group may no longer demonstrate caffeine-induced effects (i.e.URs) at a second session on the same day.

Simple and choice reaction time tasks were chosen as the cognitive measures since an extensive body of literature suggests that they are particularly sensitive to the effects of caffeine (see section 2.4.1.1). Mood and heart rate were also measured, as they are also sensitive to the effects of caffeine (Smit and Rogers 2002; Tiffin, et al.1995; Whitsett et al, 1984), and have previously been conditioned to cocaine-paired stimuli (Foltin and Haney 2000; Macfarlane and White 1989). Current mood was assessed using visual analogue scales (VASs) (adapted from Rogers et al. 1995 – see section 4.4) since the Quick Mood Scale (Woodruffe-Peacock, Turnbull, Johnson, Elahi & Preston 1998) used in the Attwood, Terry and Higgs (2004) study unexpectedly failed to detect any significant effects of caffeine. VASs have been widely used experimentally and have been shown to be sensitive to fluctuations in mood (Lieberman et al. 1987; Penetar et al. 1993; Mitchell et al. 1995; Richardson et al. 1995; Warburton 1995; Warburton & Mancuso 1998; Smit & Rogers 2000; Brice & Smith 2002a; Yeomans et al. 2002; Rogers et al. 2003). VASs are preferential to other forms of subjective measure such as “Likert” style scales as the segregation of a line into 100 mm allows small changes in mood to be recorded.

It was hypothesised that the paired group would demonstrate performance and mood benefits, and show decreases in heart rate relative to the unpaired group across the conditioning trials. Furthermore, these group differences would be maintained on the test of conditioning, at which both groups receive placebo in the test context.

5.2 METHOD

5.2.1 Participants

24 participants (14 female/10 male; mean age 23.5 years, range 18-44 years) with an average daily caffeine intake of 351.9 mg (range 280-595 mg) took part in the study for cash (£20) or equivalent course credits.

5.2.2 Design and Materials

Participants were allocated to one of two experimental groups. One group experienced the test context paired with caffeine (paired group) and the other group experienced the test context paired with placebo (unpaired). Participants were allocated pseudo-randomly to groups such that groups were matched for age and gender. The five experimental sessions were spaced such that the interval between any two did not fall below 24 hours or exceed 7 days. Task order was fully counterbalanced. 250 mg of caffeine or placebo were administered as per section 4.3.

5.2.3 Measures

Simple Reaction Time (SRT): The SRT task comprised 100 trials in which an asterisk appeared centrally on a computer screen. Participants were instructed to press the space bar on the computer keyboard as soon as they saw the asterisk. The asterisk stayed on screen for 1 s and a new stimulus did not appear until a response was made.

Choice Reaction Time (CRT): The CRT task comprised 120 trials in which a string of either “XXX” or “OOO” appeared centrally on a computer screen. Participants were instructed to press one key on the computer keyboard if they saw the X’s and another if they saw the O’s. Each string appeared on screen for 500 ms, and a new stimulus did not appear until a response was made.

Half way through each task, a message appeared on screen prompting participants to take a break, and to press any key when they felt ready to continue. To avoid anticipatory responding, the inter-stimulus interval for both tasks varied randomly between 1,000 and 2,500 ms. Both tasks were preceded by practice blocks comprising 10 trials.

Mood and Heart Rate: Participants completed the 16-item mood questionnaire (see section 4.4) immediately before, and 30 minutes after, capsule administration. Heart rate was measured (see section 4.4) immediately before and 30-55 minutes after capsule administration.

The order of the four measures was counterbalanced across sessions and across participants, so that each of the 24 potential task orders was used six times (three per group), with no participant experiencing any one task order more than once.

5.2.4 Procedure

Participants attended six sessions: a baseline session (which took place immediately prior to the first conditioning trial), four conditioning trials and a test of conditioning. All testing took place between 09:00 and 13:00 hours. The schedule for the baseline session, a typical conditioning trial and the conditioning test are shown in Table 5.1.

Table 5.1. An example schedule of a conditioning trial, plus baseline and the conditioning test, denoting one potential order of experimental measures

<u>Baseline Session (conducted immediately prior to session one.</u>	
0 mins	Standardised Instructions
4 mins	Consent form and personal details questionnaire
6 mins	Heart Rate
8 mins	Mood Questionnaire
10 mins	Choice reaction time task
19 mins	Simple reaction time task
25 mins	Taken to test cubicle for conditioning session 1
<u>Conditioning Trials (Sessions 1-4)</u>	
0 mins	Saliva Assay
1 min	Heart rate
3 mins	Mood
5 mins	Capsule
35 mins	Measure 1 (e.g. Mood)
39 mins	Measure 2 (e.g. Simple reaction time)
45 mins	Measure 3 (e.g. Choice reaction time)
54 mins	Measure 4 (e.g. Heart rate)
56 mins	Leave test cubicle – capsule 2
57 mins	Post session questionnaire
<u>Conditioning Test (Session 5)</u>	
0-55 mins	As for conditioning trials
57 mins	Post experiment questionnaire and debriefing

At the baseline session, participants received a set of standardised instructions explaining the procedure and requirements of the study, and completed a form detailing their daily consumption of caffeine as well as consumption of other psychoactive substances and medications. Written consent was also obtained. Participants then completed the mood questionnaire, simple and choice reaction time tasks and had their heart rate measured. After this, they were taken to the test cubicle to begin the first conditioning trial.

For the conditioning trials, participants were tested individually in a test cubicle containing a desk, chair and computer. Immediately after being seated in the cubicle, participants were asked to give a saliva sample by placing a small cotton wool dental roll inside the mouth for a few seconds and then removing it and placing it in a sealable plastic bag. Then heart rate was measured and the mood scales were completed. The capsule was administered, and participants were left alone for 30 minutes to allow for drug absorption. Reading material was provided (daily newspapers and current periodicals). After this interval, the experimenter returned to administer the tests. At the end of the session, the participants were taken to a different room and the paired group consumed the placebo capsule, and the unpaired group consumed the caffeine capsule. Participants then completed the post-session questionnaire.

The procedure for the final session (conditioning test) was identical to that of the previous four conditioning sessions, except that all participants received placebo in the test environment. At the end of the fifth session, participants completed the post-

experiment questionnaire. Participants were then debriefed and reimbursed for their time.

5.2.5 Data Analysis

For the SRT and CRT tasks, RTs falling below 100 ms were considered anticipatory and removed. Reaction times more than 3 standard deviations above an individual's mean score were deemed outliers and removed (2.3% and 1.4% of SRT and CRT scores respectively). Independent t-tests were used to analyse differences between the groups at baseline (SRT, CRT and heart rate). The SRT and CRT data from each of the conditioning days were split into four blocks of 25 trials and the mean reaction times (SRT, CRT) and errors (CRT) were compared over the four conditioning sessions using a three factor mixed ANOVA with session (4 levels) and block (4 levels) as within-subjects factors and group (2 levels) as a between-subject factor.

Change from baseline scores (i.e. the “before caffeine” score was subtracted from the “after caffeine” score) were calculated for heart rate and mood measures on the conditioning days and then analysed using two-way ANOVA with session and group as factors.

Between-group differences in SRT and CRT performance at test (session five) were analysed using two-way ANOVA with group and block as the between- and within-subjects factors respectively. Differences between the groups in mood and heart rate at test were analysed using independent t-tests.

A Pearson’s Chi-square was used to examine the association between group and caffeine identification on the post-session questionnaire. The number of participants in each group who identified caffeine and who identified something other than caffeine were calculated and used in the chi-square analysis.

5.3 RESULTS

There were no significant differences between groups in terms of age, habitual caffeine consumption or BMI (Table 5.2).

Table 5.2. Participant characteristics: means and t-test results of between group comparisons (caffeine-paired and caffeine-unpaired) for age, level of daily caffeine consumption and BMI. Standard deviations are given in parenthesis

	Mean		<i>t</i> value	Degrees of Freedom	<i>p</i> value
Age (years)	Paired	24.3 (7.7)	-.31	22	>0.05
	Unpaired	23.4 (7.0)			
Consumption (mg/day)	Paired	349.1 (89.4)	.18	22	>0.05
	Unpaired	354.7 (62.0)			
BMI	Paired	23.1 (3.5)	-.37	22	>0.05
	Unpaired	23.6 (2.6)			

5.3.1 Simple Reaction Time (SRT)

There was no significant difference between groups at baseline [$t = -0.30$, $df = 22$, $p > 0.05$]. For the conditioning trials, there were no significant main effects of group [$F(1,22) = 0.20$; $p > 0.05$] or session [$F(3,66) = 0.59$; $p > 0.05$] (Figure 5.1). There was a main effect of block [$F(3,66) = 5.41$; $p < 0.005$], whereby performance deteriorated across blocks, but no significant interactions between group, session and block [$F_s(3,66) < 1.76$; $p_s > 0.05$] [$F_s(9,198) < 0.90$; $p_s > 0.05$].

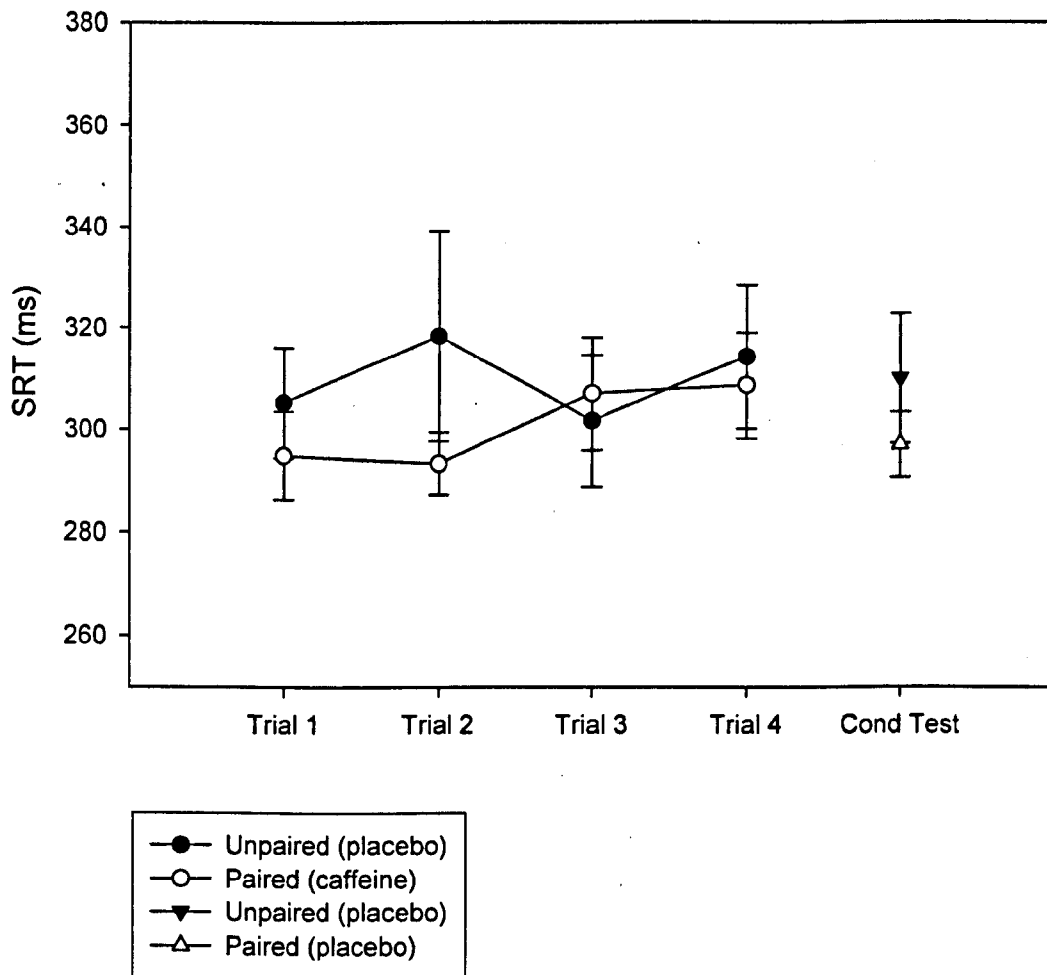


Figure 5.1. Mean SRT scores (ms) for caffeine-paired and caffeine-unpaired groups (n=12 per group) over four conditioning trials and on the conditioning test. Error bars represent SE means.

On the conditioning test (session five), there was no significant difference between the groups in SRT [$F(1,22) = 0.87$; $p > 0.05$]. There was a significant slowing of reaction time across blocks [$F(3,66) = 5.35$; $p < 0.005$] but no significant group-by-block interaction [$F(3,66) = 0.93$; $p > 0.05$].

5.3.2 Choice Reaction Time (CRT)

There was no significant difference between the groups at baseline [$t = -0.79$, $df = 22$, $p > 0.05$]. For the conditioning trials, there was no main effect of group [$F(1,22) = 0.47$; $p > 0.05$] but there was a main effect of session [$F(3,66) = 4.56$; $p < 0.01$].

Participants were slower on session one compared to subsequent sessions (Figure 5.2).

There was also a main effect of block [$F(3,66) = 12.50$; $p < 0.001$] with faster performance on block one compared to subsequent blocks. There were no significant interactions [$F_s(3,66) < 1.90$; $p_s > 0.05$] [$F_s(1,98) = 0.77$; $p_s < 0.05$].

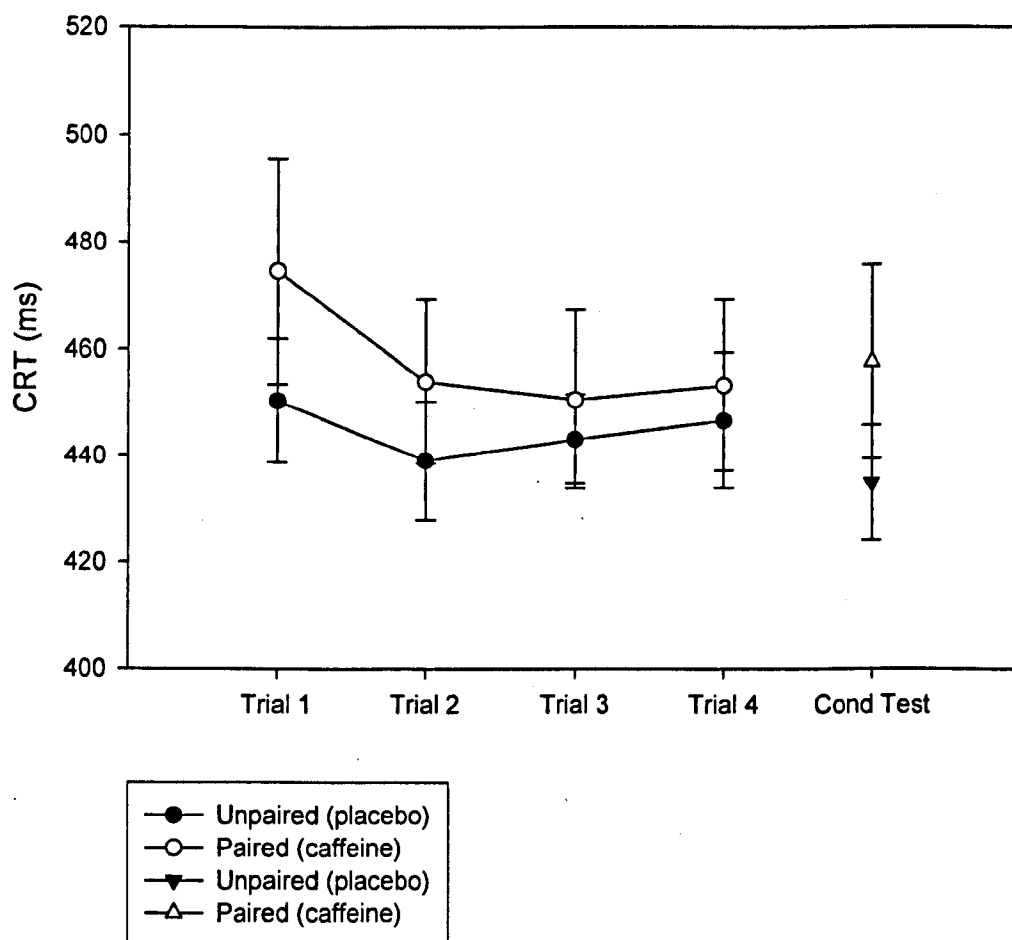


Figure 5.2. Mean CRT scores (ms) for caffeine-paired and caffeine-unpaired groups ($n=12$ per group) over four conditioning trials and on the conditioning test. Error bars represent SE means.

On the conditioning test (session five), there was no significant group difference [$F(1,22) = 0.87$; $p > 0.05$]. There was a main effect of block [$F(3,66) = 7.21$; $p < 0.0001$] with faster performance on block one relative to subsequent blocks. There was no significant group-by-block interaction [$F(3,66) = 0.78$; $p > 0.05$].

5.3.3 Mood

There were significant improvements in ratings of “Sleepiness” [$F(1,22) = 6.18$; $p < 0.05$] and “General Positive Mood” [$F(1,22) = 7.81$; $p < 0.005$] after caffeine compared to negative changes reported after placebo (see Table 3). There was also a tendency for ratings of “Mental Clarity” [$F(1,22) = 3.06$; $p = 0.097$] to decrease after placebo relative to a small increase after caffeine. There were no main effects of caffeine on any other mood factors [$F_s(1,22) < 0.65$; $p_s > 0.05$], no significant effects of session [$F_s(3,66) < 1.31$; $p_s > 0.05$] and no group-by-session interactions [$F_s(3,66) < 2.68$; $p_s > 0.05$].

On the conditioning test there was a significant group difference on ratings of “Mental Repose” [$t = 2.26$; $df = 22$; $p < 0.05$] with a decrease in the paired group versus an increase in the unpaired group (see Table 5.3). There were no significant effects on any other mood factor [$t_s < 1.27$; $dfs = 22$; $p_s > 0.05$].

Table 5.3. Mean changes (mm) for all mood factors during the conditioning trials and on the test of conditioning for the paired and unpaired groups. Standard deviations are in parenthesis

	Group	Cond. Trial 1	Cond. Trial 2	Cond. Trial 3	Cond. Trial 4	Cond. Test
Anxiety	Paired	-4.7 (11.4)	-4.0 (9.0)	6.9 (16.7)	-3.3 (15.5)	1.5 (10.6)
	Unpaired	-3.0 (12.7)	-6.4 (17.0)	0.6 (6.4)	1.5 (9.6)	-1.6 (10.5)
Mental Clarity	Paired	0.1 (21.6)	12.5 (18.1)	-0.6 (20.6)	-3.8 (28.2)	9.8 (30.1)
	Unpaired	-2.8 (21.0)	-4.3 (22.8)	-1.8 (25.2)	-15.3 (20.5)	-4.7 (27.4)
Mental Repose	Paired	4.3 (35.4)	-5.3 (21.7)	10.5 (39.9)	-7.2 (38.7)	-12.7 (22.8)
	Unpaired	4.0 (30.4)	10.4 (16.8)	-1.9 (25.5)	-1.8 (17.1)	8.7 (23.3)
Positive Mood	Paired	5.9 (17.7)	8.3 (30.0)	0.2 (17.0)	23.3 (5.6)	0.3 (37.2)
	Unpaired	-21.8 (17.9)	-4.6 (20.3)	-4.3 (21.1)	-8.5 (36.1)	-1.3 (16.4)
Tense Mood	Paired	6.3 (25.2)	2.0 (15.8)	4.3 (34.3)	-11.2 (46.2)	6.3 (16.1)
	Unpaired	-10.2 (34.6)	-2.2 (30.9)	4.8 (24.1)	4.8 (14.8)	9.3 (24.5)
Sleepiness	Paired	-1.9 (41.1)	-28.0 (38.9)	3.1 (32.8)	-2.2 (38.4)	14.8 (51.1)
	Unpaired	10.7 (30.5)	13.7 (34.0)	17.3 (36.0)	13.4 (30.4)	1.6 (25.7)

5.3.4 Heart Rate

There were no main effects of group [$F(1,22) = 1.80$; $p > 0.05$] or session [$F(3,66) = 2.00$; $p > 0.05$] and no significant session-by-group interaction [$F(3,66) = 1.40$; $p > 0.05$] during the four conditioning trials (data not shown). On the test of conditioning, there was a significant effect of group for change in heart rate [$t = -3.13$, $df = 22$, $p < 0.01$]. The paired group showed a small decrease (-2.0 bpm) compared to little change (+0.3 bpm) in the unpaired group.

5.3.5 Post-Session Questionnaires

At the end of each session, participants were asked to indicate what substance they believed that they had received that day. The responses are listed in Tables 5.4 and 5.5.

Table 5.4. Number of participants in the unpaired group who believed that they had received caffeine in each conditioning trial, compared to alternative substances, placebo or “don’t know”

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	2	2	3	2	9
Alternative substance	3	6	5	5	19
Placebo	4*	1*	2*	3*	10*
Don’t know	3	3	2	2	10

*correct choice

Table 5.5. Number of participants in the paired group who believed that they had received caffeine in each conditioning trial, compared to alternative substances, placebo or “don’t know”

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	2*	5*	4*	3*	14*
Alternative substance	3	3	2	6	14
Placebo	5	2	4	2	13
Don’t know	2	2	2	1	7

*correct choice

Pearson’s chi-square analysis showed there was no significant association between group (i.e. whether caffeine was administered) and reported identification of caffeine [$\chi^2 = 1.43$; $df = 1$; $p > 0.05$].

5.4 DISCUSSION

There were no significant effects of caffeine during the conditioning trials on reaction time performance, as the paired group did not respond faster than the unpaired group. The present results contrast with the large body of literature showing that caffeine enhances psychomotor performance, particularly on reaction time tasks (Lieberman et al. 1987; Smith et al. 1994; Attwood et al. 2004). For example, Smit and Rogers (2000) demonstrated a facilitatory effect of caffeine on simple reaction time at a dose as low as 12.5 mg. However, other studies have found no effects of caffeine on reaction time performance using higher doses (Bruce et al 1986; Kuznicki & Turner 1986; Smith, Kendrick & Maben 1994; Smith, Whitney, Thomas, Perry & Brockman 1997; Watson et al 2000). Variations in methodology and participant characteristics may explain inconsistent findings.

For example, Rogers et al. (2003) found that caffeine enhanced performance in regular caffeine consumers (>200 mg /day) only. Similarly, other studies have demonstrated significant effects of caffeine in deprived consumers with daily caffeine consumption levels in the region of 250 to 370 mg (Lieberman et al. 1987; Richardson et al; Smit & Rogers 2000; Heatherley et al. 2005). In the present study, participants were also required to be regular caffeine consumers (> 200 mg/day), based on ratings adapted from (Richardson et al. 1995), as used in Rogers et al. (2003), suggesting that habitual level of caffeine consumption is unlikely to explain why participants did not show a caffeine effect.

Other studies have suggested that the effects of caffeine on cognition are only observed when participants are in state of caffeine withdrawal (James 1994; Yeomans et al. 2002; Rogers et al. 2003; Heatherley et al 2005; James & Rogers 2005). In the present study, participants were required to abstain from consuming caffeine overnight to ensure that they were experiencing withdrawal from caffeine. In addition, the participants reported consuming amounts of caffeine that would be expected to produce withdrawal after overnight abstinence (Hughes et al. 1993; Juliano & Griffiths 2004). Confirmation of abstinence was based on self-report and so it is possible that participants had consumed caffeine prior to arrival at the test sessions. However, other studies that have taken saliva samples have found significant effects of caffeine without analysing the samples (Rogers et al. 2003; Heatherley et al. 2005), and there is no reason to assume reduced compliance by participants in the present study.

The significant effects of caffeine on some mood items also indicate that the drug was active at receptor sites following absorption. This activity of caffeine suggests that the lack of cognitive effects was not due to methodological issues such as too short an absorption interval. In addition, it is unlikely that caffeine effects were obscured by ceiling effects on the tasks. The blocked analyses showed clear performance deterioration across the tasks for both SRT and CRT, suggesting that caffeine-induced performance enhancement was still possible for both measures.

A potentially important difference between the pilot study of Attwood et al. (2004), in which significant effects of caffeine were observed, and the present study, is the information that was provided when the participants enrolled in the study. The recruitment posters for the pilot study advertised for caffeine consumers, which is likely to have primed participants that caffeine would be administered. In contrast, in the present study participants were screened using a questionnaire that asked for their consumption levels of various compounds and foodstuffs. Caffeine was not mentioned in any advertising material, so participants were unlikely to have been aware that they had been recruited on the basis of their caffeine consumer status. The active drug hypothesis asserts that drug expectancy can potentiate the active effects of a drug (Dinnerstein, Lowenthal & Blitz 1966), and such effects have been demonstrated with caffeine (Flaten & Blumenthal, 1999). Therefore, the pilot study may have inadvertently primed participants to expect caffeine, which is widely regarded as a stimulant drug, and thus potentiated its psychomotor effects.

Particularly relevant to this, the paired group correctly identified caffeine most often during the session on which they demonstrated the fastest SRT performance and significant increases in alertness (session 2), suggesting that the ability to perceive the effects of caffeine may correlate with the effects of caffeine. On other sessions, the paired group was generally unsuccessful in identifying caffeine as the substance they had received, indicating that participants were unable to correctly identify the effects of caffeine. Why participants should be better at identifying caffeine on one day rather than another is unclear, but may be related to factors affecting participant's sensitivity to the effects of caffeine (e.g. baseline arousal). In support, the paired

group reported their highest baseline “sleepiness” scores on session two relative to other sessions.

Although the paired group did differ from the unpaired group on some mood measures during the conditioning trials, no differences between the groups were observed at test. This failure to observe a conditioned effect may have been due to the drug effects during conditioning being weak and/or inconsistent. For all the mood scales, caffeine tended to offset negative effects of placebo during most sessions, with absolute improvements on only one or two sessions

A significant effect of “mental repose” was observed during the conditioning test despite no significant group effect during the conditioning trials. It is unclear why there was a group difference at this session. Examination of the means showed that both groups reported inconsistent changes during the conditioning trials.

There were no significant effects of caffeine on heart rate during the conditioning trials. There was a significant group effect on the conditioning test, although the difference was small (2 bpm). However, on several occasions the experimenter had difficulty locating and/or maintaining contact with the pulse, thereby reducing the reliability of this measure. Consequently, these scores were not analysed further. Future studies will measure cardiovascular function using a heart rate/blood pressure monitor.

In sum, no clear effects of caffeine were obtained in the present study, which is unlikely to be due to participant characteristics, drug dose or insensitivity of the performance measures. A factor differentiating the present study from the pilot study, where significant effects of caffeine were found, is the information that was available to participants at the time of enrolment. It is possible that a group of participants encouraged to believe that a study is testing the effects of caffeine will show heightened caffeine effects relative to a group of participants who are told that one of several possible substances might be administered. The aim of the next study is to test this hypothesis by varying the information provided to two groups of participants in a placebo-controlled study of caffeine effects. This line of research was investigated in order to establish the factors that produce reliable effects of caffeine.

CHAPTER 6:

EXPERIMENT 2: EFFECTS OF CAFFEINE INFORMATION/EXPECTANCY ON THE EFFECTS OF CAFFEINE

6.1 INTRODUCTION

No effects of caffeine on cognitive performance were found in Experiment 1. This contrasts with the results of the pilot study (Attwood et al. 2004), which showed clear benefits of caffeine on simple reaction time performance. One explanation for these discrepant findings relates to the different information that was presented at the time of enrolment. Although the use of caffeine was disguised in both studies by informing participants that they may receive one of several possible substances (including caffeine), the recruitment posters for the pilot study asked that only caffeine consumers sign up for the study, while the participants who signed up for Experiment 1 were not given any explicit information about caffeine consumption requirements. Consequently, the participants in Experiment 1 were unlikely to be aware that they were recruited according to their caffeine consumer status, and may have had a reduced expectation of actually receiving caffeine during the study compared with the participants in the pilot study.

There is a large body of literature suggesting that increasing the expectancy of caffeine either by information (Mikalsen, Bertelsen & Flaten 2001; Oei & Hartley 2005) or by the presence of caffeine-related cues (Flaten & Blumenthal 1999; Mikalsen et al. 2001) promotes caffeine-like physiological (Flaten, Aasli & Blumenthal 2003), subjective (Mikalsen et al. 2001) and cognitive (Fillmore & Vogel-

Sprott 1992) responses. However, expectancy effects alone cannot account for the differential results of Experiment 1 and the study of Attwood et al. (2004). This is because both the caffeine (paired) and placebo (unpaired) groups received the same information, but only the paired group showed faster reaction time performance during testing.

These results could be explained via an interaction between the expectation that caffeine had been administered and the effects of caffeine. Dinnerstein, Lowenthal and Blitz (1966) have argued that drugs potentiate placebo effects by creating an internal state that confirms the presence of an active drug. In support of this, Lotshaw, Bradley and Brooks (1996) separated the effects of caffeine and caffeine expectancy using a balanced-placebo design. Additive effects were found of caffeine and expectancy on ratings of mood and performance. Similarly, Flaten and Blumenthal (1999) reported additive effects of caffeine-associated stimuli and caffeine on mood. They reported that caffeinated coffee increased arousal more than caffeinated orange juice, suggesting an additive effect of drug and drug-associated cues (i.e. taste/smell of coffee). The authors interpreted the latter effect as a conditioned response, but a role for expectancy was not ruled out.

Furthermore, Oliveto et al. (1992) reported stimulant-like effects of caffeine on subscales of the ARCI, but only when caffeine was correctly identified as part of a drug discrimination study. When not correctly identified, scores after caffeine were similar to placebo. This is consistent with the findings of Experiment 1, in which the strongest stimulant effects of caffeine on ratings of “sleepiness” and “mental clarity”

correlated with the highest number of correct identifications of caffeine (session two). Performance on a digit symbol substitution task did not appear to be related to correct identification of caffeine (Oliveto et al.1992). In contrast, the fastest SRT performance after caffeine in Experiment 1 occurred on session two, although this did not reach significance.

The aim of the present experiment was to investigate whether information suggesting the use of caffeine would interact with the active effects of the drug on cognitive performance. Two groups attended two sessions (caffeine and placebo). One group were told that the capsule may contain caffeine (“told” group) and the other group was informed that it may contain either: paracetamol, caffeine, ibuprofen or placebo (“untold” group).

To increase the likelihood of detecting an effect of caffeine on cognitive performance several changes were also made to the battery of measures. As caffeine is particularly effective in facilitating sustained attention (Brice and Smith 2002a), the SRT task used in Experiment 1 was lengthened by 100 trials. In addition, Baddeley’s (1968) semantic verification task (SVT) replaced the CRT task used in Experiment 1. Studies have previously reported increases in accuracy on this task after caffeine (Smith, Kendrick & Maben 1994; Warburton 1995). Inclusion of the SVT also enabled the examination of cognitive responses of caffeine beyond a psychomotor response. An additional scale assessing “Headache” was added to the mood questionnaire as previous research has suggested that headache is a reliable symptom of caffeine withdrawal (see Juliano and Griffiths 2004). Finally, a cardiovascular

monitor was used to reliably measure heart rate, and systolic and diastolic blood pressure.

It was hypothesised that, relative to all other conditions, greatest task facilitation would occur in the “told” group when given caffeine, due to the combined effects of drug and expectancy.

6.2 METHOD

6.2.1 Participants

Twenty participants (11 female/9 male; mean age 22.4 years, range 19-31 years) with a mean daily caffeine intake of 232.7 mg (range 174-422 mg) took part in return for cash (£15) or course credits.

6.2.2 Design and Materials

Participants were pseudo-randomly assigned to one of two experimental groups: “untold” vs. “told”, matched for age and habitual caffeine consumption. Both drug (i.e. caffeine vs. placebo) and task (i.e. SRT, semantic verification, mood and cardiovascular measures) orders were fully counterbalanced. 250 mg of caffeine and placebo were administered as per section 4.3.

6.2.3 Measures

Simple reaction time (SRT) task: The SRT task was the same as Experiment 1, except that the number of trials was increased from 100 to 200, with a break after 100 trials (participants were prompted to press any key when ready to continue).

Semantic verification task (SVT): Two letters (“A” and “B”) appeared centrally on screen – one below the other - along with one of four possible sentences describing their configuration (“A is above B”, “B is above A”, “A is not above B” or “B is not above A”). Participants responded via the keyboard to indicate whether the statement was true or false (m for true, v for false). This task comprised 160 trials with a break after 80 trials (participants were prompted to press any key when ready to continue). The stimuli remained on screen for a maximum of 10 seconds and the next trial did not begin until a response was made. The inter-trial interval was 1000 ms. Reaction time and accuracy were recorded.

Mood Ratings and cardiovascular measures: Mood was measured by the visual analogue scales used in Experiment 1 with the addition of a “Headache” scale. A Dinamap Pro-series was used (see section 4.4) to measure heart rate and blood pressure.

6.2.4 Procedure

All participants attended two sessions (caffeine and placebo) at approximately the same time of day (between 09:00 and 13:00). Inter-session intervals did not fall below 24 hours or exceed 7 days. The “untold” group was informed that they may receive one of the following substances: paracetamol, ibuprofen, caffeine or aspirin. The “told” group was informed that they may receive caffeine.

Baseline measures were then taken: two cardiovascular measures; a mood questionnaire; and shortened versions of the SRT and semantic verification tasks,

comprising 50 and 60 trials respectively, plus an initial 10 trial practice block for each.

Immediately prior to the capsule being administered, a saliva sample was taken. Participants were then left alone in the computer cubicle to allow for drug absorption. They were instructed to sit quietly, and reading material was provided (a selection of current newspapers and periodicals). After 30 minutes, the experimenter returned and the tests were conducted. Finally, participants completed the post-session questionnaire. The told group received a modified version of this questionnaire in which the options of substances were “caffeine”, “placebo” and “other”.

Session two followed the same procedure but with the addition of the post-experiment questionnaire. Participants were then fully debriefed and reimbursed for their time.

6.2.5 Data Analysis

For both cognitive tasks, reaction time scores below 100 ms were regarded as anticipatory and removed (0.2%). For SRT, scores more than 3 standard deviations above an individual's mean score were deemed outliers and removed (1.1%).

Independent t-tests were used to test for differences at baseline. Mean reaction times (SRT, SVT) and errors (SVT) were compared using a three factor mixed ANOVA with capsule and block as within-subjects factors and information (“told” vs. “untold”) as a between subject factor. For each task, the data was split into four blocks of 50 and 60 trials for SRT task and SVT respectively.

Cardiovascular and mood measures were analysed using a two-way ANOVA with capsule and information as within- and between-subject factors respectively. Two data points were not recorded due to equipment malfunction.

6.3 RESULTS

There were no significant group differences in age, habitual consumption or BMI (see Table 6.1).

Table 6.1. Participant characteristics: means and t-test results of between group comparisons (caffeine “told” and caffeine “untold”) for age, level of daily caffeine consumption and BMI. Standard deviations are in parenthesis.

		Mean	<i>t</i> value	Degrees of Freedom	<i>p</i> value
Age (years)	Told	25.4 (6.4)	-1.83	18	>0.05
	Untold	21.5 (2.0)			
Caffeine consumption (mg/day)	Told	241.4 (76.0)	-.69	18	>0.05
	Untold	224.0 (24.9)			
BMI	Told	22.9 (2.7)	-.32	18	>0.05
	Untold	22.4 (3.7)			

6.3.1 Simple Reaction Time (SRT)

There were no significant group differences in baseline performance on either session one [$t = -0.45$, $df = 18$, $p > 0.05$] or session two [$t = -0.86$, $df = 18$, $p > 0.05$]. There were no significant main effects of drug [$F(1,18) = 0.61$; $p > 0.05$] or group [$F(1,18) = 0.28$; $p > 0.05$], and no significant drug-by-group interaction [$F(1,18) = 1.40$; $p > 0.05$]. There was a main effect of block [$F(3,54) = 7.21$; $p < 0.001$] as performance progressively deteriorated across blocks (see Table 6.2). There were no other significant interactions [$F(3,54) < 1.49$; $ps > 0.05$].

6.3.2 Semantic Verification Task (SVT)

Independent t-tests showed no significant group differences on sessions one or two in baseline reaction time [$t_s < 0.47$, $dfs = 18$, $ps > 0.05$] or number of errors [$t_s < 0.79$, $dfs = 18$, $ps > 0.05$]. There were no significant main effects of drug or group and no significant drug-by-group interactions for reaction time or number of errors [$F_s(1,18) < 1.55$; $ps > 0.05$]. There was a main effect of block on reaction time [$F(3,54) = 9.33$; $p \leq 0.001$], which was due to a improvement in performance from the first two blocks to the second two blocks (see Table 6.2). There were no other significant main effects or interactions on the blocked analysis [$F_s(3,54) < 2.06$; $ps > 0.05$].

Table 6.2. Mean (ms) (SD) SVT and SRT performance across four blocks averaged across told and untold groups (n=20)

Task	Block 1	Block 2	Block 3	Block 4
SRT	301.2 (45.3)	313.7 (38.8)	323.3 (47.7)	327.5 (43.8)
SVT	2567.4 (599.5)	2596.2 (740.3)	2297.1 (583.5)	2380.7 (631.7)

6.3.3 Mood

There were no main effects of drug [$F_s(1,18) < 0.28$; $ps > 0.05$] or group [$F_s(1,18) < 1.39$; $ps > 0.05$] and no significant drug-by-group interactions [$F_s(1,18) > 2.37$; $ps > 0.05$] on any of the mood factors (data not shown).

6.3.4 Cardiovascular Measures

There were no significant main effects of drug [$F_s(1,17) < 1.67$; $ps > 0.05$] or group [$F_s(1,17) < 1.99$; $ps > 0.05$] and no significant drug-by-group interactions [$F_s(1,17) < 1.42$; $ps > 0.05$] for systolic or diastolic blood pressure (data not shown).

There was a significant main effect of drug for HR [$F(1,16) = 6.42$; $p < 0.05$].

Reductions in heart rate occurred after both capsules (mean bpm change: told group - placebo -5.7, caffeine -7.6; untold group – placebo 1.0, caffeine -6.4), but this effect was greater following caffeine. There were no other main effects of drug [$F_s(1,17) < 1.52$; $p_s > 0.05$] or group [$F_s(1,17) < 1.97$; $p_s > 0.05$] and no significant drug-by-group interactions [$F_s(1,17) < 2.28$; $p_s > 0.05$] for HR.

6.3.5 Post-Session Questionnaires

At the end of each session, participants were asked to indicate what substance they believed that they had received that day. The responses are listed in Table 6.3.

Table 6.3. Number of participants in the “told” and “untold” groups who believed that they had received caffeine compared to an alternative substance at the caffeine and placebo sessions

	Caffeine Session		Placebo Session	
	Reported Caffeine	Reported Other	Reported Caffeine	Reported Other
Told Group	7	3	4	6
Untold Group	3	7	3	7

A greater number of participants in the told group, relative to the untold group, correctly identified caffeine as the substance they had taken during the caffeine session. A Pearson’s chi-square analysis examining the association between group (i.e. whether caffeine was expected) and reported identification of caffeine approached significance [$\chi^2 = 3.20$; $df = 1$; $p = 0.07$].

6.4 DISCUSSION

There were no significant effects of caffeine expectancy or caffeine administration on cognitive performance or mood. Importantly, there was no combined effect of information that caffeine would be received and caffeine administration. These findings suggest that the different information given to participants in the pilot study (Attwood et al. 2004) and Experiment 1 cannot account for the different outcomes of these studies.

A larger number of participants in the told group, relative to untold group, correctly identified caffeine during their caffeine session. This suggests that the manipulation to “prime” participants to expect caffeine (to some extent) was successful. However, this greater level of identification was not correlated with greater caffeine-like responding in the told group (i.e. main effect of expectancy). The present study, however, differed from those that have previously reported effects of caffeine expectancy (Fillmore and Vogel-Sprott 1992; Flaten et al. 2003 and Mikalsen et al. 2001), in that participants were not explicitly told that they would receive caffeine. Instead, to keep in line with the pilot study, participants were told that they may receive caffeine or placebo, and therefore the expectancy of caffeine was potentially weakened.

Considering the large number of studies that have reported cognitive benefits of caffeine (Lieberman et al 1987; Roache & Griffiths 1987; Jarvis 1993; Smith, Kendrick & Maben 1994; Smith, Maben & Brockman 1994; Smit & Rogers 2000; Brice & Smith 2002a), it is interesting that again no significant benefits of caffeine

were observed for the cognitive tasks in this study. Participants were generally faster following caffeine compared to placebo on SRT, although this difference did not reach significance. A lack of sensitivity of the SRT task is an unlikely explanation, as significant effects of caffeine have previously been obtained using this task (Richardson et al. 1995; Attwood et al. 2004). Limited support was found for the assertion that caffeine can offset the degradation in performance that occurs over time due to fatigue. There was a clear deterioration in performance across blocks for SRT, however this occurred to a similar extent in both the placebo and caffeine conditions.

A significant effect of block was also obtained for the SVT, however this was indicative of a practice effect, with performance improving from the first two to second two blocks. This suggests that the task was not fatiguing, and this may have reduced its sensitivity to caffeine. However, the improvement in performance on the SVT coincided with the task break (i.e. between blocks 2 and 3), suggesting that the inclusion of a break interfered with the fatiguing nature of the tasks. In support, performance slowed between consecutive blocks that were not separated by a break (i.e. blocks one to two, and three to four), although once again, this small deterioration was evident in both caffeine and placebo conditions. It is possible, considering the simplistic nature of the SRT task and the challenging nature of SVT, that although significant effects of caffeine can be obtained under certain circumstances, these tasks were not optimal to demonstrate robust effects of caffeine. Testing a wider range of tasks may increase the likelihood of detecting robust significant effects of caffeine.

There was a main effect of caffeine on HR, suggesting that the drug was active. Although a decrease in HR was observed after both caffeine and placebo, the magnitude of this decrease was greater after caffeine. This effect of caffeine was similar in size for both the told and untold groups, implying that the expectancy manipulation had no effect on this measure. Many studies have reported decreases in HR following caffeine (Strickland, Myers & Lahey 1989; Lane, Pieper, Phillips-Bute, Bryant & Kuhn, 2002), and this is often attributed to a baro-receptor mediated response to a drug-induced increase in blood pressure (Höfer & Bättig, 1994). However, similar to Kourtidou-Papadeli et al. (2002), no such corresponding blood pressure increase was found, suggesting that caffeine may be decreasing heart rate via an alternative mechanism.

Due to the difficulties encountered demonstrating clear effects of caffeine on performance and mood in Experiments 1 and 2, the next experiment tested a higher dose of caffeine (400 mg) and a new task battery (CANTABeclipse 1.0.0, Cambridge Cognition, UK), in order to ascertain whether such changes produced more reliable effects of caffeine. It is possible that, because both the present study and Experiment 1 recruited participants with a higher daily intake of caffeine than the pilot study, a higher dose of caffeine may be required to elicit clear drug effects.

CHAPTER 7:

EXPERIMENT 3: EFFECTS OF TWO DOSES OF CAFFEINE ON SELECTED TASKS FROM THE CAMBRIDGE NEUROPSYCHOLOGICAL TEST AUTOMATED BATTERY (CANTAB)

7.1 INTRODUCTION

Significant performance benefits of caffeine on tests of reaction time or on a semantic verification task were not found in Experiments 1 and 2. Reliable unconditioned effects of caffeine are required in order to test for conditioned drug effects because, in the absence of a consistent drug effect, there is no basis for a learned US-CS association.

It is possible that the dose of caffeine used in Experiments 1 and 2 was not high enough to elicit significant improvements in performance and hence a conditioned drug effect. Although psychomotor facilitation has been reported after as little as 12.5 mg of caffeine (Smit & Rogers 2000), in many other studies much higher doses have been tested (Kuznicki & Turner 1986; Jacobson & Edgley 1987; Smith et al. 1994; Kaplan et al. 1997; Kamimori et al. 2000). For example, Roache and Griffiths (1987) demonstrated greater performance enhancement after 400 mg of caffeine compared to 200 mg, and Bättig and Buzzi (1986) reported caffeine-facilitated attention at a dose of 450 mg. Although there are generally fewer reports of caffeine effects at high doses, this is in part due to recent studies predominantly testing the effects of doses of caffeine that are considered more relevant to levels of habitual use (Brice & Smith 2002a). Furthermore, Haskell et al. (2005) demonstrated significant improvements in

vigilance performance among moderate caffeine consumers (>50 mg/day) after a higher dose (150 mg) of caffeine, but not a lower dose of caffeine (75 mg) relative to placebo.

In addition, there is evidence that high consumers develop tolerance to aversive effects of the drug (see Fredholm et al. 1999) that are often associated with higher doses of caffeine, but not to the performance enhancing effects of caffeine (Jarvis 1993). This suggests that higher consumers may be able to show performance benefits at higher doses of caffeine that would otherwise be masked in lower consumers due to aversive effects. Experiment 1 recruited higher caffeine consumers (≥ 280 mg/day) than did Attwood et al. (2004) (≥ 150 mg/day), so the differences in performance may have reflected lower responsiveness to caffeine by the higher consumers.

The dose of caffeine required to elicit significant effects on cognitive performance is also likely to be influenced by a complex interaction of factors such as type of task and the vehicle used to administer caffeine. For example, Flaten and Blumenthal (1999) showed stronger effects of caffeine when administered in coffee compared to orange juice indicating additive effects of the drug and drug-related cues. This suggests a higher dose may be required when administering caffeine via capsule (as in the current series of experiments) compared with administration via caffeinated coffee.

Another possibility for the lack of effects of caffeine in Experiments 1 and 2 is that the specific tasks used were not optimal for detecting effects of caffeine on cognition. In the present study, a selection of tests was used from the Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB computerised tests have been validated in a large body of peer-reviewed publications (CANTABclipse 2006), and have been proven sensitive to the effects of drugs including MDMA (Morgan 1998), scopolamine (Robbins et al 1997), alcohol (Weissenborn & Duka 2003) and antipsychotic drugs (Tyson, Roberts & Mortimer 2004). Durlach (1998) has shown significant differential effects of (caffeinated and decaffeinated) tea on choice reaction time motor responding and decision time responding using the CANTAB battery. This may explain why measurement of total reaction time responding (both motor and decision time responding) did not show effects of caffeine in Experiments 1 and 2.

The CANTAB task battery completed by participants in the present study comprised tests of: rapid visual information processing (RVIP); match-to-sample visual search (MTS); simple reaction time (SRT); choice reaction time (CRT) and spatial recognition (SR). This enabled testing of multiple cognitive processes, including sustained attention and psychomotor responding, which have been widely reported to be improved by caffeine (see section 2.4). MTS performance was assessed because caffeine has been shown to improve reaction time performance on this task (Durlach 1998). Finally, the SR task was chosen because there are some reports that caffeine can enhance performance on memory tasks (Smith et al. 1994; Warburton 1995; Rogers & Dernoncourt 1998).

Testing was restricted to afternoons because this coincides with the reported drop in arousal that often occurs after lunch (Monk 2005), and which has been reported to be attenuated by caffeine (Smith et al. 1991). Furthermore, afternoon testing required a longer abstinence, thereby increasing the likelihood and/or severity of withdrawal (Dews, O'Brien & Bergman 2002; Juliano & Griffiths 2004). Because asking participants to remain abstinent from caffeine for longer may reduce compliance, additional procedures were included to encourage participants to remain abstinent until test. First, emails were sent the morning before each experimental session reminding participants to refrain from consuming any psychoactive substance from 11pm that night. Second, participants were asked to sign a form at the start of each session confirming that they had adhered to these requirements.

In summary, this study was designed to test the effects of a higher dose of caffeine on performance of a wider range of tasks than Experiments 1 and 2. It was predicted that the higher dose of caffeine would improve performance on the CANTAB task battery relative to placebo in a group of caffeine-withdrawn regular caffeine consumers.

7.2 METHOD

7.2.1 Participants

Twelve participants (8 female/4 male; mean age 21.5 years, range 18 to 29 years) with an average daily caffeine intake of 320.8 mg (range 280 to 490 mg) took part in return for either cash (£15) or course credits.

7.2.2 Design and Materials

A repeated-measures design was used in which participants took part in each of the three experimental conditions (0, 250 and 400 mg caffeine). The order in which the capsules were given was fully counterbalanced between participants. The interval between any two test sessions did not fall below 24 hours or exceed 7 days. 250 and 400 mg of caffeine and 250 mg placebo were administered as per section 4.3.

7.2.3 Measures

All cognitive tasks were from the CANTABclipse task battery (Cambridge Cognition, Cambridge, UK). Participants responded using a specialised press-pad, which was placed 15 cm in front of a touch-sensitive computer screen. The tasks are described below in the order in which they were completed.

Rapid Visual Information Processing (RVIP): A series of single digits appeared sequentially on the computer screen; each digit was displayed for 600 ms before being immediately replaced with the next. Participants were required to respond, via a press pad, whenever they identified one of three 3-digit number strings (3-5-7; 2-4-6; 4-6-8). The task ran for six consecutive blocks (total task time: 6 minutes), each containing nine target strings, although data from the first block were not analysed. A practice trial of 2 minutes preceded each test run.

Match to Sample Visual Search (MTS): An abstract pattern (target) appeared centrally on the computer screen. After a 1.5 second interval, distracter patterns appeared in boxes surrounding this central pattern. Within the array of distracters, one

pattern was an identical match to the central target. To initiate each trial, a press-pad key was depressed until the identical pattern was identified. At this point, the participant was required to move their hand from the press pad and touch the identical pattern on screen. If the response was incorrect (indicated by a red cross on the screen), participants were asked to continue choosing from the distracter patterns until they had chosen the correct one. There were three levels of difficulty that differed according to the number of distracters: two (level 1), four (level 2) or eight (level 3). Reaction duration, movement duration and errors were recorded. The main task was preceded by a practice block of three trials (one per level), but these data were not analysed

Simple Reaction Time (SRT): The simple reaction time task comprised two experimental blocks, each of 15 trials. The stimulus was a small yellow spot, which was presented for 250 ms inside a white circle that remained centrally on screen throughout the test. Each trial began with the participants depressing the press-pad key until the yellow spot appeared, at which point they were required to touch the screen (inside the white circle) as quickly as possible. The next trial did not begin until the participants had once again depressed the press pad key.

Choice Reaction Time (CRT): The choice reaction time task comprised four experimental blocks (15 trials each) in which five white circles were arranged around a central point on the screen. The white circles remained on screen throughout the test. For each trial, the yellow spot appeared in one of these five circles, and participants had to touch this circle as quickly as possible.

For both reaction time tests the inter-trial interval varied randomly between 750 and 2,250 ms to reduce anticipatory responding. Both tasks were preceded by a practice block of ten trials (data not analysed).

Spatial Recognition: Five squares appeared sequentially at various locations on the screen. Each square remained on screen for 3 seconds, before being immediately replaced with the next. Participants were instructed to remember the location of these squares. Once all five had been presented, there were five separate response trials. For each trial, two squares appeared simultaneously at two locations. Participants were asked to identify the square that was in an identical location to one of the previously presented five. When they had made their choice, both squares disappeared and were replaced with a new pair until all five of the original squares had been represented. There was a 1.5 second interval between each test trial. This test was performed four times, including an initial practice test (data not analysed). The measures taken were reaction time and number of errors.

Mood and Cardiovascular measures: As for Experiment 2.

7.2.4 Procedure

Participants attended three sessions on different afternoons. All testing took place between 12:00 and 18:00 hours, and participants attended each session at approximately the same time of day. Participants were seated in a small computer cubicle comprising table, chair and computer. On session one, an information sheet was provided and participants gave informed consent and completed a questionnaire

asking participants about their daily usage of various substances including caffeine. Finally, participants completed a practice run on the computer task battery.

The remainder of session one was identical to that of sessions two and three. Five minutes prior to capsule administration, cardiovascular readings were taken and the first of three mood questionnaires was completed. Participants were then asked to give a saliva sample, and sign a form confirming overnight abstinence of psychoactive substances. The cardiovascular measures were then taken again, following which a capsule was ingested. Participants were left alone in the cubicle for 30 minutes to allow for drug absorption. Reading material was provided (newspapers and current periodicals). At 30 minutes post-ingestion, the experimenter returned and the cardiovascular measures and mood questionnaire were completed. Then the participant completed the battery of computer tasks, at the end of which blood pressure, heart rate and mood were measured for the last time. Participants were then asked to complete the post-session questionnaire. Table 6.1 depicts the time course of the sessions

At the end of the final session, participants completed the post-experiment questionnaire, and were debriefed and paid or awarded equivalent course credits for their time.

Table 7.1. Schedule of experimental sessions, including a baseline session

Baseline session (conducted immediately prior to session 1):	
0 mins	Information sheet, consent, personal details form
5 mins	RVIP
12 mins	MTS
15 mins	SRT/CRT
23 mins	SR
Main Session:	
0 mins	CV and mood measures (1)
3 min	Saliva Assay and confirmation slip
4 mins	Capsule
34 mins	CV and mood measures (2)
37 mins	RVIP
43 mins	MTS
46 mins	SRT/CRT
54 mins	SR
57 mins	CV and mood measures (3)
60 mins	Post session questionnaire

7.2.5 Data Analysis

Reaction time measures for SRT, CRT and MTS were split into reaction (RD) and movement (MD) durations by the CANTABeclipse software. RD was the time taken to release the press pad following target presentation and MD was the time taken to move the hand from the press pad to the screen.

Reaction time scores below 100 ms were considered anticipatory (SRT, CRT and MTS) and removed, and scores more than 3 standard deviations above an individual's mean score were deemed outliers (SRT, CRT, MTS MD) and removed (SRT: 1.6% and 1.4%, CRT: 1.5% and 1.2%, MTS 0% and 0.1% removed from RD and MD data respectively). No upper limit was applied to the MTS RD data. However, the MD response simply requires participants to move their hand from press pad to screen (15

cm), therefore an upper limit was included for MTS MD. When a MD score was removed for MTS, the corresponding RD score was also removed (total removed <0.01%). Outliers were not considered for RVIP as participants had a limited time to respond, after which the responses were deemed errors and removed by the computer software. Three types of errors were possible on the RVIP task: missing a target (commission error); an erroneous response to a non-target (i.e. false alarm); or responding to a target too soon (i.e. an anticipatory response before all three targets were presented). All three error types were analysed separately but there was no difference in the results, so the data for total errors are reported.

The data were analysed using a one-way ANOVA with caffeine dose (0, 250 and 400 mg) as the within-subjects factor. Mean reaction and movement duration (SRT, CRT, MTS) and total reaction times (RVIP, SR) and errors (MTS, RVIP, SR) were analysed. Where a main effect of dose was found, post-hoc paired t-tests were used to determine which doses were significantly different. A Bonferroni correction was used, resulting in a p-value of 0.025 being required before significance was assumed. The RVIP reaction time data were separated into three equal blocks comprising of 15 targets per block. These data were analysed using a 3 (caffeine dose) x 3 (block) repeated measures ANOVA. Change-from-baseline scores for the mood factors and cardiovascular measures were analysed using a one-way ANOVA. In a separate pilot study, using a different participant sample (n = 6), the effects of lengthening the caffeine absorption interval were also tested. There were no additional benefits of a 45-minute absorption interval relative to a 30-minute absorption interval. Therefore, for brevity, these data are not reported.

7.3 RESULTS

7.3.1 Simple Reaction Time (SRT)

There was a main effect of caffeine dose for reaction duration [$F(2,22) = 3.60$; $p < 0.05$], with faster times after caffeine relative to placebo (see Figure 7.1). T-tests revealed that 400 mg [$t = -3.09$, $df = 11$, $p < 0.025$] but not 250 mg [$t = -1.15$, $df = 11$, $p > 0.025$] of caffeine significantly improved performance relative to placebo. For movement duration, participants were fastest following 400 mg of caffeine and slowest after placebo, but the difference was not significant [$F(2,22) = 1.45$; $p > 0.05$].

significant for $t = -1.15$, $df = 11$, $p > 0.025$

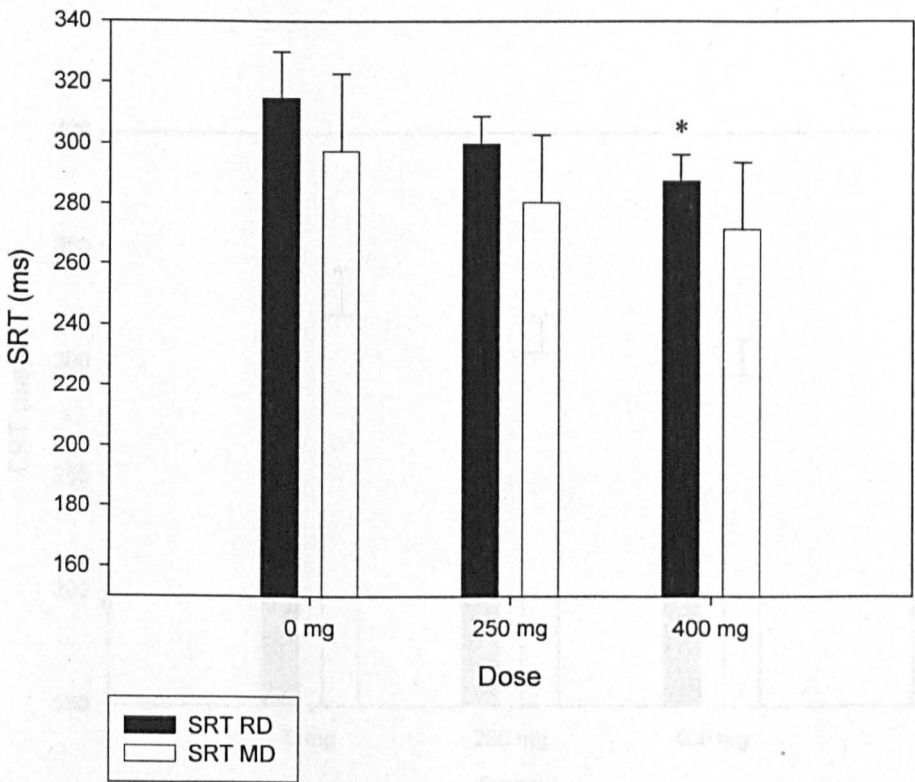


Figure 7.1. Mean SRT reaction duration (RD) and movement duration (MD) (ms) following 0, 250 or 400 mg caffeine. Error bars represent SE means.

*Significantly faster than placebo $p < 0.0025$.

Figure 7.2. Mean $F(2,22) = 1.45$, $p > 0.05$ SRT reaction duration (RD) and movement durations (MD) (ms) following 0, 250 and 400 mg caffeine. Error bars represent SE means.

7.3.2 Choice Reaction Time (CRT)

Participants responded fastest after the highest dose of caffeine and slowest after placebo for both reaction and movement durations. The capsule effect approached significance for reaction duration [$F(2,22) = 3.00$; $p = 0.07$], but was not significant for movement duration [$F(2,22) = 2.46$; $p > 0.05$] (see Figure 7.2). The comparisons between placebo and 400 mg caffeine showed a trend towards significance for reaction [$t = -1.99$, $df = 11$, $p = 0.07$] and movement [$t = -1.87$, $df = 11$, $p = 0.09$] durations. The comparisons between placebo and 250 mg caffeine were not significant [$ts < 1.43$; $dfs = 11$, $ps > 0.025$].

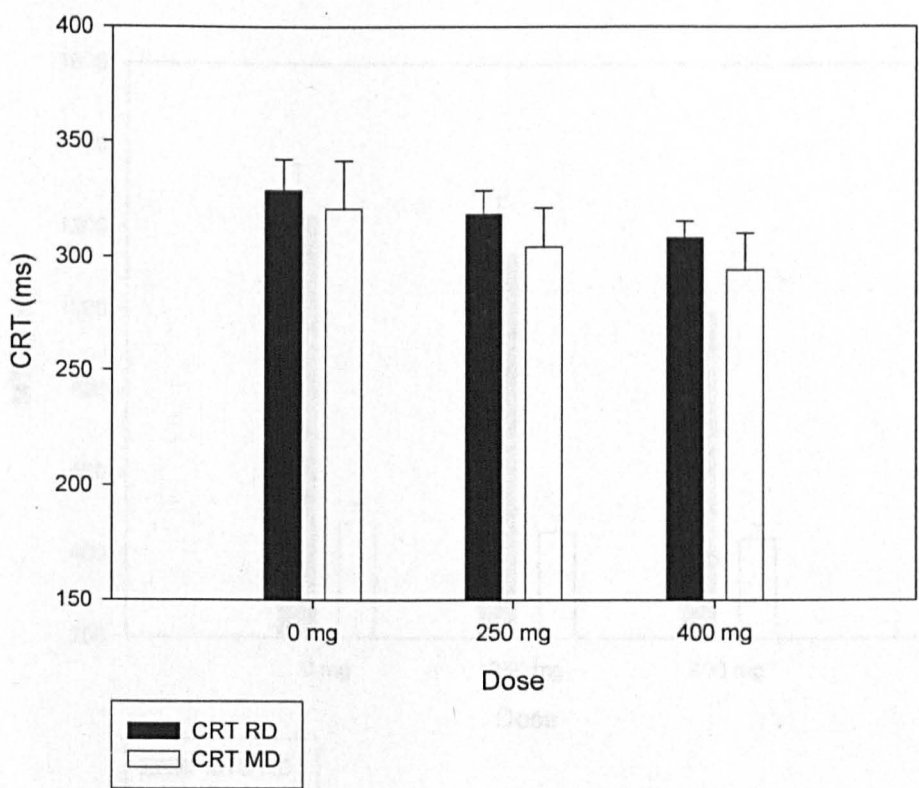


Figure 7.2. Mean ($n = 12$) CRT reaction duration (RD) and movement durations (MD) (ms) following 0, 250 and 400 mg caffeine. Error bars represent SE means.

7.3.3 Match-to-sample Visual Search (MTS)

The effect of caffeine dose approached significance for level one reaction duration (RD) [$F(2,22) = 2.81$; $p = 0.08$]. Participants were fastest after 400 mg and slowest after placebo (see Figure 7.3). The placebo and 400 mg caffeine comparison approached significance [$t = -2.37$, $df = 11$, $p = 0.04$], however the 250 mg and placebo comparison was not significant [$t = -0.77$, $df = 11$, $p > 0.025$]. Participants were also fastest after 400 mg relative to the other caffeine doses on levels two and three RD, however the effect of caffeine dose did not reach significance [$F_s(2,22) < 2.56$; $p_s > 0.05$]. There were no main effects of caffeine dose on any level for movement duration [$F_s(2,22) < 0.50$; $p_s > 0.05$] or errors [$F_s(2,22) < 1.38$; $p_s > 0.05$].

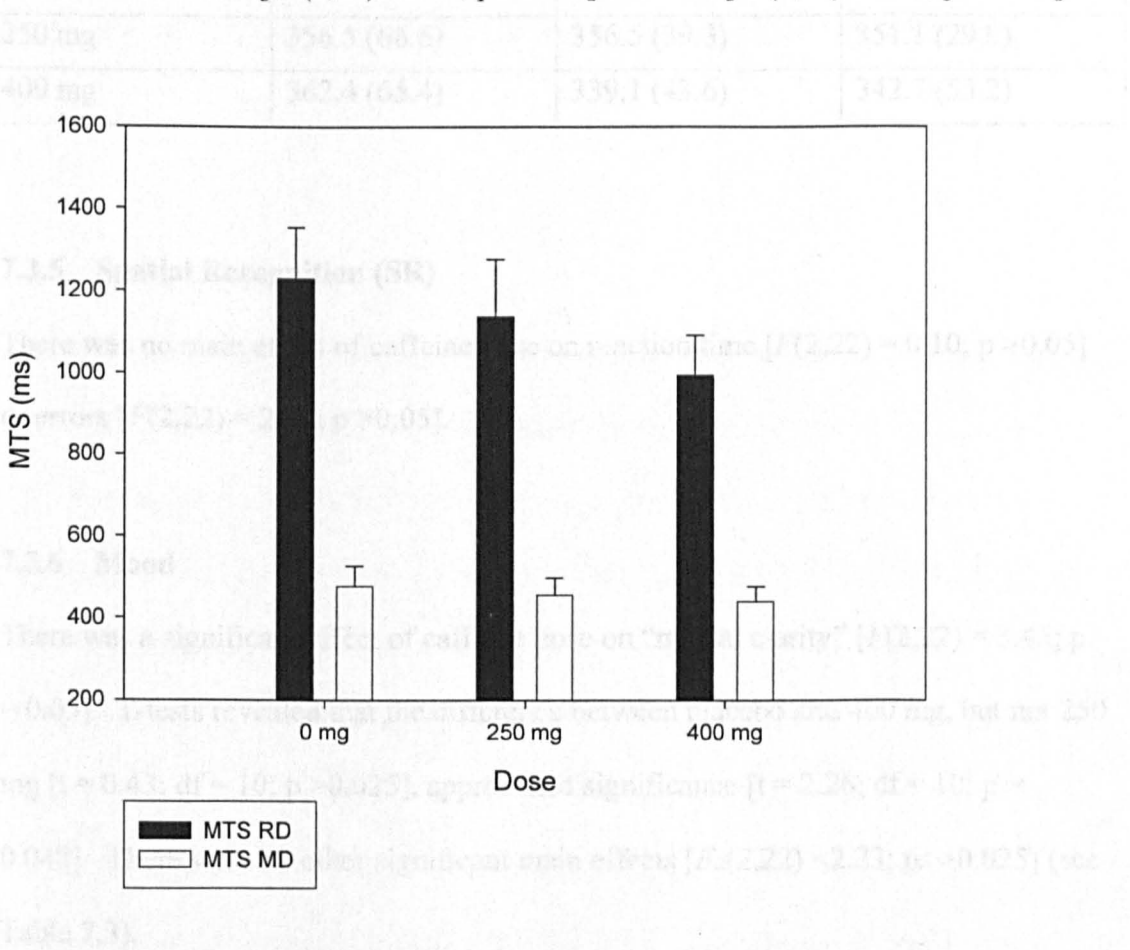


Figure 7.3. Mean reaction duration (RD) and movement duration (MD) (ms) for MTS level one following 0, 250 and 400 mg caffeine. Error bars represent SE means.

7.3.4 Rapid Visual Information Processing (RVIP)

There was no main effect of caffeine dose on RVIP reaction time [$F_s(2,22) = 0.01$; $p > 0.05$] or number of errors [$F_s(2,22) = 0.72$; $p > 0.05$]. There was evidence of a deterioration in performance across blocks after placebo, which did not occur in either caffeine conditioning, however, these effects did not reach significance [$F_s(2,22) < 0.96$; $p_s > 0.05$] (see Table 7.2).

Table 7.2. Mean ($n=12$) RVIP (ms) scores separated in to three blocks after 0, 250 and 400 mg caffeine. Standard deviations are in parenthesis.

	Block 1	Block 2	Block 3
0 mg	350.8 (44.4)	361.1 (52.1)	364.6 (60.90)
250 mg	356.5 (66.6)	356.5 (39.3)	351.1 (29.0)
400 mg	362.4 (65.4)	339.1 (43.6)	342.7 (53.2)

7.3.5 Spatial Recognition (SR)

There was no main effect of caffeine dose on reaction time [$F(2,22) = 0.10$; $p > 0.05$] or errors [$F(2,22) = 2.77$; $p > 0.05$].

7.3.6 Mood

There was a significant effect of caffeine dose on “mental clarity” [$F(2,22) = 3.45$; $p = 0.05$]. T-tests revealed that the difference between placebo and 400 mg, but not 250 mg [$t = 0.43$; $df = 10$; $p > 0.025$], approached significance [$t = 2.26$; $df = 10$; $p = 0.048$]. There were no other significant main effects [$F_s(2,22) < 2.23$; $p_s > 0.025$] (see Table 7.3).

Table 7.3. Mean changes (mm) on all mood factors following 0, 250 and 400 mg caffeine. Standard deviations are in parenthesis

	0 mg caffeine	250 mg caffeine	400 mg caffeine
Anxiety	0.0 (13.7)	-5.0 (21.5)	-2.3 (12.4)
Headache	-14.9 (30.7)	-6.3 (19.7)	-12.0 (22.2)
Mental Clarity	-0.9 (35.6)	4.8 (24.4)	24.1 (27.4)
Mental Repose	-7.2 (28.7)	7.6 (21.5)	10.2 (29.2)
Positive Mood	-10.3 (42.3)	-2.7 (34.7)	14.2 (25.3)
Sleepiness	-5.9(41.3)	-1.8 (45.4)	-28.5 (50.4)
Tense Mood	9.1 (26.8)	-0.5 (26.5)	2.4 (27.9)

7.3.7 Cardiovascular Measures

There were no significant effects for systolic and diastolic blood pressure, or heart rate [$F_s(2,22) < 2.33$, $p_s > 0.05$].

7.4 DISCUSSION

Dose-dependent improvements in performance after 400 mg but not 200 mg caffeine relative to placebo administration were observed for both the reaction and movement duration measures of SRT, CRT and MTS level one. There were no significant effects of caffeine on RVIP or on the cardiovascular measures. Finally, participants reported increases in mental clarity relative to placebo, which approached significance for the 400 mg but not 250 mg dose of caffeine.

These findings are in agreement with the many studies that have reported faster reaction times following caffeine (Lieberman et al. 1987; Roache & Griffiths 1987; Jarvis 1993; Smith, Kendrick & Maben 1994; Smith, Maben & Brockman 1994; Smit

& Rogers 2000; Brice & Smith 2002a). The optimal performance-enhancing dose of caffeine is often reported to be around 200 to 250 mg (Fredholm et al. 1999; Lorist & Tops 2003). However, performance benefits of 400 mg of caffeine have been demonstrated on psychomotor tasks (Jacobsen & Edgley, 1987; Roache & Griffiths, 1987), although there is a lack of recent data on high doses of caffeine and cognitive performance due to a trend towards using doses more consistent with every day use. It is possible that habitual caffeine consumption influences the response to acute doses of caffeine. The participants recruited in the present thesis were relatively high caffeine consumers compared to the majority of studies that recruit participants with a daily caffeine intake of around 100 to 250 mg. This may have resulted in benefits only being observed at higher doses, due to tolerance to some of the effects of caffeine in high consumers (Zahn & Rapoport 1987; Evans & Griffiths 1992).

In the present study, both reaction and movement durations were facilitated by caffeine, although only the reaction duration element of SRT was significantly improved. Caffeine is known to stimulate psychomotor activity via disinhibition of striatal motor pathways (Fenu, Cauli & Morelli 2000; Karcz-Kubicha et al. 2003; Fisone et al. 2004), and therefore caffeine's facilitation of reaction time may be mediated by effects on the motor component of the response. However, these results indicate that caffeine also has the capacity to affect speed to react to a stimulus, prior to the motor response.

Despite some studies reporting clear benefits of caffeine on RVIP performance (Frewer & Lader 1991; Hasenfratz & Bättig 1994; Warburton 1995; Warburton,

Bersellini & Sweeney 2001; Yeomans et al. 2002), no significant effects were obtained in the present study. Although it is often reported that cognitively demanding tasks show a lower sensitivity to the effects of caffeine, caffeine is thought to be particularly effective in offsetting fatigue in repetitive sustained attention tasks (see Smith 2002). Therefore, presumably, any benefits of caffeine should be particularly evident in later blocks of the task when the placebo group begin to show signs of fatigue. Consistent with this suggestion, in the present study, performance slowed across blocks after placebo, and there was a trend for this slowing to be attenuated by caffeine.

Caffeine benefits were observed for the mood factor “mental clarity” after 400 mg caffeine compared to placebo. It is possible that the VASs failed to pick up subtle changes on some mood scales. Smit and Rogers (2002) point out that even validated mood scales can be compromised by various sources of “noise” that can mask real effects. In particular, they highlight the importance of the instructions given to participants and the decrease in motivation that may occur as result of completing multiple forms leading to repetitive responses. Although clear written instructions were given to the present participants, this may not have been sufficient to ensure reliable responding. This will be addressed by providing more explicit training on the completion of the scales in future experiments.

The results of the present study suggest that CANTABeclipse is an effective tool to assess the cognitive effects of caffeine in a sample of caffeine consumers. In addition, the 400 mg dose of caffeine produced greater improvements on performance and

mood (relative to placebo) than the 250 mg dose of caffeine. Therefore, the aim of the next experiment was to assess the conditioned cognitive effects of a 400 mg dose of caffeine using the CANTAB system.

CHAPTER 8:

EXPERIMENT 4: CONDITIONED EFFECTS OF 400 mg CAFFEINE ON CANTAB TASK PERFORMANCE, AND SUBJECTIVE AND CARDIOVASCULAR MEASURES

8.1 INTRODUCTION

Due to the weak effects of caffeine observed in Experiments 1 and 2, Experiment 3 examined the effects of a higher dose of caffeine on selected tasks from the CANTABclipse task battery. Greater benefits of 400 mg of caffeine were observed relative to placebo on measures of mood and performance, compared to a lower dose of 250 mg. The present study was designed to replicate the conditioning paradigm used in Experiment 1, utilising 400 mg of caffeine and the CANTABclipse tasks that demonstrated significant effects in Experiment 3, to examine whether the cognitive and mood effects of caffeine can be conditioned to the context in which caffeine is received.

Some additional methodological changes were implemented in the present study. Firstly, the number of CRT trials was doubled by the addition of two extra blocks. Caffeine is reportedly effective in attenuating the effects of task fatigue (Smith 2002), and therefore effects should be more robust if a task is fatiguing, with evidence of performance deterioration over time after placebo. To ensure the experimental sessions were of comparable length to the previous study, the task battery was shortened by the omission of the spatial recognition task.

Secondly, additional questions were added to the post-experiment questionnaires asking participants if they experience caffeine withdrawal or craving following acute caffeine abstinence. Caffeine effects on mood and performance are reported to be particularly robust when regular caffeine consumers are tested in an acute state of caffeine withdrawal (James 1994; Rogers et al. 2003; James 2005), although not all regular caffeine consumers report withdrawal upon caffeine abstinence (Juliano & Griffiths 2001). It is therefore plausible that weak effects of caffeine may be obtained if participants do not usually experience caffeine withdrawal effects. Thus, these questions provided a gauge of the level of perceived caffeine withdrawal within the sample.

Finally, the lack of caffeine effects on several mood factors in Experiment 3, despite clear caffeine effects on performance, may have been due to insufficient training in the use of the VAS scales (Smit & Rogers 2002). Although clear instructions were provided on each VAS questionnaire, extra training was now added with the experimenter verbally reiterating these instructions, emphasising the importance of thinking of the line as a continuous spectrum of each mood trait.

It was hypothesised that the paired group who received caffeine in the test context (i.e. paired group) would demonstrate superior performance relative to the group who received caffeine in the test context (i.e. unpaired group) across the conditioning trials, and that these benefits would be maintained on the test of conditioning, at which both groups receive placebo in the test context.

8.2 METHOD

8.2.1 Participants

Twelve participants (10 female/2 male; mean age 21.2 years, range 18-26) with an average daily caffeine intake of 300.6 mg (range 184 to 545 mg) took part in this study in return for cash (£25) or course credits. Two additional participants were enrolled into the study, but failed to complete all of the experimental sessions.

8.2.2 Design and Materials

Participants were pseudo-randomly allocated to one of two experimental groups: paired vs. unpaired, such that these groups were matched for gender, age and levels of habitual caffeine consumption. Experimental sessions were spaced so that the interval between any two did not fall below 24 hours or exceed 7 days. 400 mg of caffeine and placebo were administered as per section 4.3.

8.2.3 Measures

The RVIP, MTS, SRT, cardiovascular and mood measures were as used in Experiment 3. The CRT task was as in Experiment 3, but with the addition of two experimental blocks.

8.2.4 Procedure

Participants attended five sessions: four conditioning trials and one conditioning test. In addition, session one was immediately preceded by a baseline session. Table 8.1 summarises the time course of the sessions. All testing took place between 12:00 and

18:00 hours, and participants attended each session at approximately the same time of day.

Table 8.1. Schedule of experimental sessions, including baseline and conditioning test

Baseline Session (conducted immediately prior to session one)	
0 mins	Standardised Instructions
5 mins	Consent form and personal details questionnaire
7 mins	CV measures and mood questionnaire
10 mins	RVIP
16 mins	MTS
19 mins	SRT/CRT
28 mins	Taken to test cubicle for condition session 1
Conditioning Trials (Sessions 1-4)	
0 mins	CV and mood measures (1)
3 mins	Saliva Assay
4 mins	Capsule (1)
34 mins	CV and mood measures (2)
37 mins	RVIP
43 mins	MTS
46 mins	SRT/CRT
54 mins	CV and mood measures (3)
57 mins	Leave test cubicle – capsule (2)
58 mins	Post session questionnaire
Conditioning Test (Session 5)	
0-58 mins	As conditioning trials
58 mins	Post experiment questionnaire and debriefing

At the baseline session, participants received a set of standardised instructions explaining the procedure and requirements of the study. They also completed a questionnaire, which asked about their daily consumption of various foodstuffs and medications (including caffeine), and gave their consent to continue. Baseline measures on all tasks were then taken (i.e. mood questionnaire, cardiovascular

measures, CANTAB tasks). Immediately after this session, participants were taken to the test cubicle to begin the first conditioning trial.

The conditioning trials took place in the cubicles described in Chapter 4 (Experiment 1). First, mood and cardiovascular measures were taken, and participants gave a saliva sample and signed a form confirming overnight abstinence. A capsule was then administered (caffeine – paired group; or placebo – unpaired group), and participants were left alone for 30 minutes. Reading material was provided (daily newspapers and current periodicals). After this interval, the experimenter returned to administer the tests. At the end of each session, participants were administered the converse capsule (i.e. the paired group received placebo and the unpaired group received caffeine) in a different room. Finally, participants completed the post-session questionnaire.

The final session (conditioning test) procedure was identical to the previous four conditioning trials, except that all participants received placebo in the test cubicle. At the end of the fifth session, participants completed the post-experiment questionnaire. Participants were then debriefed and paid or awarded equivalent course credits for their time.

8.2.5 Data Analysis

Reaction times below 100 ms (SRT, CRT and MTS), and more than 3 standard deviations above an individual's mean score (SRT, CRT, MTS MD) were considered outliers and removed (total removed - SRT: 1.7 and 1.5%, CRT: 1.1 and 1.7%, MTS 0% and 0%, for reaction and movement durations respectively). Mean reaction times

(SRT, CRT, RVIP) and number of errors (MTS, RVIP) were compared between groups and over the four conditioning sessions using a three-factor mixed ANOVA with session and block as within-subjects factors and group as a between-subject factor. For SRT and CRT, each block comprised 15 trials, resulting in two and four blocks respectively. RVIP was blocked into 3 blocks comprising 15 trials each. Mean reaction times of MTS were analysed using a two-way ANOVA with session and group as the within- and between-subjects factors respectively. Reaction times for SRT, CRT and MTS were split into reaction and movement durations and examined in separate analyses. Change from baseline scores were calculated for heart rate and mood factors and also analysed by a two-way ANOVA. On tests comprising a block factor (SRT, CRT, RVIP), two-way ANOVAs (with group and block as between and within subject factors respectively) were used to determine whether there were significant group differences at baseline and at the conditioning test. On all other tests, independent t-tests were used. The chi-square analysis of the post-session data was as Experiment 1.

8.3 RESULTS

There were no significant differences between groups on age, habitual caffeine consumption or BMI (see Table 8.2).

Table 8.2. Participant characteristics: mean and t-test results of between group comparisons (caffeine-paired and caffeine-unpaired) for age, level of daily caffeine consumption and BMI. Standard deviations are in parenthesis.

	Mean		<i>t</i> value	Degrees of Freedom	<i>p</i> value
Age (years)	Paired	20 (1.3)	-2.214	10	>.05
	Unpaired	22 (2.3)			
Consumption (mg/day)	Paired	310.0 (112.1)	.238	10	>.05
	Unpaired	291.3 (157.0)			
BMI	Paired	20.9 (2.7)	-1.240	10	>.05
	Unpaired	22.7 (2.3)			

8.3.1 Simple Reaction Time (SRT)

Conditioning Trials: There were no significant differences between groups for SRT reaction (RD) or movement (MD) durations at baseline [$t_s > 1.05$, $dfs = 10$, $ps > 0.05$].

There were no main effects of group, session or block and no significant interactions for either RD or MD [$F_s(1,10) < 0.98$; $ps > 0.05$] [$F_s(3,30) < 1.85$; $ps > 0.05$].

Conditioning Test: RD was significantly faster on block two compared to block one [$F_s(1,10) < 6.93$; $ps < 0.05$] (see Table 8.3). There were no other significant effects or interactions for RD or MD [$F_s(1,10) < 2.10$; $ps > 0.05$].

Table 8.3. Mean (ms) SRT reaction durations (RD) and movement durations (MD) on the conditioning trials and at the conditioning test in the paired and unpaired groups. Standard deviations are in parenthesis.

	Group	Cond. Trial 1	Cond. Trial 2	Cond. Trial 3	Cond. Trial 4	Cond. Test
SRT RD	Paired	304.1 (25.4)	308.5 (17.8)	314.8 (43.3)	284.4 (19.7)	305.4 (30.6)
	Unpaired	297.1 (27.4)	298.7 (23.5)	304.2 (29.5)	294.4 (19.0)	292.0 (32.0)
SRT MD	Paired	330.8 (86.0)	355.1 (105.7)	346.1 (89.4)	320.6 (70.1)	355.0 (122.2)
	Unpaired	293.5 (86.0)	293.5 (62.4)	276.2 (85.8)	294.0 (82.1)	285.5 (111.6)

8.3.2 Choice Reaction Time (CRT)

Conditioning Trials: There were no significant differences between groups for reaction or movement durations at baseline [$t_s < 0.27$, $dfs = 10$, $ps > 0.05$]. There were no significant main effects of group, session or block for reaction or movement durations [$F_s(1,10) < 0.77$; $ps > 0.05$] [$F_s(3,30) < 2.42$; $ps > 0.05$]. There was a significant group-by-block interaction for reaction duration with the paired group [$F(3,30) = 3.09$; $p < 0.05$], showing less deterioration across blocks relative to the unpaired group (see Figure 8.1). There were no other significant interactions for reaction or movement duration [$F_s(3,30) < 0.51$; $ps > 0.05$] [$F_s(9,90) < 1.17$; $ps > 0.05$].

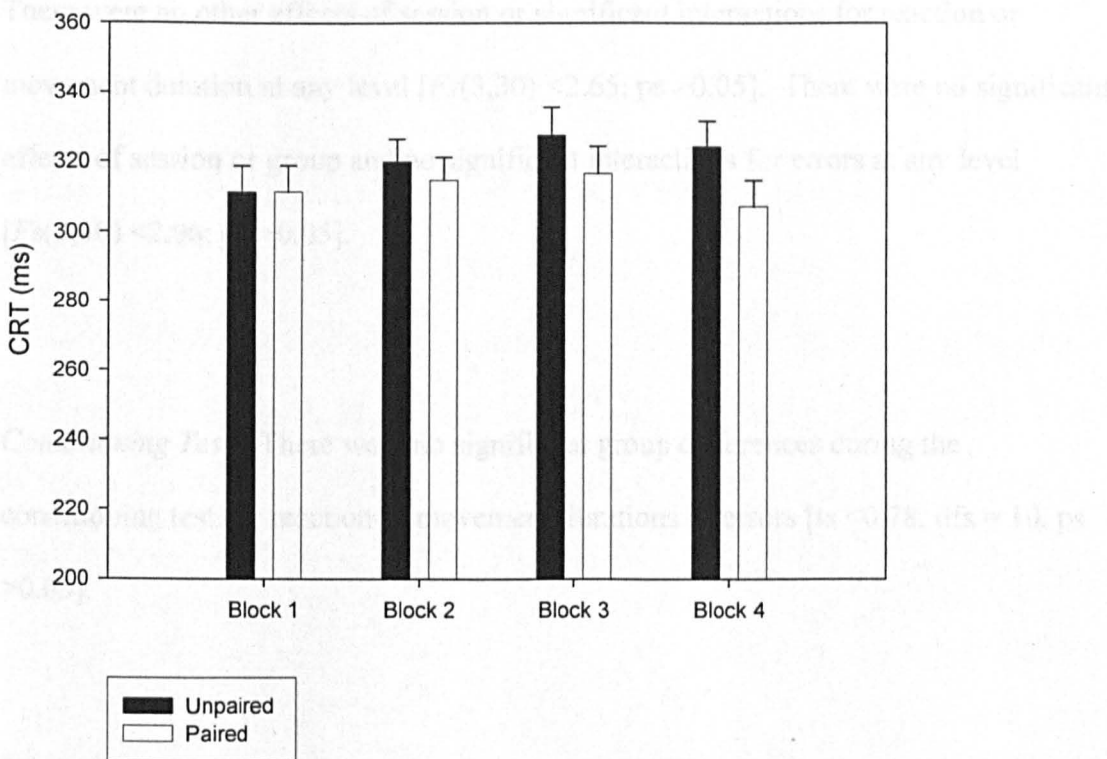


Figure 8.1. Mean ($N = 6$ per group) CRT reaction duration scores (ms) for the paired and unpaired groups. The bars represent successive blocks (15 trials each) Data are shown as the means of the four conditioning sessions. Error bars represent SE means.

Conditioning Test: There were no significant effects of group or block and no significant interactions for reaction and movement durations [$F_s(1,10) < 0.78$; $p_s > 0.05$] [$F_s(3,30) < 1.37$; $p_s > 0.05$].

8.3.3 Match-to-Sample Visual Search (MTS)

Conditioning Trials: There were no significant group differences at baseline on MTS reaction or movement duration at any level [$t_s < 1.57$, $df = 10$, $p_s > 0.05$]. There was no main effect of group for reaction or movement duration at any level [$F_s(1,10) < 0.96$; $p_s > 0.05$]. There was a significant main effect of session [$F(3,30) = 4.08$; $p < 0.05$] for level two reaction duration as reaction duration decreased across sessions. There were no other effects of session or significant interactions for reaction or movement duration at any level [$F_s(3,30) < 2.65$; $p_s > 0.05$]. There were no significant effects of session or group and no significant interactions for errors at any level [$F_s(3,30) < 2.06$; $p_s > 0.05$].

Conditioning Test: There were no significant group differences during the conditioning test for reaction or movement durations or errors [$t_s < 0.78$, $dfs = 10$, $p_s > 0.05$].

8.3.4 Rapid Visual Information Processing (RVIP)

Conditioning Trials: There were no significant group differences at baseline on RVIP reaction time [$t = 0.10$, $df = 10$, $p > 0.05$] or number of errors [$t = 0.45$, $df = 10$, p

>0.05]. There were no significant main effects of group for RVIP reaction time or errors [$F_s(1,10) < 0.33$; $ps > 0.05$]. There was a main effect of session for RVIP errors [$F(3,30) = 4.01$; $p < 0.05$] whereby participants displayed fewer errors across sessions, but no effect of session for reaction time [$F(3,30) = 2.57$; $p > 0.05$]. There were no other significant interactions for reaction time or errors [$F(2,20) = 0.16$; $p > 0.05$] [$F_s(3,30) < 0.37$; $ps > 0.05$] [$F_s(6,60) < 1.84$; $ps > 0.05$].

Conditioning Test: There were no significant main effects or interactions during the conditioning test for RVIP reaction time or errors [$F_s(1,10) < 0.59$; $ps > 0.05$] [$F_s(2,20) < 2.17$; $ps > 0.05$].

8.3.5 Mood

Conditioning Trials: There was a significant main effect of group for “Headache” (0-30 minutes) [$F(1,10) = 5.18$; $p < 0.05$]. The paired group showed an increase in headache (5.7 mm; SD = 16.7 mm) compared to a decrease in the unpaired group (-5.0 mm; SD = 16.8 mm). There were no other main effects of group [$F_s(1,10) < 3.31$; $ps > 0.05$] (see Table 3). There were no significant main effects of session or significant interactions for any of the mood factors [$F_s(3,30) < 2.29$; $ps > 0.05$].

Conditioning Test: There were no significant effects of group for any of the mood factors [$ts < 0.90$, $dfs = 10$, $ps > 0.05$] (see Table 8.4).

Table 8.4. Mean changes (mm) for all mood factors on the conditioning trials and at the conditioning test in the paired and unpaired groups. Standard deviations are in parenthesis.

Factor/Trait	Group	Cond. Trial 1	Cond. Trial 2	Cond. Trial 3	Cond. Trial 4	Cond. Test
Anxiety	Paired	-6.7 (31.7)	2.7 (3.3)	11.0 (16.7)	8.7 (9.2)	-5.8 (28.4)
	Unpaired	-1.8 (7.8)	-9.0 (13.1)	-5.2 (12.9)	4.5 (19.6)	-7.8 (16.4)
Headache	Paired	5.5 (24.8)	1.5 (39.9)	6.7 (51.3)	1.5 (29.3)	-5.7 (12.6)
	Unpaired	-6.0 (26.1)	0.5 (18.0)	-11.2 (22.8)	7.0 (11.2)	4.8 (175)
Positive Mood	Paired	5.0 (22.8)	2.1 (26.8)	-14.8 (45.2)	-6.2 (34.4)	-10.3 (35.8)
	Unpaired	-10.2 (20.7)	-10.8 (22.4)	-2.3 (33.6)	8.7 (54.7)	8.7 (45.4)
Mental Clarity	Paired	10.8 (29.3)	16.0 (51.1)	12.3 (69.2)	-13.0 (33.5)	-13.5 (48.1)
	Unpaired	16.5 (25.5)	-20.5 (11.0)	7.0 (37.3)	-4.5 (33.0)	-0.2 (28.4)
Mental Repose	Paired	12.3 (43.7)	-2.8 (46.4)	-7.5 (27.9)	12.3 (61.5)	-9.5 (40.2)
	Unpaired	-5.8 (10.8)	7.7 (35.4)	1.5 (25.7)	9.3 (21.9)	-3.2 (52.6)
Tense Mood	Paired	-14.0 (61.4)	6.3 (18.4)	-1.5 (39.9)	-2.3 (41.4)	-27.0 (47.8)
	Unpaired	4.0 (24.5)	16.2 (19.2)	1.5 (17.5)	-14.8 (27.3)	-2.7 (68.3)
Sleepiness	Paired	1.5 (36.0)	-4.2 (62.3)	11.0 (36.9)	23.8 (56.0)	-7.3 (49.4)
	Unpaired	-22.2 (52.5)	22.5 (63.9)	5.0 (65.4)	19.8 (33.5)	-0.8 (58.6)

8.3.6 Cardiovascular Measures

Conditioning Trials: There were no significant main effects of group [$F_s(1,10)$

<2.11 ; $ps >0.05$] or session [$F_s(3,30) <1.95$; $ps >0.05$] for SBP, DBP or HR. There

was a significant group-by-session interaction for SBP. Post-hoc independent t-tests

showed group differences that approached significance on session two [$t = -1.84$, $df =$

10, $p = 0.096$] and session four [$t = 1.82$, $df = 10$, $p = 0.09$]. SBP decreased in the

paired (relative to unpaired) group on session two, and increased in the unpaired

(relative to paired) group on session four. There were no significant interactions for DBP or HR [$F_{(3,30)} < 2.51$; $p_s > 0.05$].

Conditioning Test: There were no effects on any cardiovascular measure during the conditioning test [$t_s < 0.89$, $dfs = 10$; $p_s > 0.05$].

8.3.7 Post-Session Questionnaires

At the end of each session, participants were asked to indicate what substance they believed that they had received that day from a list of substances (see section 4.5).

The responses are shown in Tables 8.5 and 8.6.

Table 8.5. Number of participants in the unpaired group who believed that they had received caffeine during the conditioning trials compared to an alternative substance, placebo or “don’t know”

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	1	1	0	2	4
Other substance	2	5	5	3	15
Placebo	3*	0*	1*	1*	5*
Don’t know	0	0	0	0	0

*correct choice

Table 8.6. Number of participants in the paired group who believed that they had received caffeine during the conditioning trials compared to an alternative substance, placebo or “don’t know”

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	3*	3*	1*	2*	9*
Other substance	2	1	2	1	6
Placebo	0	2	2	2	6
Don’t know	1	0	1	1	3

*correct choice

Pearson’s chi-square analysis examining the association between group and reported identification of caffeine was not significant [$\chi^2_{(1, N=12)} = 2.64, p > 0.05$].

The post-experiment questionnaire asked participants whether they ever experienced cravings for caffeine or withdrawal symptoms following a period of no intake. If they responded “yes” to this question they were then asked whether they had felt any such withdrawal and/or craving prior to any experimental session. The results are displayed in Table 8.7.

Table 8.7. Number of participants who reported experience of withdrawal and/or craving in general and at experimental sessions in unpaired and paired groups

	No. of participants who ever experience caffeine withdrawal and/or craving (N=6 per group)	If so, no. of participants who experienced caffeine withdrawal and/ or craving at the sessions
Unpaired	2	1
Paired	3	1
Total	5	2

42% (5 out of 12) of participants reported ever experiencing caffeine withdrawal symptoms and/or caffeine craving upon abstinence, and only 17% (2 out of 12) reported feeling such symptoms during any of the experimental sessions.

8.4 DISCUSSION

No main effects of caffeine were found for any of the cognitive tasks during the conditioning trials and therefore unsurprisingly no conditioned effects of caffeine were observed at test. Choice reaction duration deteriorated across blocks in the unpaired but not the paired group, and a similar pattern was obtained for RVIP

reaction time, which is consistent with the suggestion that caffeine can offset task fatigue (see Smith 2002). However, in general there were no robust effects of caffeine on cognitive performance. In addition, there were only modest effects of caffeine on mood, as only reports of “Headache” were affected by caffeine, but in contrast to predictions suggesting that caffeine attenuates withdrawal-related headache, ratings of headache increased in paired group

In agreement with previous findings, caffeine significantly affected blood pressure but these effects were not consistent or predictable across trials (Kourtidou-Papadeli et al. 2002). Future studies should take into account factors that may affect the reliability of CV measures such as activity prior to the test sessions.

These results contrast with the significant effects of caffeine found in Experiment 3. One possibility for the contrasting findings is that the sample size (which was smaller in the present study than those previously reported in this thesis) was too small to detect statistically reliable results. However, on many of the cognitive measures, the direction of the effects was opposite to those predicted suggesting that the effects would not have strengthened with additional participants.

Alternatively, because only five of the 12 participants reported ever experiencing caffeine craving or withdrawal upon abstinence, and only two of these reported being aware of caffeine craving and/or withdrawal during the study, it is possible that the modest effects of caffeine were due to low responsiveness to caffeine due to lack of withdrawal effects. To address this issue, a more rigorous screening procedure could

be introduced to identify participants who demonstrate performance benefits of caffeine.

It may also be significant that the present study was a between-subjects design whereas Experiment 3 used a within-subjects design. Although in the present study groups did not significantly differ on the basis of BMI, age and levels of habitual consumption, other factors which may have influenced responses to caffeine such as: personality (Gilliland 1980); anxiety (Bruce, Scott, Shine & Lader 1992); pharmacokinetics (Lader, Cardwell, Shine & Scott 1996); co-committant drug use (Patwardhan, Desmond, Johnson & Schenker 1980); and beliefs about the effects of caffeine on performance (Oei & Hartley 2005) were not controlled for. Furthermore, significant effects may be masked by inter-subject variability in baseline responding. A within-subject design reduces such variability and has the added advantage of increasing the statistical power to detect effects without increasing the number of participants. This is particularly advantageous for studies in which suitable participants (e.g. non-smoking, high caffeine consumers) are difficult to recruit. Therefore, the next experiment implemented a within-subjects design and included a pre-experiment screening procedure to increase the likelihood of observing significant effects of caffeine.

CHAPTER 9:
EXPERIMENT 5: CONDITIONED PERFORMANCE AND SUBJECTIVE
EFFECTS OF CAFFEINE USING A DIFFERENTIAL CONDITIONING
PARADIGM

9.1 INTRODUCTION

No significant effects of caffeine on mood or performance were observed in Experiment 4 despite the fact that clear effects of the same dose of caffeine were observed in Experiment 3. These contrasting findings may relate to the fact the participants in Experiment 3 received both caffeine and placebo according to a within-subjects design whereas a between-subjects design was used in Experiment 4.

There is considerable experimental evidence suggesting that responses to caffeine are affected by individual differences (Lader et al. 1996). Although the two groups in Experiment 4 were similar in age, habitual consumption, cigarette smoking and BMI, there are other factors that are known to affect responding to caffeine that could not easily be controlled. For example, there can be substantial inter-individual variability in the rate at which caffeine is absorbed and metabolised, due to both physical (e.g. concomitant drug-use, food in gastrointestinal tract) and genetic factors (Lader et al. 1996; Magkos & Kavouras 2005). Furthermore, between-subject variability in baseline responding may mask the effects of caffeine.

The present study used a within-subjects differential conditioning paradigm to reduce inter-subject variability in responding. For half of the conditioning trials, one context

(CS+) was paired with caffeine, and for the other half of the trials placebo was administered in different context (CS-). These sessions were followed by two tests of conditioning, in which responses to placebo were assessed in the CS+ and the CS- contexts. Foltin and Haney (2000) used a similar procedure to examine the effects of pairing two sets of neutral cues with either placebo or cocaine smoking, and demonstrated cocaine-like physiological responding and craving to the cocaine-paired, but not placebo-paired, cues. In addition, differential procedures have successfully demonstrated conditioning to the physiological and behavioural effects of alcohol (Glautier, Drummond & Remington 1994), physiological effects and craving responses to nicotine (Lazev, Herzog & Brandon 1999; Field & Duka 2001) and the rated pleasantness of caffeine (Yeomans, Durlach & Tinley 2005).

Because learning in one context may affect learning in the other context, the temporal arrangement of the trials in differential conditioning paradigms requires consideration. Both alternating (i.e. a CS+ always followed by a CS- trial) and randomised procedures have been used previously (Glautier et al. 1994; Field & Duka 2001; Yeomans, Durlach & Tinley 2005). While it may be argued that alternating procedures promote expectancy effects, there are also potential problems with fully or quasi-randomised procedures. For example, if multiple CS- sessions occur at the beginning or at the end of the learning phase this can inhibit conditioning through processes of latent inhibition or extinction, respectively (Maes & Vossen 2000). Therefore, an alternating procedure was used in the present study but the conditioning trials were separated by at least 24 hours to reduce expectancy effects. Information

about the previous session is less likely to be remembered after 24 hours than after shorter inter-session intervals (Foltin & Haney 2000; Field & Duka 2002).

To maximise the likelihood of obtaining clear caffeine effects on performance, participants were screened before the main experiment to identify caffeine consumers displaying caffeine-induced performance enhancements. Participants attended two 60-minute experimental sessions (caffeine versus placebo). Only participants who showed reaction time benefits of caffeine relative to placebo were offered a place on the main study. Similar procedures have been used in caffeine discrimination studies to identify participants who are able to discriminate drug from placebo. For example, Oliveto et al. (1992) attempted to establish reliable discrimination of caffeine (versus placebo) capsules in a group of caffeine consumers. After an initial training phase, participants were tested for discrimination, and only participants who achieved a performance criterion were enrolled into the final experimental phase of the study. Participants performing below this criterion were discontinued from the study.

It was predicted that participants would display mood and performance benefits of caffeine relative to placebo. Furthermore, these benefits would be maintained in the caffeine-paired context relative to placebo-paired context when placebo was administered in both contexts.

9.2 METHOD

9.2.1 Participants

9.2.1.1 Screening

49 participants (18 male; mean age 23.2 years) participated in the screening procedure. A comprehensive report of the procedure, demographics and results is presented in Chapter 11. 45 participants attended two sessions (caffeine vs. placebo) on separate afternoons at approximately the same time of day. Capsule order was counterbalanced across participants and capsule administration was double blind.

Caffeine administration, the task battery and the experimental procedure were identical to the main study (see 9.2.4), with the following exceptions: a) the sessions took place in a general usage laboratory so that there was no pre-exposure to the conditioned stimuli prior to the conditioning trials; b) practice versions of the SRT and CRT tasks (two blocks of 15 trials) were included at the beginning of each session.

Participants were included in the main study if their reaction times following caffeine administration were consistently faster than their reaction times following placebo administration. To maintain the double blind procedure, two additional experimenters examined the data and selected the participants. Of the twenty participants who were accepted from the screening procedure, twelve agreed to participate in the main study.

9.2.1.2 Main Study

Two participants failed to complete all of the sessions, as their data was omitted from the analysis. The remaining ten participants (7 female/3 male; mean age of 22 years, range 18-29 years; BMI mean 22.5, range 20.7-25.8) had an average daily caffeine intake of 251 mg/day (range 144 to 450 mg), and participated in return for cash (£50) or course credits.

9.2.2 Design and Materials

This within-subject design required participants to attend 10 sessions comprising four trials in which caffeine was paired with one context (CS+) and four trials in which placebo was paired with a different context (CS-). These conditioning trials were followed by two tests of conditioning, in which placebo was administered in both contexts. CS- and CS+ sessions were alternated (i.e. CS+1/CS-1, CS+2/CS-2, CS+3/CS-3 and CS+4/CS-4) and capsule order (placebo/caffeine vs. caffeine/placebo) and the context which served as the CS+ were counterbalanced across participants. The sessions were spaced so that the interval between any two did not fall below 24 hours. Although it was originally stipulated that the interval between sessions should not exceed 7 days, illness in one participant and the Christmas vacation (2 participants) resulted in some longer inter-session intervals (up to 28 days). 400 mg of caffeine and placebo were administered as per section 4.3.

Conditioned Stimuli: The test rooms that served as either the CS+ or the CS- were matched in size and both contained a table, chair and computer. They were differentiated by colour (black versus white painted walls and ceiling), and contained

distinct olfactory and visual cues. A distinct odour was added to each cubicle by using a Glade plug-in diffuser (SC Johnson, Surrey). Fragrances were chosen based on the results of a pilot questionnaire that asked participants (N=20) to rate four sample odours from the Glade plug-in range. Ratings were made using 100 mm visual analogue scales with the anchors “not at all” (0) and “extremely” (100). The two fragrances chosen for the study were “Bamboo and White Fresa” (added to the white cubicle) and “Smells of the Orient” (added to the black cubicle), on the basis that they were well matched for pleasantness [$t = 0.22$, $df = 19$, $p > 0.05$], pungency [$t = 0.36$, $df = 19$, $p > 0.05$] and novelty [$t = 0.85$, $df = 19$, $p > 0.05$], yet were also considered relatively distinct (similarity score of 30 mm on a 100 mm VAS).

The same 20 participants also completed a VAS (100 mm) asking them to rate eight size A6 prints (GB poster.com) for “liking”, “familiarity” and “interest” (“not at all” to “extremely”). The two best matched were pictures of Neuschwanstein Castle, Germany and Bora Bora Island, which did not significantly differ on any of the ratings (liking [$t = -0.34$, $df = 21$, $p > 0.05$]; familiarity [$t = 0.39$, $df = 21$, $p > 0.05$]; interest [$t = 0.87$, $df = 21$, $p > 0.05$]). Full-size (91 x 61 cm) versions of these prints were subsequently placed in the white and black cubicles respectively.

9.2.3 Measures

The CANTAB tasks and mood measure were as used in Experiment 4, but the cardiovascular measures were omitted due to lack of consistent effects observed in previous studies.

9.2.4 Procedure

All testing took place between 12:00 and 18:00 hours, and participants attended each session at approximately the same time of day. The individual test sessions followed the same procedure as Experiment 4 (see section 8.2.4), with the exception that the cardiovascular measures were no longer taken (see Table 9.1).

The conditioning tests also followed the same procedure, except that all participants received placebo in each test environment. At the end of the tenth session, participants completed the post-experiment questionnaire and were then debriefed and paid or awarded course credits for their time.

Table 9.1. Schedule of conditioning trials and conditioning test

<u>Conditioning Trials (Sessions 1-8)</u>	
<i>0 mins</i>	Mood measures (1)
<i>3 min</i>	Saliva Assay
<i>4 mins</i>	Capsule
<i>34 mins</i>	Mood measures (2)
<i>37 mins</i>	RVIP
<i>43 mins</i>	MTS
<i>46 mins</i>	SRT/CRT
<i>54 mins</i>	Mood measures (3)
<i>57 mins</i>	Leave test cubicle – capsule 2
<i>58 mins</i>	Post session questionnaire
<u>Conditioning Test (Session 5)</u>	
<i>0-58 mins</i>	As conditioning trials
<i>58 mins</i>	Post experiment questionnaire and debriefing

9.2.5 Data Analysis

Reaction times below 100 ms (SRT, CRT and MTS), and more than 3 standard deviations above an individual's mean score (SRT, CRT, MTS MD) were considered

outliers and removed (SRT: 1.5% and 1.2, CRT: 1.7% and 1.0%, MTS: 0% and 0% removed for reaction and movement duration respectively). For the conditioning trials, mean reaction times (RVIP), reaction and movement durations (MTS, SRT and CRT) and errors (RVIP, MTS) were compared using repeated measures ANOVAs with drug (caffeine versus placebo) and conditioning trial (1 to 4) and block as within subject factors. ANOVAs for RVIP, SRT and CRT included an additional within-subjects factor of block. Change from baseline scores for the mood factors were analysed using two-way ANOVAs comprising drug and session within-subject factors. Where appropriate, post-hoc paired t-tests (using the Bonferroni correction) were used to examine the drug effects across the conditioning trials. The effects of drug order and room were also analysed, and significant effects or interactions are reported in the relevant sections; non-significant effects are not reported.

Paired t-tests (CS+ compared with CS-) or 2-way ANOVA with conditioned stimulus type and block as factors were used to determine whether the responses to placebo in the CS+ context differed from responses to placebo in the CS- context.

Repeated measure ANOVAs (CS condition and block as within-subjects factors) were used to determine whether there were significant differences between CS conditions on the conditioning test for all measures comprising a block factor. For all other measures, paired t-tests were used. The chi-square analysis of the post-session data was as Experiment 1.

9.3 RESULTS

9.3.1 Simple Reaction Time (SRT)

Conditioning Trials: For reaction duration, there was a trend towards a significant effect of drug [$F(1,9) = 3.76$; $p = 0.085$] and a significant main effect of block [$F(1,9) = 5.59$; $p < 0.05$], with quicker performance after caffeine relative to placebo and marginally (mean: 4.7 ms) faster performance on block one versus block two. There was also a significant interaction between drug and trial [$F(3,27) = 3.08$; $p < 0.05$]. Participants were faster after caffeine on sessions one and two, but this difference was lost on later trials due to faster responding after placebo (see Figure 9.1).

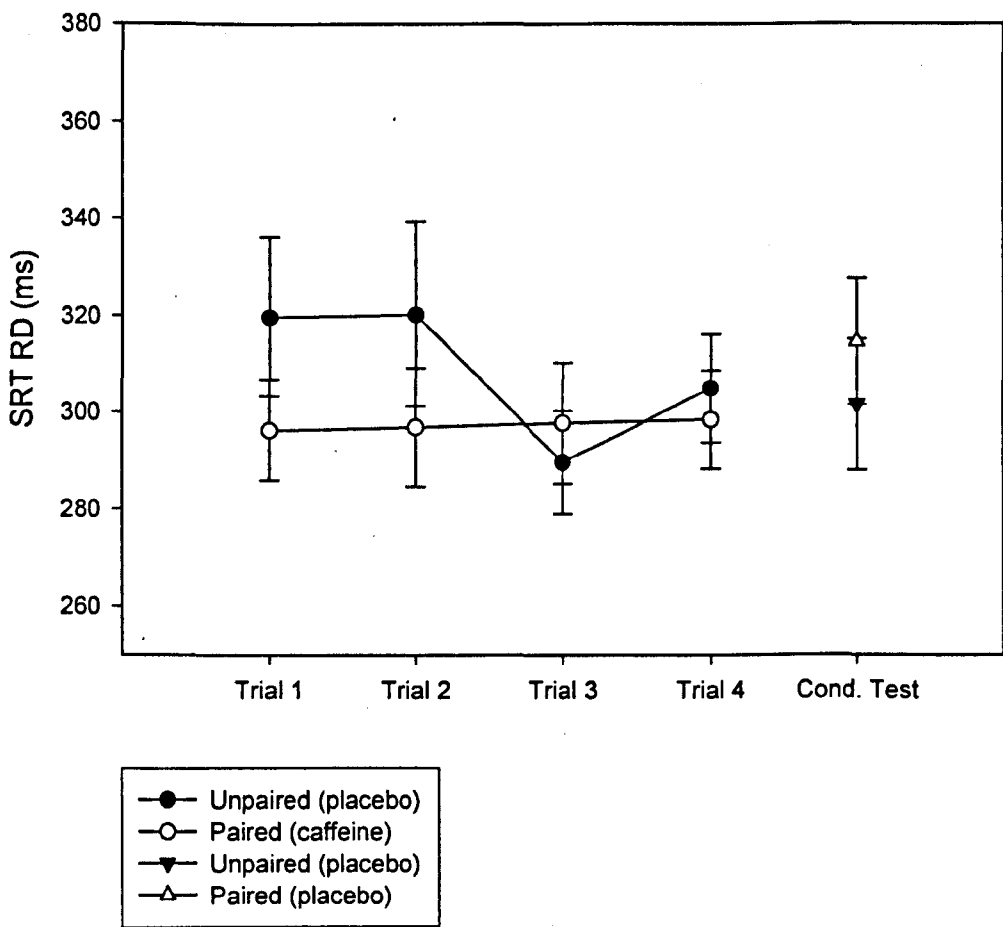


Figure 9.1. Mean SRT reaction duration scores (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and tests. Error bars represent SE means.

Post-hoc t-tests revealed that the difference between caffeine and placebo RD performance was significant for trial 2 only [$t = 3.26$, $df = 9$, $p < 0.0125$]. There were no other significant interactions [$F(1,9) = 0.39$; $p > 0.05$] [$F_s(3,27) < 1.63$; $p_s > 0.05$].

For movement duration, there was a significant main effect of drug $F(1,9) = 10.43$; $p < 0.05$] with participants responding faster after caffeine relative to placebo. There was no significant effect of trial [$F(3,27) = 1.69$; $p > 0.05$] or block [$F(3,27) = 1.16$; $p < 0.05$] and no significant caffeine-by-trial [$F(3,27) = 0.73$; $p > 0.05$] (see Figure 9.2) or block interactions [$F(1,9) = 0.01$; $p > 0.05$] [$F_s(3,27) < 1.49$; $p_s > 0.05$].

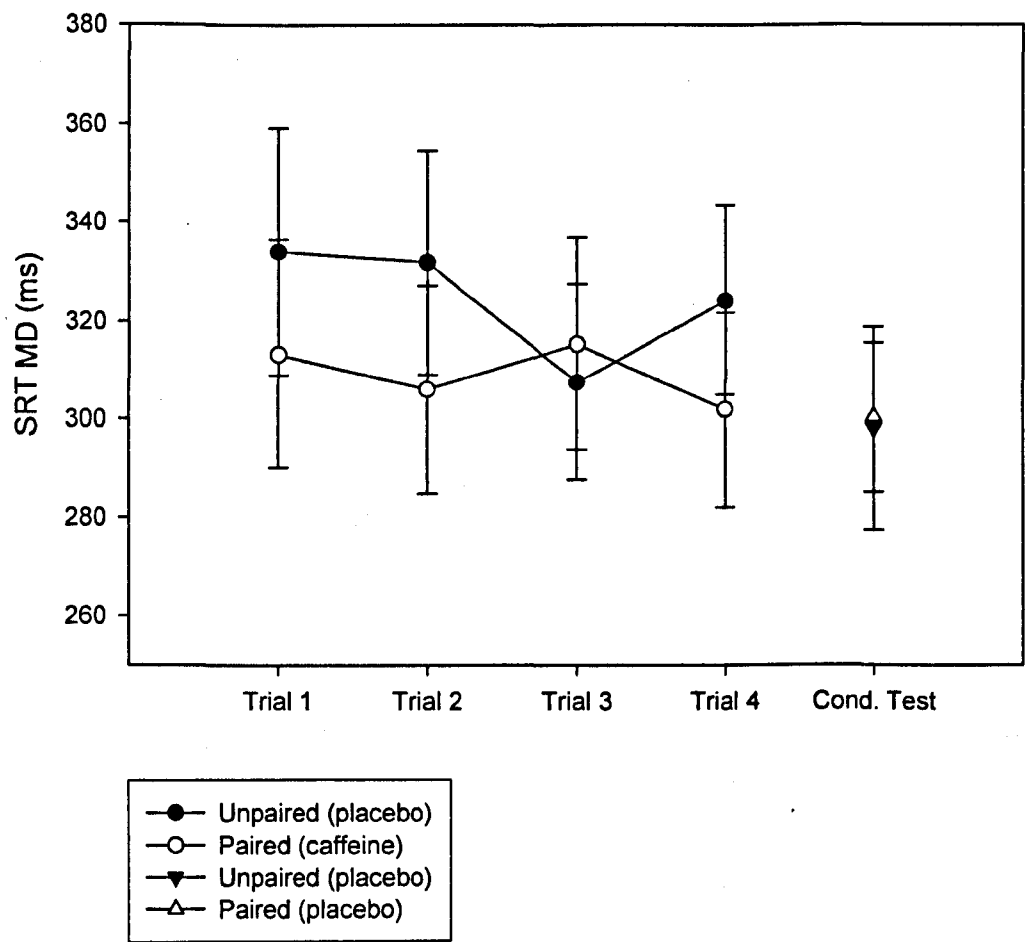


Figure 9.2. Mean SRT movement duration scores (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and tests. Error bars represent SE means.

There was a main effect of room [$F(1,8) = 6.0$; $p < 0.05$] with responding faster in the white cubicle (310.6 ms) compared to the black cubicle (323.3 ms). There was a significant drug-by-drug order interaction [$F(1,8) = 28.9$; $p < 0.02$], in which participants assigned to the placebo-caffeine order were faster after caffeine (308.4 ms) relative to placebo (344.1 ms), compared to little difference between caffeine (303.7 ms) and placebo (305.0 ms) for participants who were assigned to the caffeine-placebo order.

Conditioning Test: There were no significant effects of conditioned stimulus type or block and no significant interactions between these factors for reaction and movement [$F_s(1,9) < 1.77$; $p_s > 0.05$]. There was a main effect of block for movement duration [$F(1,9) = 12.05$; $p > 0.05$] with performance slowing from block one (279.6 ms) to block two (291.1 ms).

9.3.2 Choice Reaction Time (CRT)

Conditioning Trials: For reaction duration, there was a significant main effect of drug [$F(1,9) = 8.02$; $p < 0.03$] and trial [$F(3,27) = 4.67$; $p < 0.01$], and a trend towards a main effect of block [$F(3,27) = 2.38$; $p = 0.092$]. Participants were faster overall after caffeine relative to placebo, and there was an improvement in performance across trials (see Figure 9.3), and a slowing across blocks. The means show that the caffeine benefit was largest for the first trial [$t = 2.37$, $df = 9$, $p = 0.04$]. There were no significant interactions for reaction duration [$F(3,27) < 1.72$; $p > 0.05$] [$F(9,81) < 1.56$; $p > 0.05$].

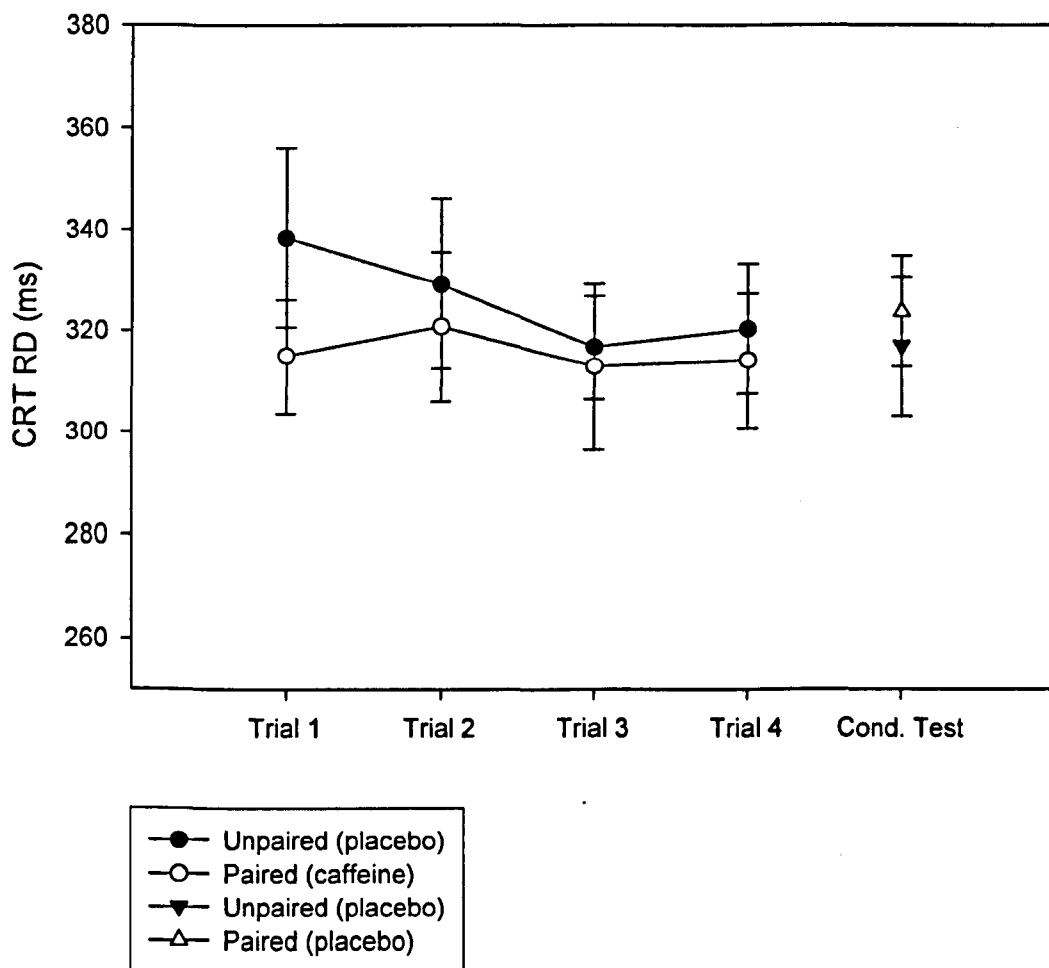


Figure 9.3. Mean CRT reaction duration scores (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and conditioning tests. Error bars represent SE means.

For movement duration, there was a significant main effect of drug [$F(1,9) = 9.06$; $p < 0.02$] and an effect of trial that approached significance [$F(3,27) = 2.67$, $p = 0.067$]. Participants were faster after caffeine and showed a general improvement across trials (see Figure 9.4). There was no significant effect of block [$F(3,27) = 0.72$; $p < 0.05$] and no significant interactions [$F_s(3,27) < 0.19$; $p_s > 0.05$] [$F_s(9,81) < 0.87$; $p_s > 0.05$].

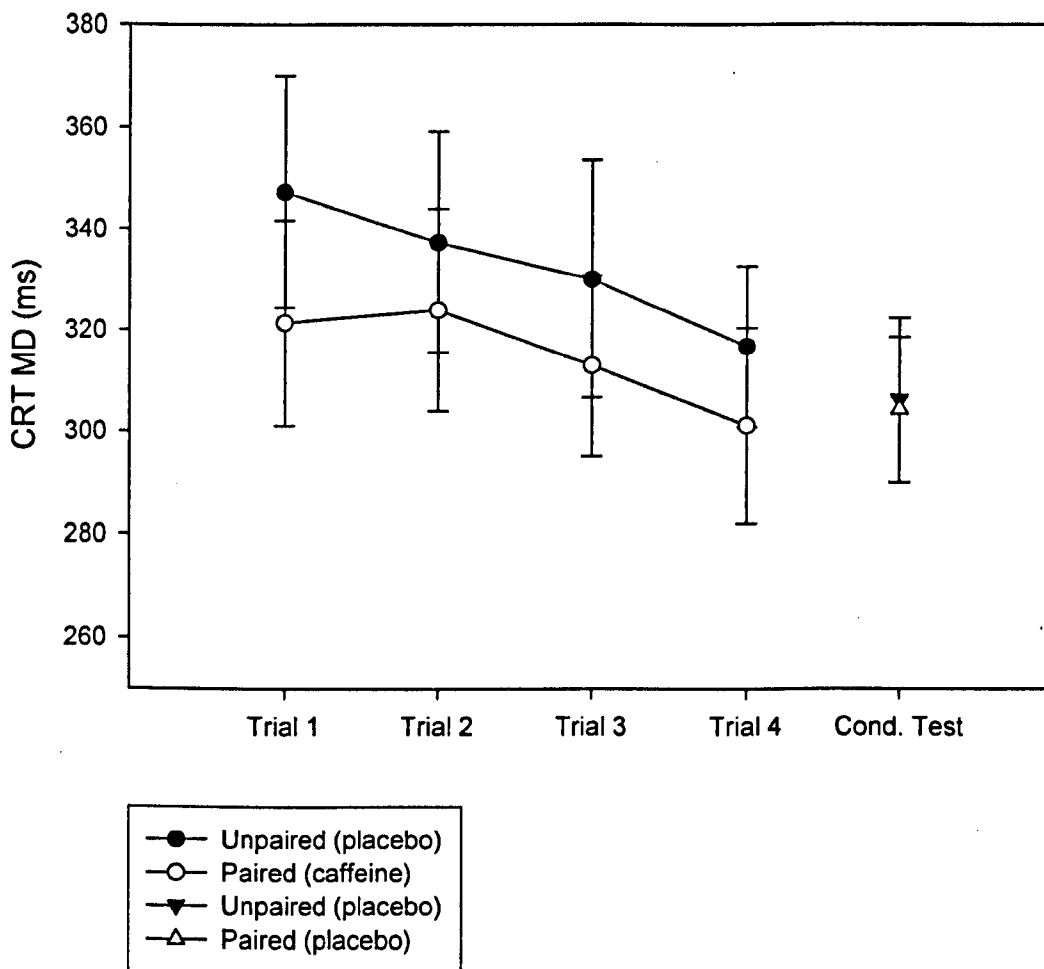


Figure 9.4. Mean CRT movement duration scores (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and conditioning tests. Error bars represent SE means.

Conditioning Test: There were no main effects of conditioned stimulus type or any interactions for reaction or movement durations [$F_s(3,27) < 1.47$; $p_s > 0.05$] [$F_s(9,81) < 0.38$; $p_s > 0.05$].

9.3.3 Match-to-Sample Visual Search (MTS)

Conditioning Trials: There was a significant main effect of drug [$F(1,9) = 5.19$; $p < 0.05$] and trial [$F(3,27) = 6.74$; $p < 0.005$] for level one reaction duration. Overall performance was faster after caffeine and improved across trials. However, there was also a significant drug-by-trial interaction [$F(3,27) = 3.57$; $p < 0.05$] with faster times after caffeine compared to placebo on trials 1 and 2 only. Post-hoc t-tests revealed a significant effect of drug on trial two only [$t = 3.31$, $df = 9$, $p < 0.01$] (see Figure 9.5).

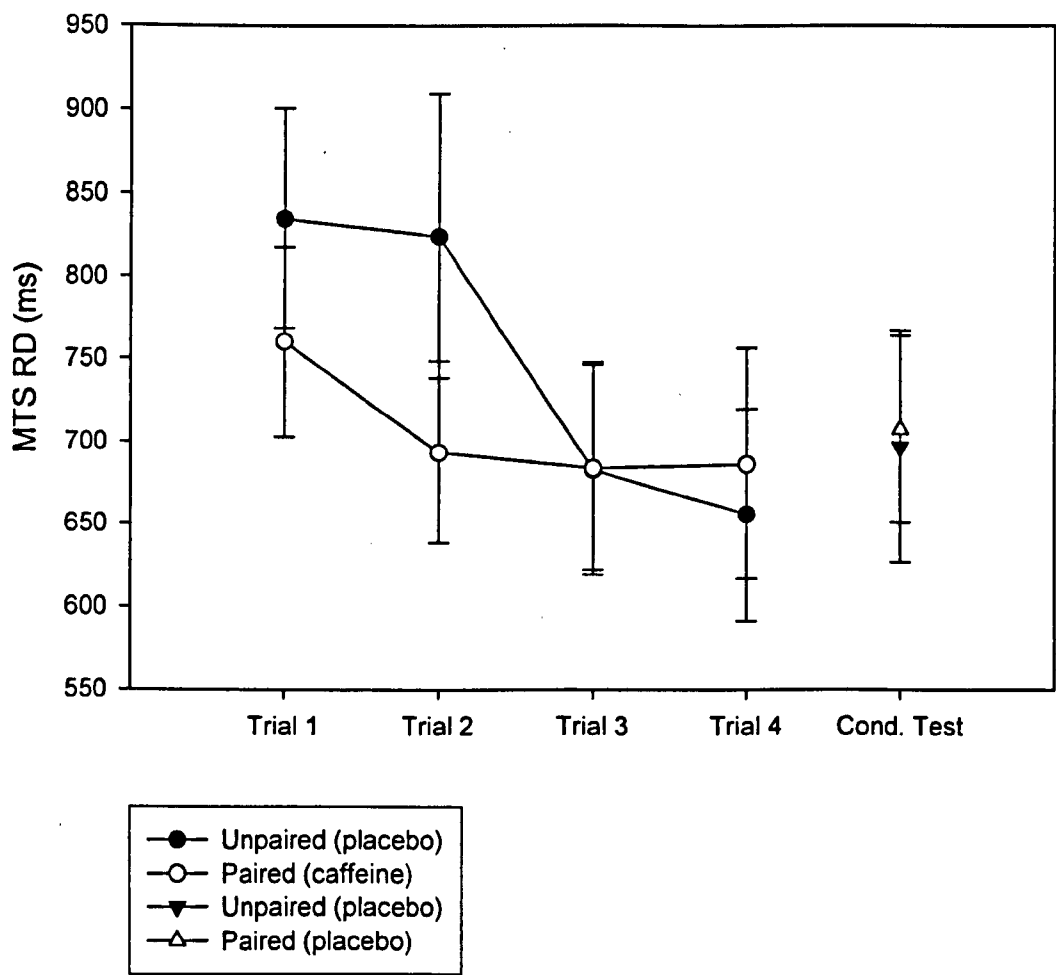


Figure 9.5. Mean reaction duration (ms) on level one of MTS for caffeine and placebo conditions across four conditioning trials and conditioning tests. Error bars represent SE means.

Participants did not make any errors for level one and therefore only error data for levels two and three are reported. There were no significant effects of drug or trial and no significant interactions for reaction duration on levels two or three [$F(3,27) < 2.14$; $p > 0.05$]. There was a significant effect of room order for all levels of reaction duration [$F(1,8) > 5.60$; $p < 0.05$], with participants experiencing the rooms in the order black/white displaying significantly slower performance relative to those allocated to the order white/black. In addition there were no significant main effects or interactions on movement duration or errors at any level [$F(3,27) < 2.13$; $p > 0.05$].

Conditioning Test: There were no significant effects of conditioned stimulus type for reaction or movement durations at any level [$t < 1.69$, $df = 9$, $p > 0.05$]. There were no significant effects of conditioned stimulus type on errors for levels 2 or 3 [$t = 2.24$, $df = 9$, $p > 0.05$]. There was a main effect of room for level one reaction duration [$t = 3.80$, $df = 9$; $p < 0.005$] with faster performance in the white cubicle (652.6 ms) relative to the black cubicle (752.1 ms).

9.3.4 Rapid Visual Information Processing (RVIP)

Conditioning Trials: There were no significant main effects of drug [$F(1,9) = 2.44$; $p > 0.05$], trial [$F(3,27) = 0.28$; $p > 0.05$] or block [$F(2,18) = 0.13$; $p > 0.05$], and no significant interactions [$F(2,18) = 2.25$; $p > 0.05$] [$F(3,27) = 0.55$; $p > 0.05$] [$F(6,54) < 1.43$; $p > 0.05$] for reaction time.

There was a near significant effect of drug [$F(1,9) = 5.07$; $p = 0.051$] on errors, with fewer errors after caffeine, and a significant effect of trial [$F(3,27) = 3.30$; $p < 0.05$], with fewer errors across trials (see Figure 9.6). There was a significant trial-by-block interaction [$F(6,54) = 2.60$; $p < 0.05$] with fewer errors across blocks on earlier trials but participants making more errors across blocks on later sessions. There was no main effect of block [$F(2,18) = 2.15$; $p > 0.05$], nor any other significant interactions [$F(2,18) = 0.18$; $p > 0.05$] [$F(3,27) = 0.44$; $p > 0.05$] [$F(6,54) = 0.71$; $p > 0.05$].

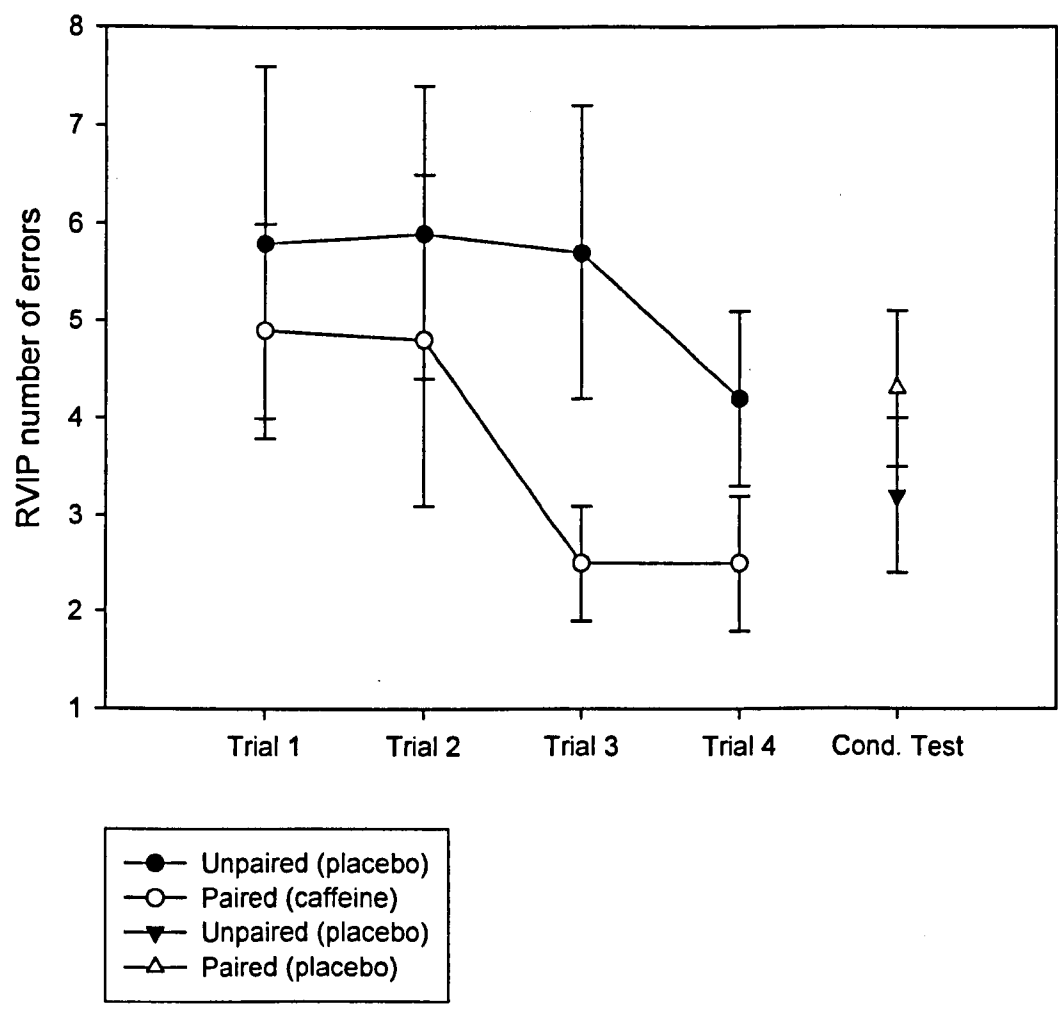


Figure 9.6. Mean number of errors on RVIP for the CS+ (caffeine) and CS- (placebo) conditioning trials and conditioning tests. Error bars represent SE means.

Conditioning Test: There were no main effects or interactions for reaction time or errors [$F_s(3,27) < 1.47$; $p_s > 0.05$] [$F_s(9,81) < 0.38$; $p_s > 0.05$].

9.3.5 Mood

Conditioning Trials: Placebo produced significant increases in “sleepiness” [$F_s(1,9) > 15.38$; $p_s < 0.004$] and significant decreases in “positive mood” [$F(1,9) = 11.41$; $p < 0.008$] and “mental clarity” [$F(1,9) = 24.42$; $p < 0.008$], relative to little change after caffeine. Following caffeine, there were decreases in “mental repose” [$F(1,9) = 4.88$; $p = 0.06$] and increases in headache (30-50 mins) [$F(1,9) = 3.65$; $p = 0.09$] that approached significance (see Figure 9.7). There were no significant main effects of trial [$F_s(3,27) < 2.46$; $p_s > 0.05$].

There was a significant drug-by-trial interaction for “general positive mood” [$F(3,27) = 5.39$; $p < 0.008$], and a trend towards an interaction for “sleepiness” (30-50 mins) [$F_s(3,27) = 3.52$; $p = 0.03$] (see Table 9.2). For “general positive mood”, the effect of drug was significant for trial one [$t = -4.16$; $df = 9$; $p < 0.005$], but not for subsequent sessions [$t_s < 1.39$; $dfs = 9$; $p_s > 0.05$]. For “sleepiness”, comparisons were significant for both trial one [$t = 5.62$; $df = 9$; $p < 0.0001$] and trial two [$t = 2.24$; $df = 9$; $p < 0.05$], but not for subsequent trials [$t_s < 0.31$; $dfs = 9$; $p_s > 0.05$] (see Table 9.2). In addition, there were significant session-by-drug-by-drug order interactions for “sleepiness” [$F(3,24) = 4.17$; $p < 0.05$] and “general positive mood” [$F(3,24) = 5.35$; $p < 0.05$]. The interaction appears to be due to one instance (trial two) when the placebo/caffeine ordered participants reported increases in sleepiness and decreases in general positive mood after caffeine relative to placebo.

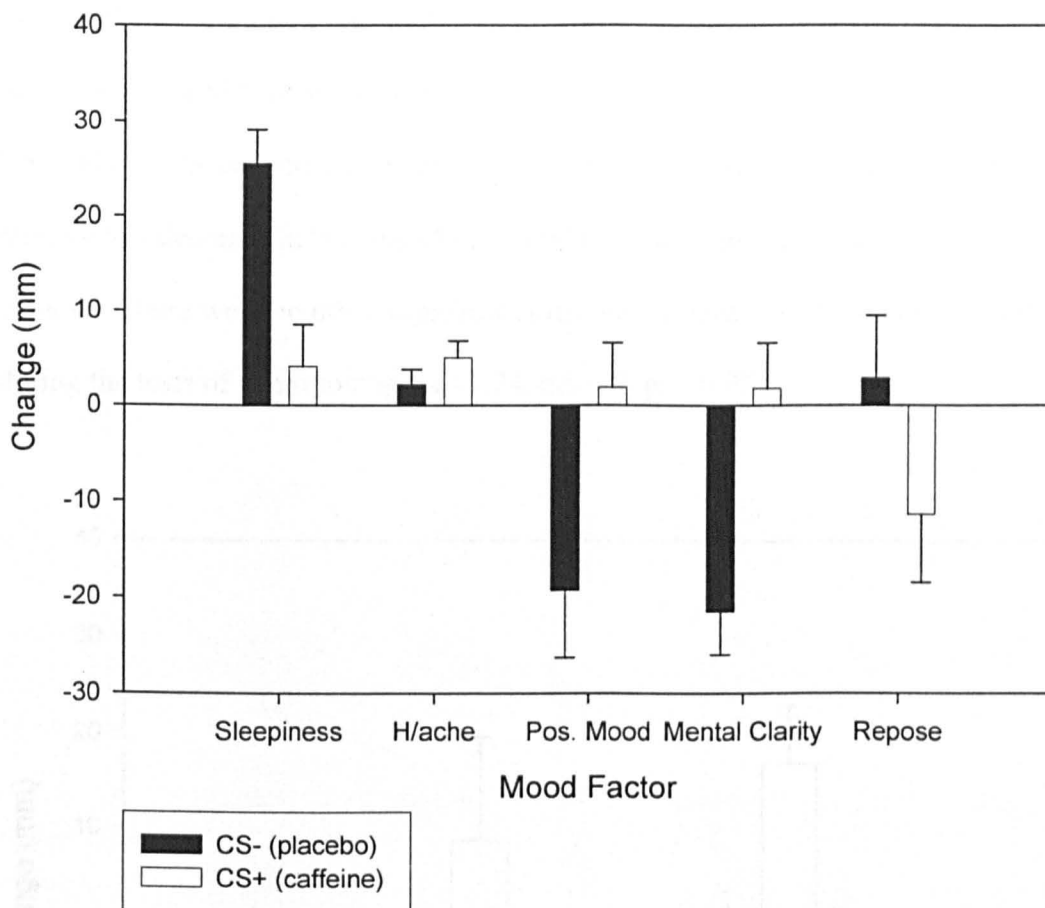


Figure 9.7. Mean change (mm) in rated “sleepiness”, “general positive mood”, “mental clarity”, “mental repose” and “headache” (30-50 mins) combined over the placebo (CS-) and caffeine (CS+) conditioning trials. Error bars represent SE means.

Table 9.2. Mean changes (mm) in “positive mood” and “sleepiness” across the four conditioning trials in the CS- (placebo) and CS+ (caffeine) conditions. Standard deviations in parenthesis

	Condition	Trial 1	Trial 2	Trial 3	Trial 4
Positive Mood	CS-	-41.7 (38.9)	-6.0 (36.3)	-18.4 (17.3)	-11.0 (32.9)
	CS+	17.2 (25.5)	-3.8 (35.7)	-10.4 (26.3)	4.3 (37.2)
Sleepiness	CS-	43.7 (17.2)	42.2 (40.0)	4.1 (33.1)	11.9 (38.4)
	CS+	-3.3 (24.0)	8.7 (46.9)	2.5 (23.8)	8.0 (31.5)

Conditioning test: There was a significant difference between CS+ and CS- responding on ratings of “headache” [$t = -2.42$, $df = 9$, $p < 0.05$] and a trend approaching significance on “mental clarity” [$t = 1.89$, $df = 9$, $p = 0.09$] (see Figure 9.8). There was an increase in “mental clarity” and “headache” in the CS+ context relative to a decrease in “mental clarity” and no change in “headache” in the CS- context. There were no other significant differences between CS+ and CS- conditions during the tests of conditioning [$ts < 1.74$, $dfs = 9$, $ps > 0.05$].

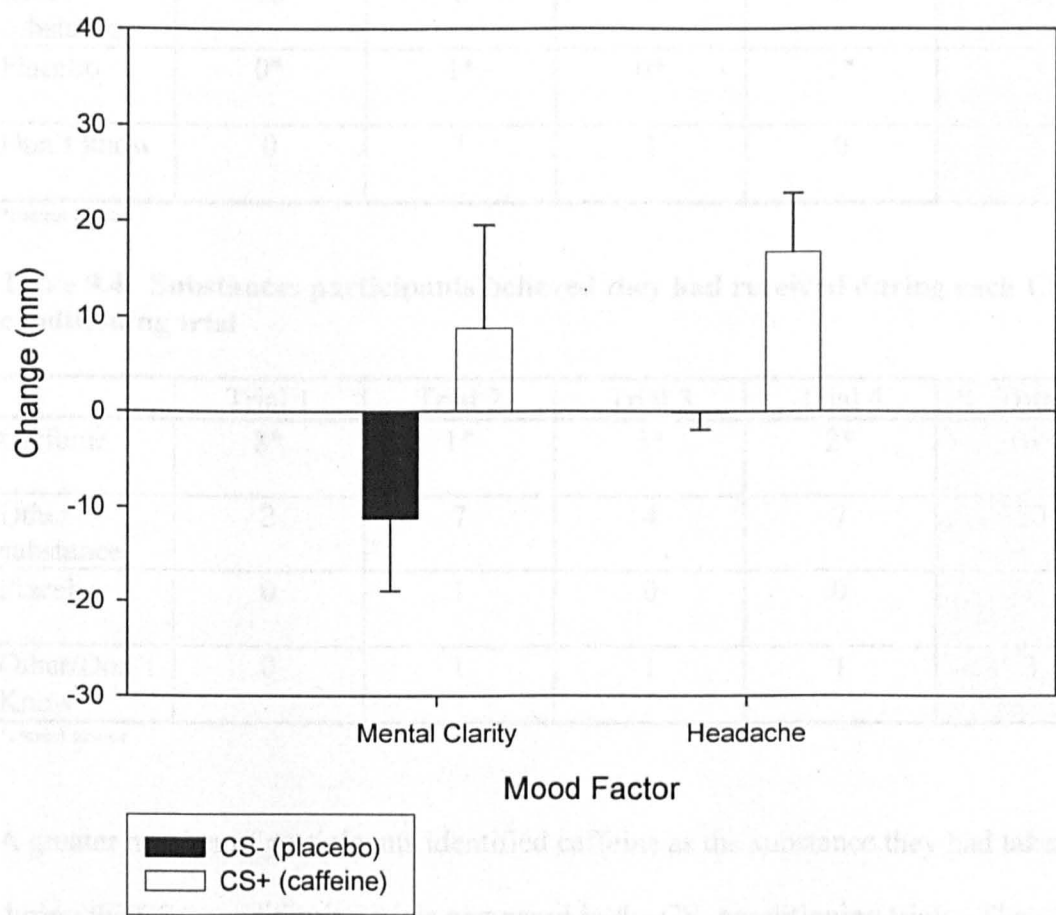


Figure 9.8. Mean change (mm) in rated “mental clarity” and “headache” on the tests of conditioning in the CS+ and CS- contexts. Error bars represent SE means.

9.3.6 Post-session Questionnaires

At the end of each session, participants were asked to indicate what substance they believed that they had received that day. The responses are listed in Tables 9.3 and 9.4.

Table 9.3. Number of participants who believed that they had received caffeine during the conditioning trials in the CS- context, compared to alternative substances, placebo or “don’t know”

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	0	2	0	1	3
Other substance	10	6	9	8	33
Placebo	0*	1*	0*	1*	2*
Don’t know	0	1	1	0	2

*correct choice

Table 9.4. Substances participants believed they had received during each CS+ conditioning trial

	Trial 1	Trial 2	Trial 3	Trial 4	Total
Caffeine	8*	1*	5*	2*	16*
Other substance	2	7	4	7	20
Placebo	0	1	0	0	1
Other/Don’t Know	0	1	1	1	3

*correct choice

A greater number of participants identified caffeine as the substance they had taken during the CS+ conditioning trials compared to the CS- conditioning trials. The pearson’s chi-square analysis examining the association between condition (i.e. whether caffeine was administered) and reported identification of caffeine was significant [$\chi^2 = 11.67$; $df = 1$; $p < 0.005$]. On the test of conditioning in which

placebo was administered in the context paired with caffeine (CS+), two out of ten participants reported that they had received caffeine and two reported placebo. The remainder stated that they thought they had received one of the other substances.

The post-experiment questionnaire asked participants whether they ever experienced cravings for caffeine or withdrawal symptoms following a period of no intake. If they responded “yes” to this question they were then asked whether they had felt any such withdrawal and/or craving prior to any experimental session. The results are displayed in Table 9.5. 70% of the participant sample reported ever experiencing caffeine withdrawal and/or craving during periods of acute caffeine abstinence. Of these, 43% reported feeling such effects during the experimental sessions.

Table 9.5. Number of participants who reported experience of withdrawal and/or craving in general and at experimental session

	No. of participants who ever experience caffeine withdrawal and/or craving	If so, no. of participants who experienced caffeine withdrawal and/ or craving at the sessions
Count	7	3

9.4 DISCUSSION

Benefits of caffeine were observed for SRT and CRT and for MTS reaction time and RVIP accuracy. Furthermore, caffeine attenuated the negative changes in mental clarity, sleepiness and general positive mood that were reported after placebo. There was also a trend for caffeine to decrease ratings of mental repose. These clear effects of caffeine suggest that the use of the within-subjects design and the screening procedure were effective in increasing the likelihood of detecting effects of caffeine. This implies that previous inconsistencies in reports of the effects of caffeine on

cognition may be in part due to inter-subject variability in responding and sensitivity to caffeine.

There was also some evidence of conditioned responding to the context previously paired with caffeine (CS+). Participants reported greater mental clarity and headache after placebo in the CS+ context compared to decreases or no change (respectively) after placebo in the CS- context. However, there was no difference in responding to the CS+ compared with the CS- for the cognitive measures, despite the detection of effects of caffeine on cognition during conditioning. It is important to note that for the majority of cognitive measures there was no effect of caffeine at the end of the conditioning trials, apparently due to placebo performance improving across conditioning trials. A similar pattern was also observed for “general positive mood” and “sleepiness”.

One explanation for this pattern of results is that practice effects were operating in the placebo but not the caffeine trials. However, this seems unlikely, especially since robust practice effects are not observed for simple tasks such as SRT. In addition, the similar pattern of results found for some mood factors further undermines an explanation based on practice effects alone. It is also unlikely that the effects are due to demand characteristics relating to the substance the participants thought they had received on the placebo trials. When asked to identify the substance that they had received at the latter CS- trials, participants were not more likely to choose caffeine, compared to earlier trials, indicating that the performance and mood improvements were not subjectively evaluated as “caffeine-like”. Alternatively, the improvements

in performance and mood in the placebo group be explained by a generalisation of an early drug-like conditioned response from the CS+ to the CS- context. Conditioning literature has showed that conditioned effects often generalise to stimuli that are similar to the original CS (Pavlov 1927; Sandoz, Pham-Deleque, Renou & Wadhams 2001). Moreover, Godoy & Delius (1999) reported that a conditioned response generalised from a CS+ to a CS- context in an animal study examining apomorphine sensitisation (Godoy & Delius 1999). If the findings of the present study were due to a generalised CR, then it is predicted that a CR would be observed in the CS+ context after fewer conditioning trials, i.e. prior to its generalisation to the CS- context.

The reason why learning may have generalised is unclear but there are two possible explanations: Firstly, the effects of caffeine may have conditioned to a cue present in both contexts (e.g. the experimenter, computer hardware, capsule), and this may have overshadowed less salient cue elements of the context that were distinctive to each context (e.g. fragrance). Overshadowing effects of salient discrete cues over context cues has been confirmed experimentally (Walter & Riccio 1983; Hall & Symonds 2006). For example, Hall & Symonds (2006) found that a particularly salient CS (flavoured drink) inhibited the aversive conditioning to a compound context cue. It is plausible that the capsule itself may have been a particularly salient cue, given the UR is a post-ingestive consequence of the capsule, and post-ingestive aversions have been shown to condition more readily to a vehicle-related cue (e.g. taste) than to a visual or auditory cue (Domjan, 2005).

Alternatively, the CS+ and CS- may not have been sufficiently distinct. In the real world, stimuli are not constant, therefore the ability to generalise learning from one context to another is an important adaptive mechanism. For example, when conditioning is established to a tone CS, similar tones acquire the ability to elicit CRs spontaneously (Pavlov, 1927). Although the contextual elements of the CS differed in the present study (i.e. different posters, different fragrances, different coloured tables and chairs), the fundamental elements were the same (i.e. they both comprised posters, fragrances, tables and chairs). Such generalisation of conditioned responding has been reported in autonomic conditioning preparations, whereby a cue with similar sensory (phonetic) properties to an established CS+ elicited conditioned galvanic skin responses, despite no prior pairing with the US (shock) (Mandel & Bridger 1973).

It is noteworthy that the magnitude of improvement in the CS- context (i.e. to a level comparable to the caffeine context) is greater than would be predicted by Pavlovian theory. Findings from generalisation gradient studies report that CR strength is directly related to the level of proximity or similarity between the CS and the generalised stimuli (Pavlov 1927; Gutman and Kalish 1956); therefore the generalised CR is expected to be of a lesser magnitude than the original CR (Pavlov 1927; Domjan 1986). However, previous studies have employed discrete CS such as a tone or tactile stimulation of the skin. Pavlov (1927) claimed that the CS produced localised cortical activation, which “irradiated” to surrounding cortical areas relating to sensorily similar stimuli. However, it is unclear how this relates to compound cues comprising many discrete elements. The irradiation argument would suggest multiple sites of activation, and these may summate to produce higher levels of responding

than would be predicted with discrete cues. It is also interesting that performance in the CS+ context did not improve during the latter trials (i.e. no evidence of an additive relationship between CR and drug effect), although there are claims that the UR and CR may not interact in a simple additive manner (Carey & Gui 1997).

The pattern of data favours generalisation of conditioned effects rather than discrimination. It would be reasonable to expect that across the course of the conditioning trials, differentiation of the two contexts would increase as participants have more time to develop coherent representations of the complex compound cues (Fanselow 1990). However, if the current findings are a result of contextual generalisation of a CR then it appears that participants formed gestalt-like configural representations of the context (i.e. a computer cubicle, scented with poster) rather than differentiating on the basis of sensory differences (i.e. white computer cubicle, floral scented with poster of a castle).

When asked what substance they thought that they had received, participants opted for caffeine significantly more often on caffeine trials than on placebo trials. This suggests that participants were better able to detect caffeine in the present study compared to Experiments 1 and 4, in which the number of identifications of caffeine did not significantly differ depending on whether caffeine or placebo was received. However, in the present study this appeared to be due to a large number of correct identifications of caffeine occurring on the first caffeine trial, after which identification of caffeine dropped substantially, despite significant mood and

performance effects being observed, suggesting that participants' explicit awareness that they had received caffeine did not influence the effects of caffeine.

Furthermore, the within-subjects design and alternating trial procedure did not seem to promote contingency awareness, as no participant correctly reported the nature of the study, and when told that they had consistently received caffeine in one context and placebo in another, only 30% correctly identified the room in which they had received caffeine. Therefore, the current effects were acquired without explicit awareness of the CS-US contingency.

In sum, the effects of caffeine were more robust in the present study than in previous experiments in this thesis. This may have been mediated by the screening procedure that was designed to identify "caffeine responders". It is interesting that 70% of the present sample reported that they experience caffeine withdrawal symptoms and/or craving during periods of caffeine abstinence (compared to 42% in Experiment 4). This suggests that the participants identified in the screening procedure may have shown effects of caffeine as they were also those who most readily experienced caffeine withdrawal. This is consistent with the large body of literature indicating that acutely deprived caffeine consumers are particularly sensitive to the effects of caffeine (James 1994; Lane 1997; Yeomans et al. 2001; Rogers et al. 2003)

The present study demonstrated significant cognitive effects of caffeine relative to placebo that did not condition to the test context. However, early performance (and mood) benefits of caffeine declined due to systematic improvements in the placebo

condition. It was hypothesised that this may have been due to the acquisition of an early CR that generalised to the CS- context. This would suggest that a CR could be observed if tested earlier in the procedure, before stimulus generalisation occurs. Thus, the aim of Experiment 6 is to replicate Experiment 5 with fewer conditioning trials, in order to test for a conditioned response prior to any generalisation.

CHAPTER 10:

EXPERIMENT 6: CONDITIONED PERFORMANCE AND SUBJECTIVE EFFECTS OF CAFFEINE WITH TWO CONDITIONING TRIALS

10.1 INTRODUCTION

Experiment 5 examined whether the effects of caffeine on performance and mood could be conditioned to a previously-neutral context using a differential conditioning paradigm. Despite significant effects of caffeine across the conditioning trials, no conditioned cognitive effects were observed. However, further analysis of the data revealed that while caffeine improved performance on the early conditioning trials, these benefits were lost during the later conditioning trials due to systematic improvements in performance after placebo. One explanation of this pattern of results is that a conditioned association was formed between the effects of caffeine and the test context (CS+) that generalised to the CS- environmental context, resulting in the expression of a conditioned response in the placebo-paired context on later trials.

The aim of Experiment 6 was to test this hypothesis by reducing the number of conditioning trials so that testing occurs before any possible generalisation to the placebo-paired context. The number of conditioning trials was reduced to four (two per CS). Although there was evidence of generalisation by trial three in Experiment 5, it was felt that a single CS+/caffeine pairing may be insufficient to support conditioning. Although there is evidence of conditioned taste aversions after a single trial (see Domjan 1986), these are presumed to be particularly specialised examples of conditioning due to the significant biological importance associated with avoidance of

toxins (Domjan 2005). Animals have shown limited evidence of conditioning after a single conditioning trial (Cepeda-Benito, Davis, Reynoso, Harraid 2005; Cepeda-Benito & Tiffany 1992), and a human conditioning study demonstrated strong conditioned effects after three, compared on one, conditioning trials (Barrett, King & Pang 2000). Furthermore, due to the counterbalanced trial ordering in Experiment 5 (i.e. CS+/CS- vs. CS-/CS+), half of the participants had received three CS+/caffeine pairings by the third CS- session, which may have provided more time and learning to promote the generalised effect.

A screening procedure was also used in the present study to identify caffeine responders. In Experiment 5, less than half of the screening sample (44%) was faster after caffeine than placebo. Examining the reported average caffeine intake of the sample revealed that the higher consumers (>200 mg/day approximately) were more likely to show caffeine-induced performance benefits than the lower consumers (<200 mg/day). Therefore the minimum level of caffeine intake required for enrolment into the screening procedure for Experiment 6 was increased to 200 mg/day.

10.2 METHOD

10.2.1 Participants

10.2.1.1 Screening

21 participants (6 male, 15 female; mean age 21.3 years, range 18-29 years; mean BMI 22.7, range 19.0-26.4) with an average daily caffeine intake of 262.3 (range 175-625 mg) took part in the screening procedure, in return for cash (£10) or course credits.

Screening materials and procedure: The experimental design, measures, caffeine administration and procedure were all identical to the screening procedure for Experiment 5. Twelve participants were accepted from the screening procedure and all took part in the main study.

10.2.1.2 Main Study

Twelve participants (4 male, 8 female; mean age 22 years, range 18-29 years; mean BMI 22.7, range 19.0-26.3) with an average daily caffeine intake of 256.8 mg (range 175-625 mg) took part in the study in return for cash (£30) or course credits.

10.2.2 Design and Materials

This study used the same differential conditioning paradigm as Experiment 5, with the exception that in the present study there were two CS- and two CS+ conditioning trials prior to the tests of conditioning. Capsule order, room order and the room in which caffeine was received were fully counterbalanced. All testing took place between 12:00 and 18:00 hours, and participants attended each session at approximately the same time of day. Experimental sessions were spaced so that the interval between any two did not fall below 24 hours or exceed 7 days.

All test materials, including the caffeine and placebo capsules, the to-be-conditioned stimuli (e.g. cubicles, visual and olfactory cues) and dependent measures (e.g. cognitive test battery and mood VAS) were identical to those used in Experiment 5.

10.2.3 Procedure

The procedure (both screening and main) was identical to Experiment 5.

10.2.4 Data Analysis

Reaction times below 100 ms (SRT, CRT and MTS), and more than 3 standard deviations above an individual's mean score (SRT, CRT, MTS MD) were considered outliers and removed (SRT: 1.8% and 1.0, CRT: 1.3% and 1.8%, MTS: 0.2% and 0.2% removed for reaction and movement duration respectively).

All data analyses were as per Experiment 5. Due to a technical error, the task battery data for one experimental session for one participant was lost. Linear regression was used to estimate the missing data points (Tabacknick and Fidell, 2001). Each complete data set was used as the IV in turn, and the regression equation yielding the best significant fit was used to estimate the missing data point.

10.3 RESULTS

10.3.1 Simple Reaction Time (SRT)

Conditioning Trials: There was a significant effect of drug for reaction duration [$F(1,11) = 11.09$; $p < 0.01$], with faster responding after caffeine relative to placebo (see Figure 10.1). There was no main effect of trial [$F(1,11) = 0.12$; $p > 0.05$] or block [$F(1,11) = 1.73$; $p > 0.05$] and no significant interactions [$F_s(1,11) < 1.36$; $p_s > 0.05$].

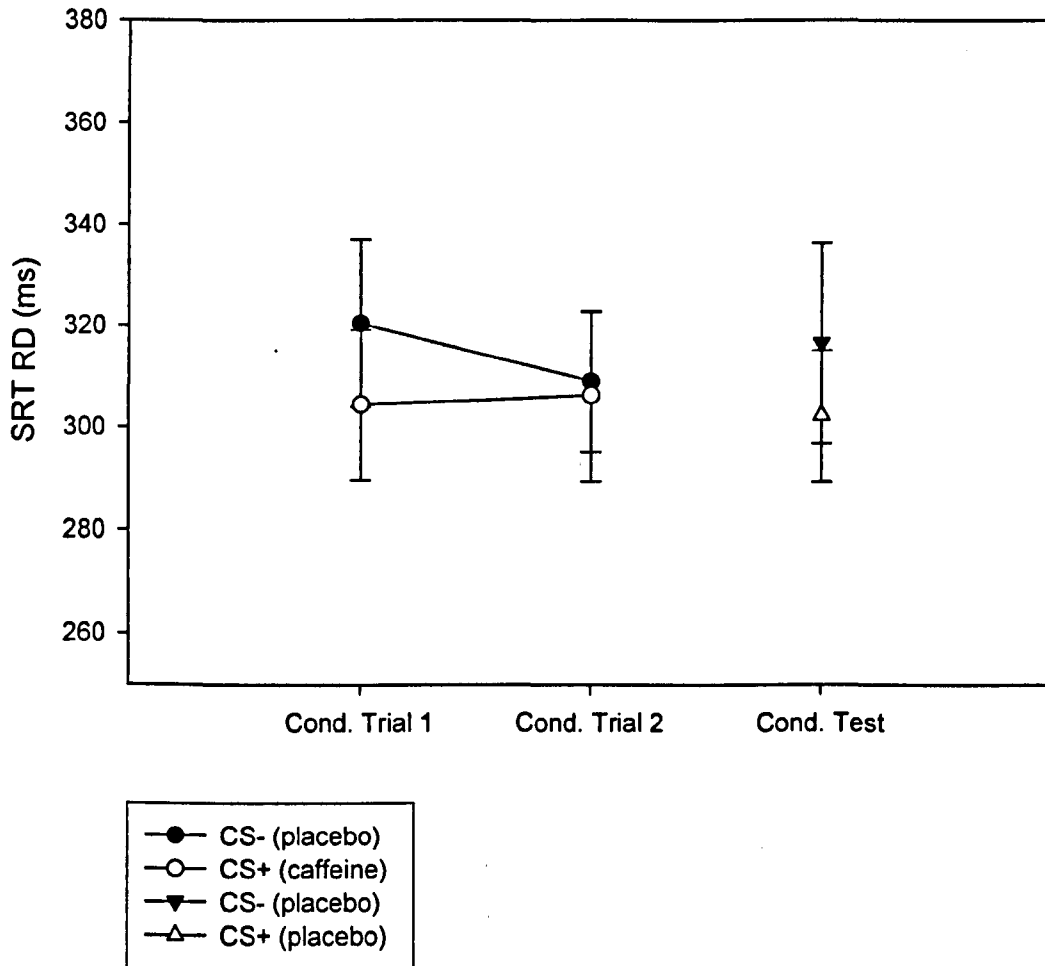


Figure 10.1. Mean SRT reaction duration (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and conditioning tests. Error bars represent SE means.

There were no main effects of drug, trial or block and no significant interaction [$F_s(1,11) < 2.30$; $p_s > 0.05$] for movement duration (see Figure 10.2).

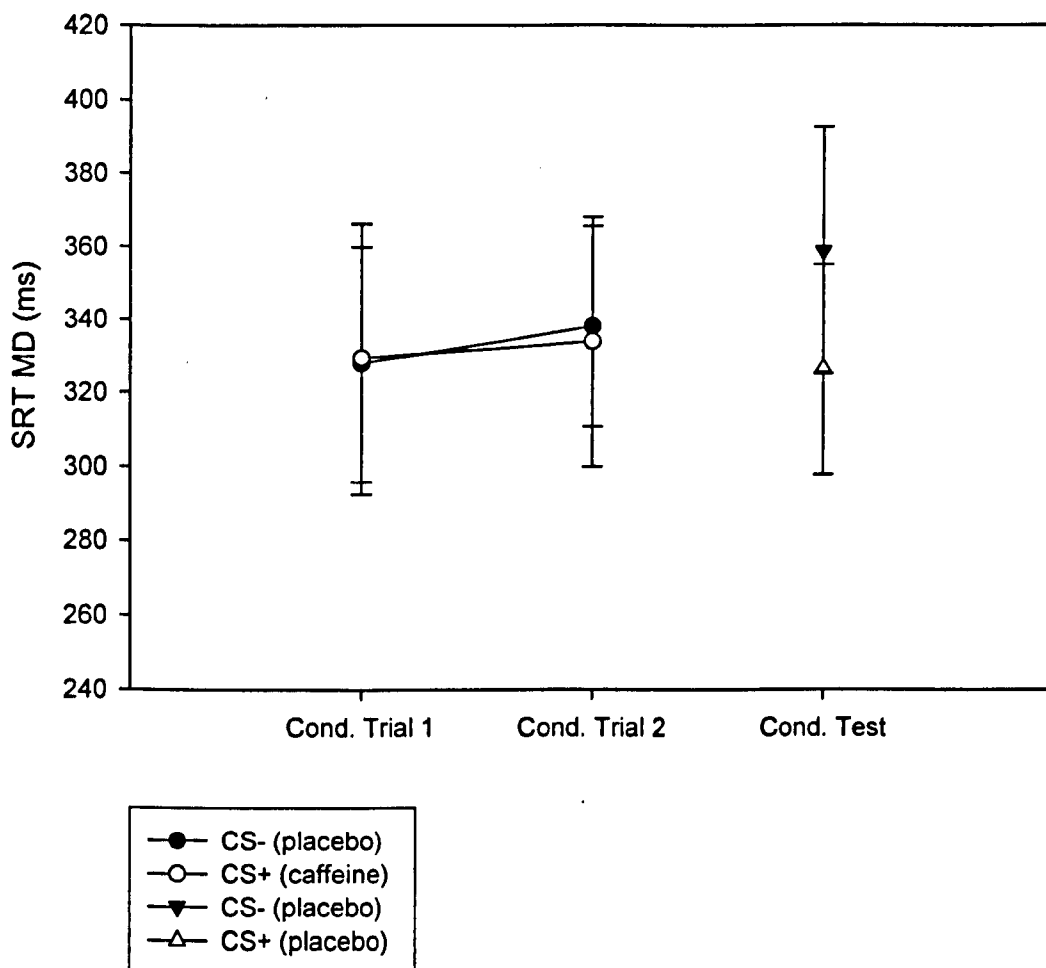


Figure 10.2. Mean SRT movement duration (ms) for the CS+ (caffeine) and CS- (placebo) conditioning trials and conditioning tests. Error bars represent SE means.

Conditioning Tests: Participants were faster in the CS+ context compared to the CS- context for both reaction and movement durations (see Figures 10.1 and 10.2). This difference was significant for movement duration [$t = -3.03$, $df = 11$, $p < 0.02$], but did not reach significance for reaction duration [$t = -1.47$, $df = 11$, $p > 0.05$].

10.3.2 Choice Reaction Time (CRT)

Conditioning Trials: There was a significant effect of drug [$F(1,11) = 9.12; p < 0.05$] for reaction duration, with faster responding after caffeine relative to placebo (see Figure 10.3). There was no main effect of trial [$F(1,11) = 0.22; p > 0.05$] or block [$F(3,33) = 1.07; p > 0.05$] and no significant interactions [$F(1,11) = 2.60; p > 0.05$] [$F_s(3,33); p < 1.07$].

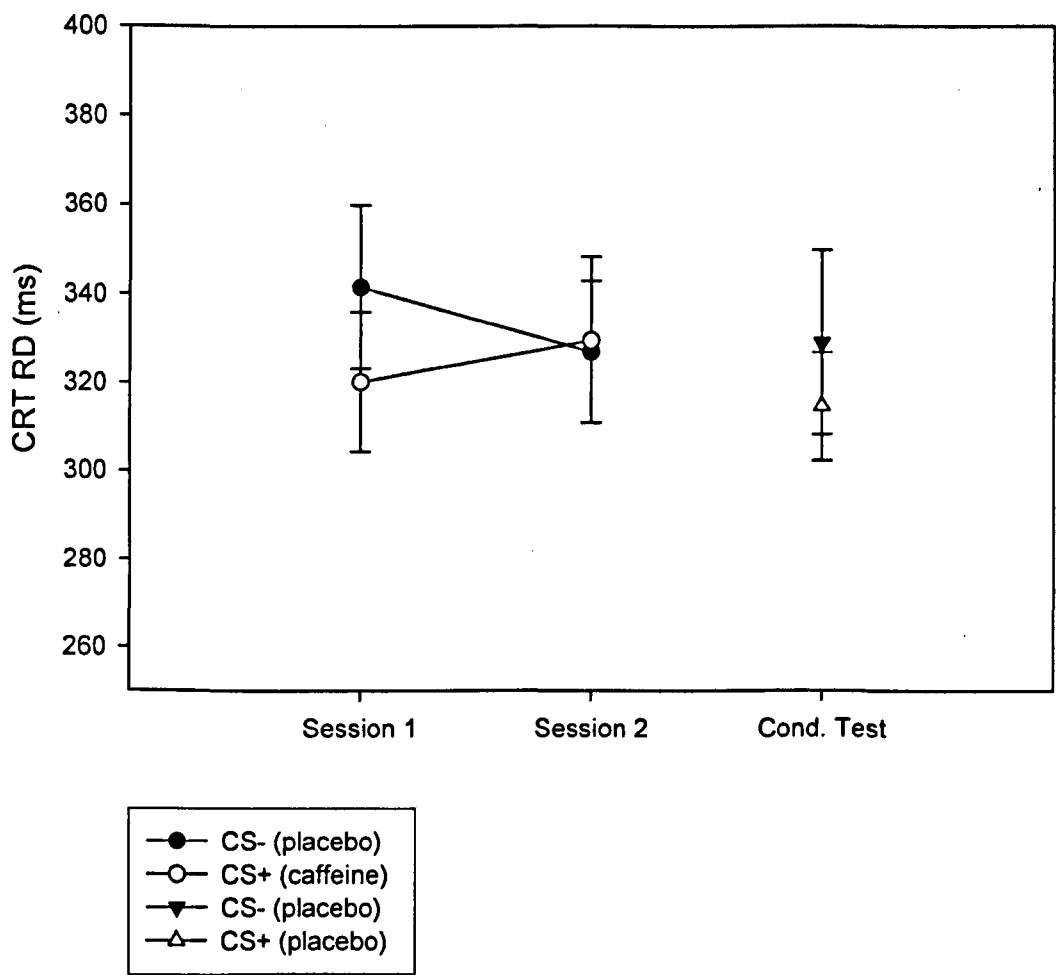


Figure 10.3. Mean CRT reaction duration (ms) in the CS- and CS+ contexts during the conditioning trials and test. Error bars represent SE means.

There was a trend towards a drug effect for movement duration [$F(1,11) = 4.67; p = 0.054$], with faster responding after caffeine relative to placebo (see Figure 10.4).

There was no main effect of trial [$F(1,11) = 3.15$; $p > 0.05$] or block [$F(1,11) = 0.31$; $p > 0.05$] and no significant interaction [$F(1,11) = 1.90$; $p > 0.05$].

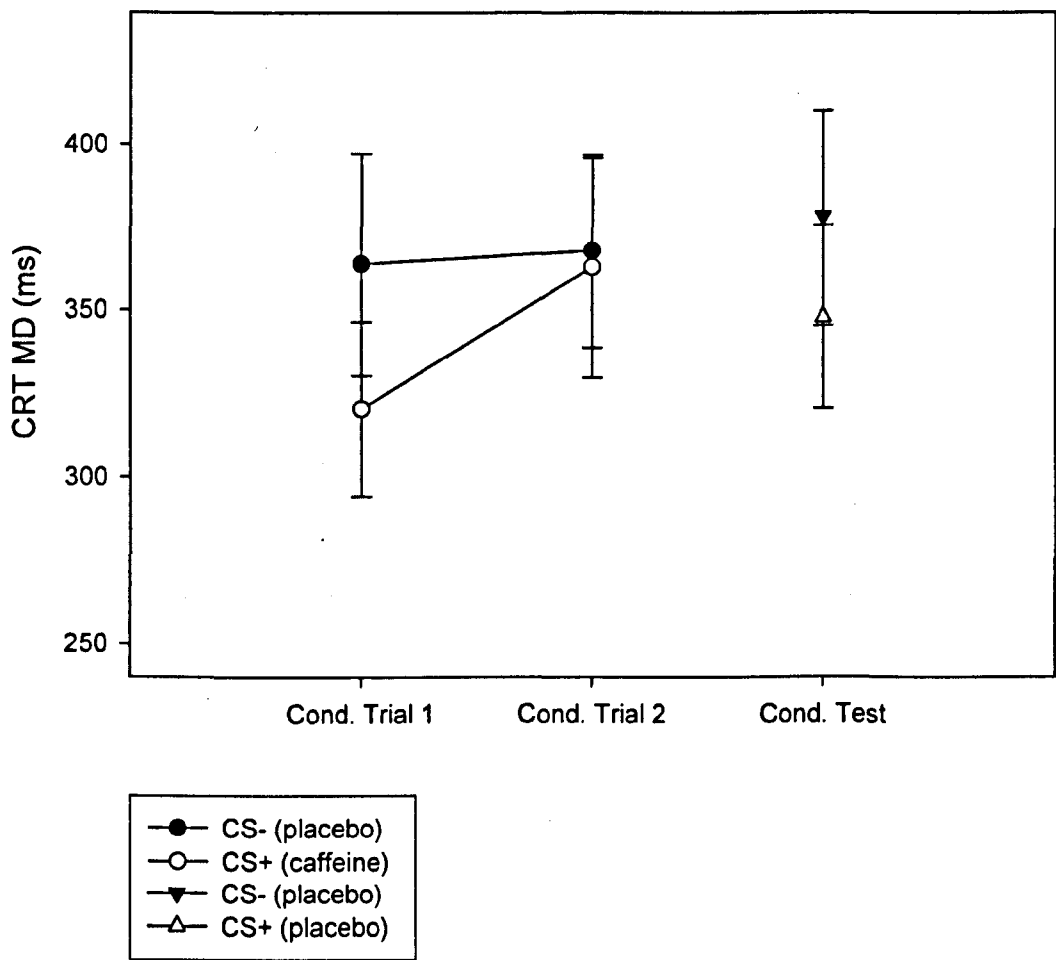


Figure 10.4. Mean CRT movement duration (ms) in the CS- and CS+ contexts during the conditioning trials and test. Error bars represent SE means.

Conditioning Test: Participants responded faster in the caffeine-paired (CS+) context compared to the placebo-paired (CS-) context. This difference was significant for movement duration [$F(1,11) = 8.03$; $p < 0.05$], but did not reach significance for reaction duration [$F(1,11) = 1.31$; $p > 0.05$].

10.3.3 Rapid Visual Information Processing (RVIP)

Conditioning Trials: For reaction time, there were no main effects of drug, trial [$F_s(1,11) < 0.80$; $p_s > 0.05$] or block [$F_s(2,22) = 1.10$; $p > 0.05$]. There was a significant drug-by-trial interaction [$F(1,11) = 5.23$; $p < 0.05$]. Post-hoc tests revealed a significantly faster responding after caffeine relative to placebo on trial one [$t = -2.30$, $df = 11$, $p < 0.05$], compared to faster responding after placebo on trial two (non-significant) [$t = 1.03$, $df = 11$, $p > 0.05$]. There were no other significant interactions [$F_s(2,22) < 0.82$; $p_s > 0.05$].

There were no main effects of drug or trial [$F_s(1,11) < 0.44$; $p_s > 0.05$] on RVIP errors. There was a significant drug-by-trial interaction [$F(1,11) = 8.16$; $p < 0.02$]. Post-hoc tests revealed significantly fewer errors after caffeine relative to placebo on the trial one [$t = -3.29$, $df = 11$, $p < 0.01$], and fewer errors after placebo during trial two (non-significant) [$t = 1.41$, $df = 11$, $p > 0.05$]. There were no main effects or significant interactions of block [$F_s(2,22) < 1.11$; $p_s > 0.05$] for reaction time or errors.

Conditioning tests: There was no significant difference between responding in the two contexts during the tests of conditioning for reaction time or errors [$F_s(1,11) < 0.87$; $p_s > 0.05$]. There were no main effects of block for reaction time or errors [$F_s(2,22) < 1.89$; $p_s > 0.05$] and no context-by-block interactions. There was a significant drug-by-drug order interaction [$F(1,10) = 11.6$; $p < 0.01$] with participants assigned to the CS+/CS- order displaying faster performance in the CS+ context (365.1 ms) relative to the CS- context (398.7) compared to participants assigned to the CS-/CS+

order who demonstrated faster performance in the CS- context (403.0 ms) relative to the CS+ context (471.3 ms).

10.3.4 Match-to-Sample Visual Search (MTS)

Conditioning Trials: There were no main effects of drug [$F(1,11) < 1.12$; $ps > 0.05$] for any level of MTS reaction duration. For all levels, there was a reaction time decrease across trials which approached significance for level one [$F(1,11) = 4.34$; $p = 0.061$], and reached significance for levels two [$F(1,11) = 12.09$; $p < 0.01$] and three [$F(1,11) = 5.93$; $p < 0.037$]. There were no significant drug-by-trial interactions [$F(1,11) < 1.80$; $ps > 0.05$]. Participants did not make any errors for level one and therefore only error data for levels two and three were analysed. There were no significant main effects or interactions for movement duration or errors on any level of MTS [$F(1,11) < 2.47$; $ps > 0.05$].

Conditioning test: There were no significant effects for reaction durations, movement durations or errors [$ts < 1.81$; $dfs = 11$, $ps > 0.05$].

10.3.5 Mood

Conditioning Trials: There were significant increases in ratings of “headache” (0-30 mins) after caffeine relative to decreases after placebo [$F(1,11) = 13.07$; $p < 0.005$]. There were no other significant effects of drug or trial [$F(1,11) < 3.16$; $ps > 0.05$]. There was a near significant drug-by-trial interaction for “mental clarity” [$F(1,11) = 3.60$; $p = 0.08$], with increases after caffeine on trial one compared to a decrease after

placebo [$t=1.39$, $df = 11$, $p > 0.05$]. In contrast, there was a decrease after caffeine and no change after placebo on trial two [$t=-0.78$, $df = 11$, $p > 0.05$] (see Table 10.1). There were no other significant interactions [$F_s(1,11) < 2.40$; $p_s > 0.05$].

Table 10.1. Mean changes (mm) on all mood factors across the two conditioning trials in the CS- (placebo) and CS+ (caffeine) conditions and at the conditioning test. Standard deviations are given in parenthesis.

	Condition	Cond. Trial 1	Cond. Trial 2	Cond. Test
Anxiety	CS-	-6.0 (27.4)	2.5 (7.3)	-1.8 (6.4)
	CS+	1.3 (14.8)	0.1 (8.9)	-2.9 (14.3)
Headache	CS-	-0.9 (28.8)	2.9 (12.0)	-3.0 (11.2)
	CS+	9.4 (20.2)	4.0 (9.5)	4.3 (7.8)
General Positive Mood	CS-	-14.8 (53.7)	-14.1 (24.0)	-22.0 (23.4)
	CS+	-11.4 (56.9)	-18.9 (27.9)	-17.9 (45.8)
Mental Clarity	CS-	-14.5 (22.9)	0.5 (24.0)	2.75 (16.0)
	CS+	6.0 (38.9)	-6.5 (26.0)	-17.2 (24.8)
Mental Repose	CS-	-14.3 (22.2)	-2.0 (19.7)	1.8 (23.9)
	CS+	-12.7 (36.4)	1.7 (31.2)	14.1 (27.4)
Tense Negative Mood	CS-	4.0 (25.0)	-14.3 (39.2)	-8.1 (23.9)
	CS+	3.8 (42.8)	-5.7 (43.8)	1.6 (21.1)
Sleepiness	CS-	4.3 (43.6)	11.0 (57.2)	31.5 (42.3)
	CS+	-3.2 (19.5)	7.2 (22.0)	-6.1 (50.0)

Conditioning test: Despite no significant differences between conditions at the conditioning test [$t_s < 1.80$; $dfs = 11$; $p_s > 0.05$], there were trends that approached significance. There were substantial increases in “sleepiness” in the CS- context relative to decreases in the CS+ context [$t = -1.98$, $df = 11$, $p = 0.07$]. “Mental clarity” decreased in the CS- context compared to a small increase in the CS+ context [$t = 2.10$, $df = 11$; $p = 0.06$].

10.3.6 Post-session Questionnaires

At the end of each session, participants were asked to indicate what substance they believed that they had received that day. The responses are listed in Tables 10.2 and 10.3.

Table 10.2. Number of participants who believed that they had received caffeine during the conditioning trials and conditioning test in the CS- context, compared to alternative substances, placebo or “don’t know”

	Conditioning Trial 1	Conditioning Trial	Cond. Test
Caffeine	1	4	3
Other substance	8	5	8
Placebo	1*	1*	1*
Don’t know	2	2	0

*substance that was actually administered

Table 10.3. Number of participants who believed that they had received caffeine during the conditioning trials and conditioning test in the CS+ context, compared to alternative substances, placebo or “don’t know”

	Conditioning Trial 1	Conditioning Trial	Cond. Test
Caffeine	5*	2*	2
Other substance	6	8	9
Placebo	0	0	1*
Don’t know	1	2	0

*substance that was actually administered

The Pearson’s chi-square analysis examining the association between condition (i.e. whether caffeine was administered) and reported identification of caffeine (during conditioning trials) was not significant [$\chi^2 = 0.44$, $df = 1$; $p > 0.05$].

Half of participants (six out of twelve) reported ever experiencing caffeine withdrawal and/or craving upon abstinence. Of these, three reported experiencing craving at some point during the experimental procedure (see Table 10.4).

Table 10.4. Number of participants who reported experience of withdrawal and/or craving in general and at the experimental sessions

	No. of participants who ever experience caffeine withdrawal and/or craving	If so, no. of participants who experienced caffeine withdrawal and/ or craving at the sessions
Count	6	3

10.4 DISCUSSION

Across the conditioning trials, participants were faster after caffeine compared to placebo on all aspects of SRT and CRT reaction duration. Furthermore, there was evidence of caffeine-like facilitation in the CS+ context at the conditioning tests when placebo was administered in both contexts. In addition, “mental clarity” was increased and “sleepiness” reduced in the CS+ context at test compared with the CS- context. Taken together, these data provide evidence of conditioned caffeine effects to stimuli previously-paired with caffeine administration.

Shapiro and Nathan (1986) have shown that conditioned compensatory responses develop to the detrimental effects of alcohol on performance. However, this is one of the first studies to examine explicitly whether drug-like facilitation of task performance can be conditioned to previously neutral stimuli. The majority of previous studies have focused primarily on conditioning of the physiological, reinforcing and/or subjective effects of caffeine. Zwyzghuizen-Doorenboos et al. (1990) reported conditioned effects of caffeine on a vigilance task but coffee was used

as the vehicle for caffeine, thus the results may also be explained by interactive effects of caffeine and expectancy or effects of constituents in coffee other than caffeine.

An alternative explanation is that participants may have associated the negative effects of caffeine withdrawal with the CS- context. Thus the conditioned effects observed may have been conditioned withdrawal effects to the CS- context rather than conditioned stimulant effects to the CS+ context. However, several observations make this interpretation unlikely. Firstly, reaction time performance in the CS+ context did not deteriorate from the last conditioning trial to the conditioning test when caffeine was replaced with placebo. Thus, at the test of conditioning participants were demonstrating a caffeine-like level of performance in the CS+ context, supporting an interpretation of conditioned stimulant effects. Secondly, participants tended to show an improvement in reaction time performance in the CS- context from session one to two. Conditioning theory would predict that a CR would strengthen with additional CS-US pairings and therefore a conditioned withdrawal response would produce weaker conditioning on session two compared to session one. Thirdly, the self-reported level of withdrawal during the experimental sessions was low (three out of twelve), implying that withdrawal effects may have been absent in most participants or at least, too small to be explicitly identified. Finally, the nature of the studies was such that only caffeine administration was explicitly paired with a test context. The withdrawal effects of caffeine have a slow onset and are persistent suggesting that they would have been present outside of the experimental sessions. Thus, withdrawal effects would not have been explicitly paired with any experimental cue and conditioning theory would not predict conditioning in these circumstances.

Pavlovian theory (1927) claims that the CR should be similar to the UR, with the magnitude of the CR dependent on CS intensity (Domjan 1986). The current findings suggest that the CR was of a similar magnitude to the UR. On most measures responses were of a comparable level during the conditioning test (CS+ session 3) to the responses on the second CS+ conditioning trial. Although for both SRT and CRT, performance was faster in the CS+, compared to CS-, context throughout testing, there was significantly faster responding on reaction durations (SRT and CRT) during the conditioning trials, but the benefits of the CS+ (relative to CS-) context during the conditioning test reached significance on movement duration elements of the response. As previously mentioned, few studies have separated the reaction and movement response times, and therefore fine delineation of effects has not been possible. These results imply that during the CS+ conditioning trial a general conditioned improvement may have been elicited that benefited all aspects of reaction time responding to some extent.

The CRs elicited during the conditioning test may have been weakened by the fact that the main effect of caffeine appeared to be weaker on session two compared to session one on all reaction time elements. Based on the findings of Experiment 5, it was plausible that some level of generalisation would recur and obscure conditioned effects. However, although the difference between conditions is attenuated on session two, this is less obviously a result of generalisation of an excitatory conditioned response, as for some elements of the response (CRT RD and MD), this effect is partially mediated by somewhat slower performance after caffeine from CS+ sessions one to two. In contrast, the pattern of SRT RD data appears to replicate the

Experiment 5 generalisation hypothesis, with faster responding occurring in CS- from sessions one to two. This suggests some conditioning, albeit weak, may occur after a single conditioning trial.

Alternative interpretations suggest mechanisms other than conditioning may explain the heightened responding that occurs to drug-paired stimuli. Animal models of conditioned locomotion to psychostimulants (e.g. Tirelli & Heidbreder 1999) have noted that the CR was often of a similar magnitude to the response made by saline-treated animals during their first exposure to the test context. This led to the contention that the drug attenuated the natural habituation that occurs over time to a novel environment. Therefore, the CR was actually a heightened exploratory response due to the novelty of experiencing the context in a drug-free state for the first time. In support, studies have shown that psychostimulant drugs, such as cocaine and amphetamine, interfere with an animal's ability to habituate to a novel environment (File 1977; Stähle 1992). How exploratory behaviour in rats translates to human information processing is unclear, but is likely to be related to heightened attention invoked by an "unexplored" context (Balkenius 2000), which progressively decreases over time (habituation). On this basis, the faster responding to the CS+ (relative to CS-) at the conditioning test, would be a novelty-induced facilitation in performance, possibly mediated by an increase in attentional processes that did not occur to the CS- due to habituation across the conditioning trials. However, two observations make the anti-habituation hypothesis an unlikely explanation of the present findings. Firstly, unlike the animal data, the "CR" was not similar to the responses prior to habituation, i.e. to the CS- on session 1. On most reaction time measures (SRT/CRT RD, CRT

MD) the response to CS+ on the final session (i.e the CR) was substantially faster than the response made after to the CS- (placebo) on trial one. Secondly, there is no evidence of habituation in the placebo condition. A decrease in arousal due to habituation would be expected to slow reaction time performance, however, on all measures, performance improved from the first to second placebo session.

In a similar vein, the CR may have been an increase in arousal due to “surprise” induced by the change in procedure from CS+ trials two to three (i.e. change from caffeine to placebo) (Robbins & Ehrman 1992). In support, ratings of sleepiness decrease during the conditioning test relative to the second CS+ trial, indicative of heightened arousal at the conditioning test. However, when asked if they had noticed any change in the procedure at any time on the post-experiment questionnaire, no participants mentioned being aware of a change in substance, thereby undermining the suggestion that they were “surprised” by the dose of placebo at the conditioning test.

Alternatively, expectancy of caffeine could induce caffeine-like effects during the conditioning tests, if participants have learned to expect caffeine in a particular room. In this case, the CS+ does not have any intrinsic ability to elicit a response. However, the identification of caffeine in the CS+ context did not differ significantly from the CS- context (i.e. where participants actually received placebo), suggesting little basis for a learned expectancy effect. Furthermore, when asked in the post-experiment questionnaire, if they could identify the room in which they had received caffeine, only three out of twelve identified the CS+ correctly. From the remaining nine participants, three guessed incorrectly (choosing the CS- context), and six stated that

they did not know. Therefore, participants were generally unaware of which context had been paired with caffeine.

Interestingly, conditioned effects were obtained despite this lack of contingency awareness. A large body of literature suggests that explicit knowledge of the CS-US contingency is necessary for the acquisition of a CR (Hogarth, Dickinson, Hutton, Bamborough & Duka 2006; Hogarth & Duka 2006). However, these data suggest that performance effects may be conditioned to a caffeine-paired context without such explicit understanding. It is plausible that participants may have acquired implicit knowledge of the CS-US contingency, which they were unable to report explicitly, however this could not be ascertained from the present data, and is a potential avenue for future research.

Despite, benefits of caffeine over placebo in the present study, the effects of caffeine were somewhat weaker than those obtained in Experiment 5. In some instances, the effects of caffeine did not reach significance, implying that perhaps the study was underpowered. However, Experiment 5 showed stronger effects despite a smaller sample size than the present study, indicating that an issue of power is unlikely. Alternatively, individual differences in responsiveness to caffeine may have affected the strength of the effect. Despite showing caffeine responses during the screening procedure, responsivity to caffeine is unlikely to be an all-or-none phenomenon, and the participants recruited for this study may simply be less responsive than those in Experiment 5, showing benefits on some measures but not others. The PSQ data showed that participants were relatively poor at identifying caffeine in the present study, with no significant differences in caffeine identification between caffeine and

placebo sessions. In Experiment 5, participants were more likely to correctly identify caffeine, implying they were more likely to subjectively experience a caffeine-like effect. Furthermore, a lower percentage of participants report ever experiencing withdrawal or craving for caffeine in this study compared to Experiment 5, which has been heavily implicated as an important factor in determining the reliability and robustness of caffeine effects.

In sum, this study found some evidence of conditioned caffeine-like effects during the conditioning test. However, the somewhat weak effects of caffeine (i.e. not significant on all sessions and no effects on mood, MTS or RVIP) may have limited conditioning. Clearly, further research is required to develop these early findings, but the implication is that performance facilitation of psychostimulant drugs may be conditioned to stimuli present at the time of drug administration.

CHAPTER 11:

EXPERIMENT 7: DIFFERENTIAL RESPONSIVENESS TO CAFFEINE AND PERCEIVED EFFECTS OF CAFFEINE IN MODERATE AND HIGH REGULAR CAFFEINE CONSUMERS

11.1 INTRODUCTION

In previous experiments reported in this thesis, there were problems obtaining reliable effects of caffeine. However, data from the screening procedure of Experiment 5 provided some insights into between-subjects variability of caffeine responses, which are reported in this chapter. The screening procedure involved participants attending two sessions, at which they were administered either caffeine or placebo and completed the mood and cognitive measures. Only participants who displayed faster reaction times after caffeine relative to placebo were offered a place on the main study. In earlier experiments reported in this thesis, only relatively high caffeine consumers were recruited, based on the assumptions that higher caffeine intake may result in greater withdrawal symptoms, which are known to promote the effects of caffeine, and tolerance to some of the aversive effects of caffeine. However, these were merely assumptions based on previous findings, and the screening procedure provided the opportunity to test the effects of 400 mg of caffeine in a large sample of caffeine-consumers who differed in their level of habitual caffeine consumption.

The mood-enhancing effects of caffeine have been well documented (e.g. see Smith 2002 for review). However, there is evidence to suggest that not all habitual caffeine consumers report such effects. Evans and Griffiths (1992) stratified a group of regular caffeine consumers into caffeine “choosers” and “non-choosers” based on a

caffeine-versus-placebo choice procedure. In tests of the subjective effects elicited by caffeine, the “choosers” reported more positive effects of caffeine on mood (e.g. increased vigor, friendliness, energy) in comparison with the “non-choosers” who tended to report negative subjective effects (e.g. tension, anxiety, jitteriness). The observation that the “choosers” tended to show negative subjective effects of placebo (e.g. headache and fatigue) relative to “non-choosers” indicates that the “choosers” might have experienced greater caffeine withdrawal symptoms compared with the “non-choosers” and this influenced their responding. This is consistent with the observation that the facilitatory effects of caffeine are often observed in deprived consumers of caffeine, but not in non-consumers (Smit & Rogers 2002; Rogers et al. 2003; James & Rogers 2005). However, it is also possible that the caffeine “choosers”, but not “non-choosers” had developed tolerance to the aversive anxiety-related effects of caffeine that occur at high doses (Jacobson & Thurman-Lacey 1992).

Smit and Rogers (2000) have also argued that levels of daily caffeine intake may influence the effects of caffeine on cognitive performance. They reported that the beneficial effects of caffeine on cognitive performance were more robust in a group of high caffeine consumers (>200 mg/day) relative to a group of moderate consumers (<100 mg/day). However, since all participants were overnight-withdrawn from caffeine, it was not possible to determine whether this was due to a greater negative impact of withdrawal in the high consumers, or to differences in sensitivity to the effects of caffeine.

It has also been shown that individuals consuming high levels of caffeine (≥ 350 mg/day) demonstrate an attentional bias for caffeine related words, whereas moderate consumers (100-250 mg/day) and non-consumers do not (Yeomans et al. 2005). Moreover, Yeomans et al. (2005) found that the attentional bias in the high consumers correlated both with reported levels of habitual caffeine intake and caffeine craving.

Hence, while there is evidence to suggest that individual differences in habitual levels of caffeine consumption influence the response to caffeine, the factors that mediate this differential responsiveness remain unclear. The present study tested the effects of 400 mg caffeine (or placebo) in participants deemed as either moderate (< 200 mg/day) or high (> 200 mg/day) caffeine consumers, based on a criterion adapted from Smit & Rogers (2000).

11.2 METHOD

11.2.1 Participants

49 students and staff from the University of Birmingham, UK (18 male, 31 female; mean age 23.2 years, range 18-41 years) were recruited by means of an e-mailed intake questionnaire to ensure that all were non-smoking, habitual caffeine consumers. Three participants were excluded for not attending both sessions, and one participant was excluded for not being able to swallow the capsule; therefore data for 45 participants are included in the analyses. The participants were separated into two groups (moderate versus high consumers) whose daily caffeine intake was less than or greater than 200 mg/day respectively (see Table 1).

11.2.2 Design and Materials

Each participant attended two sessions (caffeine and placebo). Inter-session intervals did not fall below 24 hours or exceed 14 days. Capsule order was counterbalanced across participants and capsule administration was double blind. Prior to each session, participants were asked to abstain from all psychoactive substances from 23:00 hrs the previous night. 400 mg of caffeine and placebo were administered as per section 4.3.

11.2.3 Measures

All measures were identical to those used in Experiment 5, with the addition of baseline SRT and CRT tasks. Each baseline task comprised two blocks of 10 and 15 trials.

11.2.4 Procedure

All testing took place between 12:00 and 18:00 hrs, and participants attended their sessions at approximately the same time of day. On arrival, participants confirmed overnight abstinence from any psychoactive substances and provided a saliva sample. A baseline mood questionnaire and practice on the SRT and CRT (two blocks each) were completed immediately before capsule ingestion. An interval of 30 minutes then elapsed to allow for drug absorption, during which time the participants were asked to sit quietly (reading material, e.g. current periodicals, was provided). After this interval, the experimenter returned to administer the mood and cognitive measures. Finally, participants completed the post-session questionnaire.

11.2.5 Data Analysis

Reaction times below 100 ms (SRT, CRT and MTS), and more than 3 standard deviations above an individual's mean score (SRT, CRT, MTS MD) were considered outliers and removed (SRT: 0.9% and 1.0, CRT: 1.9% and 1.5%, MTS: 0% and 0.4% removed for reaction and movement duration respectively).

Independent *t*-tests examined between group differences at baseline for reaction time performance and mood. Gender and capsule order were also analysed but did not influence results; therefore these data are not reported.

Two-way ANOVAs with group (high consumer, moderate consumer) and drug (caffeine, placebo) as the between and within-subject factors respectively were conducted on data from the cognitive tasks. Baseline scores were found to correlate with the test measures, and therefore baseline was included as a covariate where possible. The number of errors was analysed for the RVIP and MTS tasks. *A priori* planned comparisons were conducted to analyse the effect of capsule on mood and performance scores in the high and moderate consumer groups. A Pearson's Chi-square was employed to examine the association between group and responses on the post-session questionnaire.

11.3 RESULTS

Some BMI data were missing due to six participants choosing not to complete the personal details form in full, therefore the BMI analysis comprised data from 39 participants. There were no significant differences between groups for age or BMI,

however there was a significant group difference in levels of habitual caffeine consumption (see Table 11.1).

Table 11.1. Participant characteristics: means and t-test results of between group comparison - moderate consumers (MC) (intake <200 mg/day) and high consumers (HC) (intake >200 mg/day) of caffeine – for gender, age, level of daily caffeine consumption and BMI. Standard deviations are given in parentheses.

	Mean		t value	Degrees of Freedom	p value
Gender (m:f)	MC	9:15	N/A	N/A	N/A
	HC	9:12			
Age (years)	MC	23.7 (4.3)	0.45	43	>0.05
	HC	22.6 (5.8)			
Consumption (mg/day)	MC	150.7 (44.2)	-7.50	43	>0.0001
	HC	304.8 (76.4)			
BMI	MC	22.3 (3.2)	0.44	37	>0.05
	HC	21.8 (3.3)			

11.3.1 Simple Reaction Time (SRT)

At baseline, there were no significant differences between groups on reaction or movement duration [$t_s < 0.42$, $dfs = 41$, $p > 0.05$]. There were no significant main effects of caffeine or group on reaction duration [$F_s(1,40) < 2.97$; $p > 0.05$] or movement duration [$F_s(1,40) < 0.61$; $p > 0.05$]. However, there were significant caffeine-by-group interactions for both reaction [$F(1,40) = 4.59$; $p < 0.05$] and movement durations [$F(1,40) = 4.57$; $p < 0.05$]. Paired t-tests revealed that the high consumers [$t = 2.80$, $df = 20$, $p < 0.05$], but not the moderate consumers [$t = 0.16$; $df = 21$; $p > 0.05$], were significantly faster on reaction duration after caffeine compared with placebo (see Figure 11.1). For movement duration, the moderate consumers were significantly slower after caffeine relative to placebo [$t = 2.78$, $df = 21$, $p < 0.05$].

In contrast, the high consumers were marginally faster after caffeine, although this difference was not significant, [$t = 0.74$, $df = 20$, $p > 0.05$].

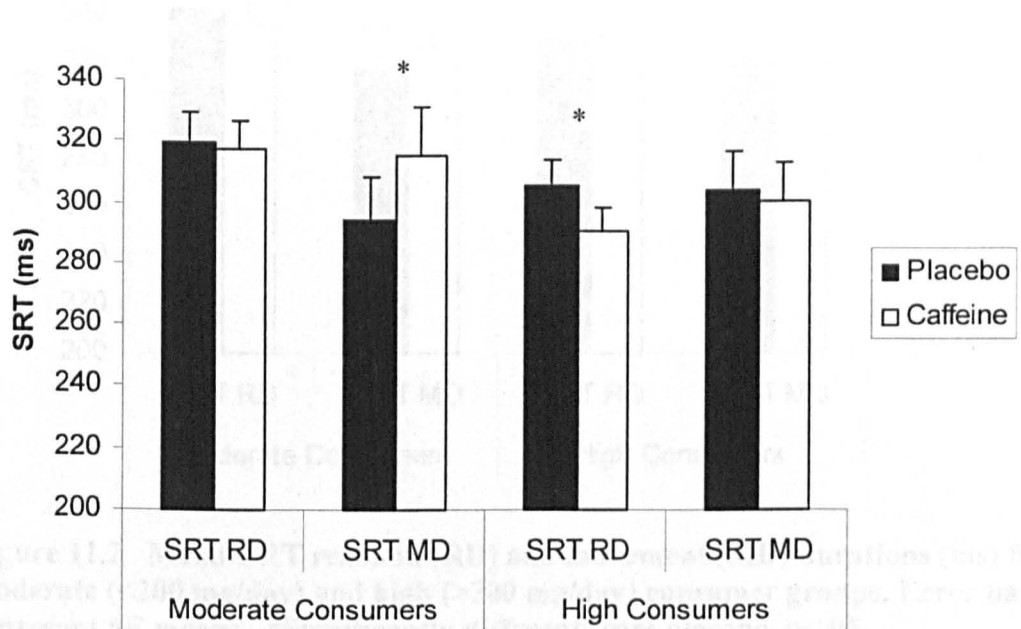


Figure 11.1. Mean SRT reaction (RD) and movement (MD) durations (ms) for moderate (<200 mg) and high (>200 mg/day) consumer groups. Error bars represent SE means. *Significantly different from placebo, $p < 0.05$.

11.3.2 Choice Reaction Time (CRT)

There were no significant differences at baseline between groups for either reaction or movement durations [$ts < 0.86$; $dfs = 41$; $p > 0.05$]. There was a significant main effect of caffeine for reaction duration [$F(1,40) = 8.58$; $p < 0.01$], but no effect of group [$F(1,40) = 1.56$; $p > 0.05$], nor a significant interaction [$F(1,40) = 1.12$; $p > 0.05$]. Paired t-tests showed that only the high consumers were significantly faster after caffeine compared with placebo [$t = 2.78$, $df = 20$, $p < 0.05$] (see Figure 11.2). For movement durations, there were no significant main effects of caffeine or group, nor was there a significant interaction between group and capsule [$F_s(1,40) < 1.24$; $p > 0.05$].

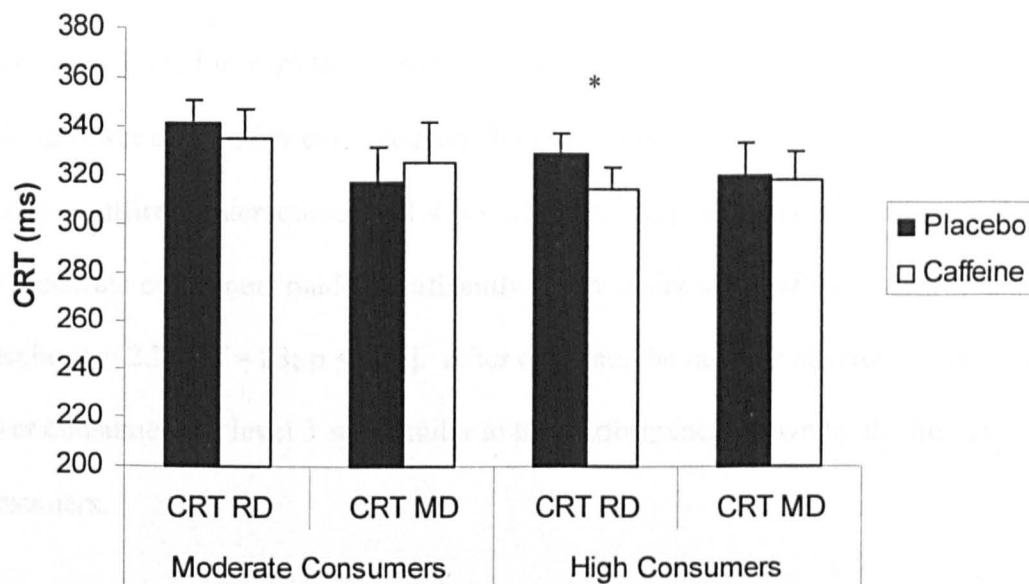


Figure 11.2. Mean CRT reaction (RD) and movement (MD) durations (ms) for moderate (<200 mg/day) and high (>200 mg/day) consumer groups. Error bars represent SE means. *Significantly different from placebo, $p < 0.05$.

11.3.3 Match-to-sample Visual Search (MTS)

There were no significant main effects of capsule or group on any level of MTS reaction and movement durations, [$F_s(1,43) < 3.13$; $p > 0.05$]. There was one significant interaction between group and capsule for level three movement duration [$F(1,43) = 4.42$; $p < 0.05$]. The group means suggested that the moderate consumers were slower after caffeine relative to placebo, with little difference for the high consumers, however this difference did not reach significance [$t = -1.84$, $df = 23$, $p > 0.05$]. There were no other significant interactions [$F_s(1,43) < 3.49$; $p > 0.05$].

There were no errors for level one performance, therefore only errors for levels two and three were analysed. There were no significant main effects or significant

interaction for level two errors [$F_s(1,43) < 0.62$; $p > 0.05$]. There was a significant effect of caffeine for level three errors [$F(1,43) = 5.17$; $p < 0.05$] due to participants making fewer errors after caffeine than placebo. There was no main effect of group and no significant interaction [$F_s(1,43) < 2.54$; $p > 0.05$]. Paired t-tests revealed that the moderate consumers made significantly fewer errors after caffeine relative to placebo [$t = 2.72$, $df = 23$; $p < 0.02$]. After caffeine, the number of errors made by the lower consumers on level 3 was similar to the performance shown by the higher consumers.

11.3.4 Rapid Visual Information Processing (RVP)

There were no significant main effects or significant interactions for reaction time or errors [$F_s(1,43) < 2.09$; $p > 0.05$]. A blocked analysis was conducted by splitting the data into two equal blocks of 18 targets. For reaction time, there was a significant effect of block [$F(1,43) = 4.46$; $p < 0.05$] and a trend towards a block-by-capsule interaction [$F(1,43) = 2.90$; $p = 0.10$] suggesting that the deterioration in performance across blocks was most prominent in the placebo condition. Although the caffeine-by-block-by-group interaction did not reach significance [$F(1,43) = 2.30$; $p > .05$], inspection of the means suggests that this deterioration was counteracted by caffeine in the high but not moderate consumers.

11.3.5 Mood

There were no significant group differences on any of the mood factors at baseline [$t_s < 1.79$, $dfs = 43$; $p_s > 0.05$]. Caffeine, relative to placebo, significantly increased

ratings of “tense negative mood” [$F(1,43) = 9.30$; $p < 0.005$] and “anxious” [$F(1,43) = 4.68$; $p < 0.05$], and decreased ratings of “mental repose” [$F(1,43) = 9.31$; $p < 0.005$], “sleepiness” [$F(1,43) = 6.88$; $p < 0.02$]. There were no other significant main effects of caffeine [$F_s(1,43) < 1.05$; $p > 0.05$]. There were no significant main effects of consumer group [$F_s(1,43) < 3.74$; $p > 0.05$]. There was a near significant caffeine-by-group interaction for ratings of “sleepiness” [$F(1,43) = 7.25$; $p < 0.05$]. A priori t-tests showed that the high consumers, but not moderate consumers, reported significant reductions in “sleepiness” [$t = -3.54$, $df = 20$, $p < 0.003$] relative to placebo (see Figure 11.3).

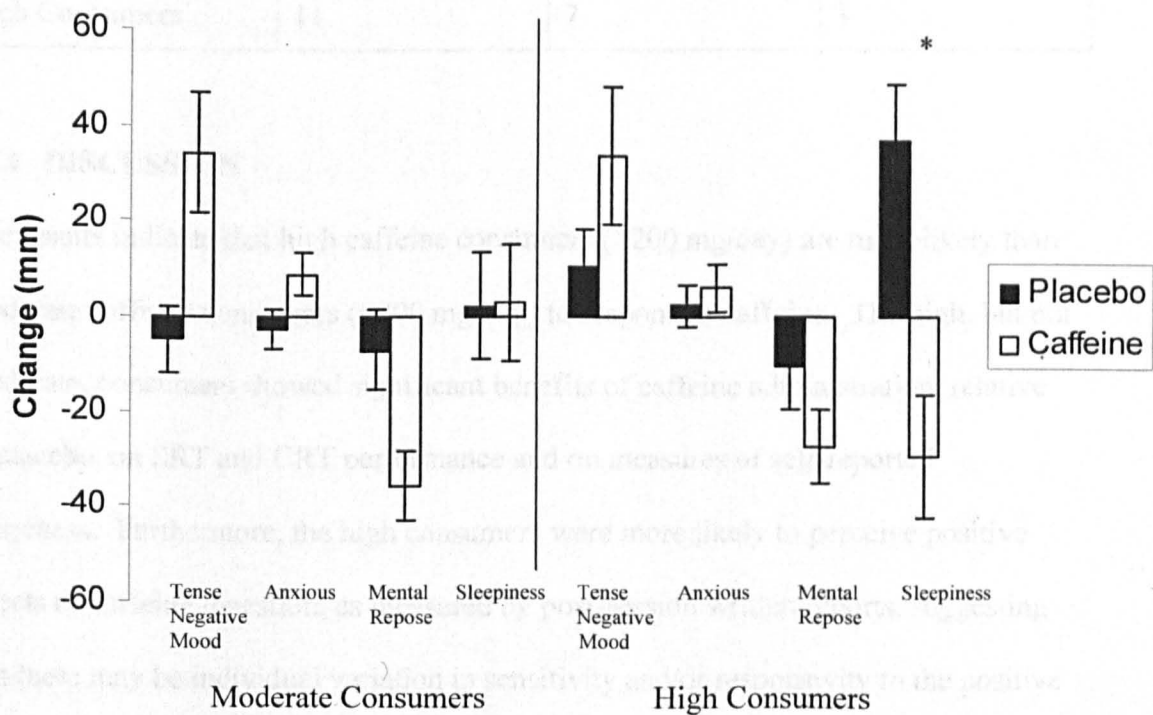


Figure 11.3. Change (mm) in ratings of “tense negative mood”, “anxious”, “mental repose” and “sleepiness” for the caffeine and placebo sessions in the moderate and high caffeine consumer groups. Error bars represent SE means. *Significantly different from placebo, $p < 0.01$.

11.3.4 Post-session Questionnaires

There were no differences between groups in terms of the number of participants reporting negative effects of caffeine. In contrast, the high consumers were significantly more likely to report positive consequences of caffeine and the moderate consumers were more likely to report “no effect” [$X^2_{(2, N=45)}=7.92$; $p < 0.05$; see Table 11.2].

Table 11.2. Numbers of moderate (intake <200 mg/day) and high (intake >200 mg/day) caffeine consumers who reported positive, negative or no effects after caffeine.

	Positive Effects	Negative Effect	No Effect
Moderate Consumers	4	9	11
High Consumers	11	7	3

11.4 DISCUSSION

The results indicate that high caffeine consumers (>200 mg/day) are more likely than moderate caffeine consumers (<200 mg/day) to respond to caffeine. The high, but not moderate, consumers showed significant benefits of caffeine administration, relative to placebo, on SRT and CRT performance and on measures of self-reported sleepiness. Furthermore, the high consumers were more likely to perceive positive effects of caffeine ingestion, as measured by post-session written reports, suggesting that there may be individual variation in sensitivity and/or responsivity to the positive effects of caffeine, which may be mediated by level of habitual intake.

There are several possible explanations for the differential responsiveness of high and moderate consumers to caffeine. First, the high consumers may experience greater

levels of withdrawal during abstinence, which is reversed by caffeine (James & Rogers 2005). However, analyses of the baseline data found no significant differences between moderate and high consumers on any of the subjective or cognitive measures. Griffiths et al. (1990) suggest that measurement of caffeine withdrawal should involve several different measures and should take place over different time points, perhaps implying that more extensive measurement of pre-session mood may have identified withdrawal symptoms. However, considering the sensitivity of the VAS scales to pick up post-caffeine mood fluctuations, there is no reason to expect sensitivity shortcomings with these measures at baseline. Furthermore, evidence of a caffeine withdrawal state has previously been detected using these questionnaire items (Rogers et al. 1995).

Alternatively, the moderate consumers may have experienced aversive effects of caffeine, to which the high consumers had developed tolerance (Evans & Griffiths 1992). However, the post-consumption mood measures demonstrated that both consumption groups showed increases in anxiety and tense negative mood after caffeine, and analysis of the post-session questionnaire (which examined participants' perceptions of the effects of capsule ingestion) showed that the two groups did not differ in their reports of negative effects. This suggests that the group differences were not due to stronger experiences of aversive effects among the moderate consumers. In fact, compared with the high consumers the moderate consumers were less likely to report positive effects and more likely to report perceiving no effect of the capsule. This suggests that the stronger responses of the high consumers may be due to greater sensitivity to the positive effects of caffeine, and this in turn might

influence their level of caffeine consumption. Why individuals should differ in their sensitivity to the positive effects of drugs is not clear, but evidence suggests that individual variations in metabolism could be important (e.g. Grant et al. 1987).

Whether chronic daily dosing of caffeine can sensitise people to the effects of caffeine has not been studied explicitly, but sensitisation could contribute to differential sensitivity.

The present data suggest that experiencing positive effects of caffeine is not necessary for the maintenance of caffeine use, since the moderate consumers still drink caffeinated beverages every day. For these moderate consumers, consumption might be maintained by other factors, for example the association of the drink with environmental reinforcers such as social interactions or breaks from work, or perhaps simple exposure to tea or coffee is sufficient to generate liking (Zajonc 1968) and maintain consumption. Alternatively, the moderate consumers may have expectations regarding the positive effects of caffeine that influence their choice of beverage.

There were significant effects of caffeine on level 3 of the match-to-sample task in the moderate consumers: relative to placebo, the moderate consumers made fewer errors after caffeine. In comparison, the high consumers did not show effects of caffeine on any level of the task. However, further analysis revealed that the moderate consumers were also slower after caffeine on level 3 of this task, and the number of errors made was reduced only to the level of the high consumers, suggesting that their increased accuracy on the caffeine session may have reflected a speed-accuracy trade-off. The moderate consumers also demonstrated slower performance than high consumers on

CRT movement duration, indicative of deleterious effects of caffeine on motoric control. This may have been mediated by the increase in tense negative mood and anxiety reported by both groups, which was offset by positive effects in the high consumers.

A screening procedure was also included prior to enrolment into Experiment 6. However, due to the findings reported here, only relatively high (>200 mg/day) consumers were recruited. This resulted in a smaller sample being required (21 compared to 45 in Experiment 5) to recruit 12 participants who demonstrated performance benefits after caffeine. These data are not reported.

These findings have implications for caffeine researchers who group caffeine consumers together as a single homogenous sample, and may explain some of the variability in reports of the effects of caffeine. Although many studies have reported significant effects of caffeine on mood and cognitive performance, some studies have found little or no effect on these measures (e.g. Bruce, et al. 1986; Kuznicki & Turner 1986; Smith et al. 1994, 1997; Watson et al. 2000). Methodological issues have often been cited to explain these null effects. However, the present data suggest that not taking account of consumer's habitual level of caffeine consumption may decrease the likelihood of observing significant effects of caffeine. In addition, the present results suggest that data collected from high caffeine consumers are not necessarily representative of the effects of caffeine in moderate consumers, and vice versa.

In summary, the findings suggest that variations in the psychomotor and subjective responses to caffeine among habitual caffeine consumers may be related to levels of regular caffeine consumption. High consumers may display a heightened responsiveness and perception of the positive effects of caffeine, which in turn may drive their high levels of caffeine use. Further research is required to elucidate whether this heightened responsiveness of caffeine in high consumers is a consequence of chronic administration, or whether the high levels of caffeine consumption are driven by a heightened sensitivity to the positive effects of caffeine.

CHAPTER 12:

GENERAL DISCUSSION

12.1 OVERVIEW OF CONDITIONING FINDINGS

The initial aim of the present thesis was to investigate whether caffeine-induced benefits of performance and mood could be conditioned to stimuli present at the time of caffeine ingestion, and to conduct a series of studies examining whether the apparent conditioned responses conformed to conditioning principles. However, unexpected difficulties in obtaining reliable effects of caffeine meant that additional studies were conducted to explore some of the factors affecting the responses to caffeine and although evidence of conditional responding was found in Experiments 5 and 6, there was not time to conduct the studies examining the associative nature of these effects.

In Experiment 5, caffeine-like effects on mental clarity were observed, with participants showing reduced decrements in mental clarity in the CS+ context during the conditioning trials and at the conditioning test compared to the CS- context. However, there were no significant differences in reaction time in the presence of the CS+ and CS- at the test of conditioning in Experiment 5. Benefits of caffeine on several performance and mood measures relative to placebo were evident during the early conditioning trials in Experiment 5 but these disappeared on later trials due to improvements in responding placebo (CS-). It was hypothesized that a conditioned association between drug and context had been acquired during the initial trials, which generalised to the CS- context. Therefore, in Experiment 6, the number of

conditioning trials was reduced and the test of conditioning was carried out prior to this hypothesised contextual generalisation. As predicted, the participants in Experiment 6 displayed enhanced performance and mood responses in the CS+ relative to CS- context at test.

These results support the suggestion that cognitive and mood effects of caffeine can be conditioned to contextual stimuli. They further suggest that conditioning to stimuli paired with caffeine can occur rapidly (within one or two CS-drug pairings) and may generalise to stimuli similar to the CS.

The number of trials required to produce conditioned effects appears to be in part dependent on the response being conditioned. For example, conditioning of the eyeblink response in humans and animals is slow and often requires in excess of 100 reinforced trials (Gormezano, Kehoe & Marshall 1983). Pavlov (1927) reported that a conditioned salivation response to a tone paired with food reached an asymptote after 60 trials. In contrast, conditioned fear and taste aversion responses can be acquired with as little as one conditioning trial (see Domjan 1986). In human drug conditioning research, most studies have examined responses to stimuli such as drug paraphernalia, smell of coffee, packets of cigarettes, which have been paired with drug effects prior to the experiment and the number of previous CS-US pairings is unknown. Nevertheless some studies have examined responses to previously neutral stimuli and reported conditioned effects after three (Flaten et al. 1997), four (Staiger & White 1988; Clements, Glautier, Stolerman, White & Taylor 1996; Stockhorst et al. 1999; Foltin & Haney 2000; Field & Duka 2002), five (Field & Duka 2001) and six to

eleven (O'Brien 1976) conditioning trials. The present results suggest that conditioning to drug related contextual stimuli can occur earlier than first thought after just two CS-US pairings. The reason why conditioning appeared to occur after fewer trials than has previously been reported is unclear, however factors such as novelty of conditioned stimuli may facilitate conditioning. This effect of novelty may be due to greater attention being paid to novel stimuli, or due to attenuated influences of latent inhibition to novel stimuli, i.e. conditioned stimuli that have been experienced before are less likely to acquire conditioned associations due to latent inhibition (see Section 3.2.2.3). Therefore, the nature of the contexts in the present conditioning studies that comprised multiple cues, some of which were rated for their novelty (olfactory and visual cues) may have facilitated early conditioning.

The lack of contingency awareness in the Experiments 5 and 6 contradicts the large body of literature suggesting awareness is a necessary factor in both pharmacological and non-pharmacological conditioning (Dawson & Biferno 1973; Dawson & Schell 1985; Newlin 1986; Shapiro & Nathan 1986; Davey 1987). However, there are drug conditioning studies that have also demonstrated conditioned effects in the apparent absence of contingency awareness. Staiger & White (1988) reported conditioned heart rate and temperature responses in the presence of alcohol-paired stimuli. However, these responses occurred irrespective of participant's ability to identify having received alcohol. Furthermore, Field & Duka (2001) reported conditioned effects to stimuli-paired with cigarette smoking without contingency awareness. However, these effects were weaker than those observed in a group of "aware" participants. The heightened CRs in the aware group were attributed to expectancy

effects of receiving drug. These data suggest that CS-US contingency knowledge produces more robust conditioned effects, although it is not necessary in all conditioning situations. Therefore, the lack of conditioning in participants classified as contingency unaware, may be due to the effects being weak and easily masked by other factors, and contingency awareness may strengthen conditioned effects (possibly by the addition of expectancy effects) that may otherwise not have been observed.

Taken together these findings support those of the pilot study in demonstrating conditioned cognitive effects of caffeine. The next section reviews the caffeine-related findings from this thesis and then discusses the transient nature of caffeine effects.

12.2 OVERVIEW OF CAFFEINE FINDINGS

12.2.1 Main effects of caffeine

12.2.1.1 Cognitive Performance, Mood and Cardiovascular Measures

Relative to placebo, caffeine facilitated simple and choice reaction and movement durations (Experiments 3, 5 and 6), MTS level one reaction duration (Experiments 3 and 5) and RVIP accuracy (Experiment 5). There was evidence of caffeine-induced attenuation of task fatigue on RVIP reaction time (Experiment 5). In addition, caffeine increased mental clarity and general positive mood (Experiment 3), and decreased mental repose (Experiments 3 and 5). Furthermore, caffeine attenuated the decreases in sleepiness (Experiments 3 and 5) general positive mood and mental clarity (Experiments 1 and 5) observed after placebo.

The results of these studies are consistent with other reports that caffeine facilitates reaction time performance (see section 2.4.1.1). However, it has been postulated that caffeine may facilitate performance of cognitive tasks via speeding of the motor response that is often required in such tasks, rather than a by direct effect on cognitive performance. In the experiments presented in this thesis, reaction times were divided into reaction and movement durations (Experiments 3-6), and significant benefits of caffeine were obtained on both elements of the response, indicating that caffeine facilitated performance beyond a simple motor response.

These effects of caffeine on SRT and CRT, along with those on MTS level one were the most reliable performance effects obtained in this thesis. Less reliable effects were observed on RVIP and the higher levels of MTS. This is consistent with the contention that tasks of lower cognitive demand are most sensitive to the effects of caffeine, relative to more complex tasks, such as problem solving or memory (James 1991; Nehlig & Debry 1994; Rusted 1994). This has been attributed to caffeine's stimulatory effect on general arousal, which, according to the Yerkes-Dodson principle (1908), is expected to facilitate simple tasks, yet have less effect or even degrade performance on complex tasks by interfering with cognitive processing. In support, caffeine decreased ratings of mental repose suggesting an agitating effect.

Although effects of caffeine on higher cognitive function have not been reliably observed, caffeine is known to be particularly effective in maintaining vigilance in long and/or fatiguing tasks (Frewer & Lader 1991; Robelin & Rogers 1998).

However, in the experiments reported in this thesis, no significant effects of caffeine were found on RVIP speed. However, it is possible that the 6 minute RVIP task used was not long enough to demonstrate significant effects of caffeine on sustained attention and vigilance. Effects of caffeine have been observed using longer versions of the task (Smit & Rogers 2000; Yeomans et al. 2002). In addition, Frewer and Lader (1991) found a significant interaction of caffeine and time on a 15 minute RVIP task, whereby caffeine offset the deterioration in performance that occurred in the placebo condition. Similarly, in the present studies, the blocked analyses revealed patterns of a caffeine-by-block interaction (Experiments 3 and 5). The mean scores showed deterioration in performance across blocks after placebo, which was offset by caffeine. Blocked analyses were also run on SRT, CRT and SVT in Experiments 1 and 2, however no effects were found, despite the fact these lasted longer than the RVIP task. However, these tasks incorporated breaks, allowing participants to recover from any decrement due to task fatigue. Therefore, to maximise the likelihood of observing caffeine effects on vigilance, future studies should ensure that the attentional task is of sufficient duration to detect performance deterioration under normal test conditions.

The mood findings in this thesis were consistent with effects of caffeine reported previously in regular consumers, such as improved mental clarity and general mood (Smit and Rogers 2002). Caffeine also decreased ratings of relaxation and tiredness, but despite the relatively high dose in the later studies presented in this thesis, caffeine did not produce consistently significant increases in anxiety or tense negative mood compared to placebo. Therefore, participants did not appear to experience the

aversive effects sometimes associated with high doses of caffeine, indicating that 400 mg does not have significant adverse effects in groups of moderate to high regular caffeine consumers. This may be in part due to tolerance to the aversive effects of caffeine that is reported to occur following regular consumptions (Evans & Griffiths 1992).

Caffeine's stimulation of arousal has been identified as a potential mechanism by which it improves cognitive performance. In support, the benefits of caffeine have been shown to be dependent on the extent to which performance on a particular task is benefited by increases in arousal (Anderson & Revelle 1983). In the present studies, the benefits of caffeine on performance were often paralleled with beneficial effects on sleepiness and mental clarity (comprising an alertness rating). However, the differences were largely driven by negative effects after placebo that did not occur after caffeine, rather than absolute benefits of caffeine. Examination of participants' baseline reaction time data from the screening procedure, highlighted a similar pattern for SRT reaction duration, i.e. participant scores deteriorate from baseline after placebo but not after caffeine. Therefore, caffeine may decrease reaction time by offsetting the tiredness, which often occurs after placebo. However, findings showing performance effects (albeit of a lesser magnitude) without corresponding mood effects (Experiment 6), and mood effects without performance facilitation (Experiment 1) suggest that caffeine may stimulate performance by mechanisms other than general alerting responses, and that although alerting effects may potentiate performance after caffeine, they are not necessary to produce performance enhancement.

The studies in this thesis failed to find reliable effects of caffeine on cardiovascular measures, which led to the omission of these measures in later chapters. Baseline recordings were taken only 5 minutes after arrival, which may not have been sufficient time to obtain a reliable resting measure, thus compromising the data. Ideally there should be a longer interval of inactivity before the baseline readings are taken but due to time constraints, this was not feasible in the present studies. In any case, reports of the effects of caffeine on cardiovascular measures are mixed, with some studies failing to replicate the significant findings of others (see Smit & Rogers 2002). In sum, the present results suggest that caffeine effects on cardiovascular parameters are small and difficult to detect.

12.2.1.2 Post-session data

Participants in the present studies were poor at identifying when they had received caffeine despite the fact that the dose used was relatively high and all participants were high habitual caffeine consumers. Only one study (Experiment 5) found a significant association between receiving caffeine and correctly identifying it. This study also produced the strongest effects of caffeine, highlighting a possible association between experiencing subjective feelings of caffeine and its cognitive effects. One explanation for these results is that the within-subjects design used in Experiment 5 gave the participants the opportunity to discriminate between the active and non-active capsules and that the participants formed expectancies about how they should be affected by the drug based on this learned discrimination. However, Experiment 6 was also a within-subjects experiment but participants in this study did not successfully identify caffeine as the substance that they had consumed.

Furthermore, drug discrimination effects would be expected to strengthen across trials as there is more time to learn to discriminate between the two capsules, however correct identification of caffeine was more likely during the earlier trials in Experiment 5. Therefore, it is unlikely that the caffeine effects observed were due to a learned ability to differentiate the effects of the caffeine capsule from those of the placebo capsule.

However, the significant effects of caffeine found in other studies in this thesis in the absence of correct identification of caffeine ingestion suggest that subjective awareness of caffeine is not necessary for cognitive effects. The weak ability to identify caffeine as the substance consumed may have been influenced by the nature of the post-session questionnaire (PSQ) and the information given about the study. Participants were told that they may receive one of several possible substances at each session, and this may have led participants to believe that each substance would be tested at some point. Therefore, participants may have felt caffeine-like effects yet opted for an alternative substance on the PSQ if they had chosen caffeine on a previous session. To examine this, an open-ended question was added to the PSQ (Experiments 3-6) asking participants to report any feelings they had after consuming the capsule. It is interesting that on 60% of occasions when a substance other than caffeine was chosen on a caffeine trial, participants reported feeling no effect of the capsule. This would suggest that participants were not subjectively feeling a caffeine effect and opting for an alternative substance due to the nature of the PSQs.

From Experiment 4 onwards, questions were added to the post-experiment questionnaire (PEQ) asking participants whether they ever experienced symptoms of caffeine withdrawal upon abstinence, and if they had been aware of such symptoms during the course of the study. This was included in order to provide an indication of whether participants experience caffeine withdrawal, as caffeine withdrawal state has been argued to be a pre-requisite for experiencing cognitive effects of caffeine (see section 2.6). The highest rate of reported withdrawal (70% of the sample) was found in Experiment 5 and the most robust effects of caffeine were also observed in this experiment compared to other studies presented in this thesis. Although these findings are suggestive of a relationship between caffeine withdrawal and positive cognitive and mood effects of caffeine, it should be noted that the questionnaire was a measure of the participant's perception caffeine withdrawal. It is possible that some participants experience caffeine withdrawal symptoms upon acute caffeine abstinence, but do not attribute these effects to caffeine withdrawal. In contrast, the results from the screening data reported in Chapter 11 suggested greater sensitivity to caffeine in the higher caffeine consumers compared to the moderate consumers that appeared to be independent of caffeine withdrawal. Taken together, these findings suggest caffeine withdrawal is sufficient but not necessary to demonstrate subjective benefits of caffeine.

12.2.2 Inconsistencies in the effects of caffeine

Experiments 1 and 2 failed to find clear effects of caffeine and despite observing significant caffeine effects in Experiment 3, significant effects of caffeine were not observed in Experiment 4. However, significant effects were observed in Experiments 5 and 6.

The difficulties of observing reliable cognitive effects of caffeine were unexpected considering its widely reported stimulant effects and its ability to enhance performance on a wide range of cognitive tasks (see section 2.4). However, there is also some inconsistency in the caffeine literature regarding caffeine's ability to enhance cognitive and psychomotor performance. For example, although reaction time measures are relatively sensitive to the effects of caffeine (compared to higher cognitive functions such as memory for example), null effects of caffeine have also been reported (Bruce et al. 1986; Kuznicki & Turner 1986; Smith, Kendrick and Maben 1994; Smith et al. 1997; Watson et al. 2000).

It is likely that inconsistencies in the observed effects of caffeine on performance are related to methodological differences across experiments. For example, Wenzel and Rutledge (1962) argued that the wide variation of doses used in different studies is a potential source of these inconsistencies. Similar to the studies reported in this thesis, Roache and Griffiths (1987) found greater enhancement at 400 mg than at 200 mg. In addition, the current studies, the most reliable effects of caffeine were obtained on reaction time tasks as opposed to higher level visual search (MTS) suggesting that the

nature of the task used is also important. Even tasks that ostensibly measure the same cognitive process often differ on features such as duration, sensory modality, type of response, inter-stimulus interval and stimulus intensity. For example, as a test of vigilance, RVIP has previously been reported to be effective in demonstrating effects of caffeine (Frewer & Lader 1991; Warburton 1995). However, no significant effects were obtained on RVIP in any of the studies reported in this thesis; an effect that is plausibly due to the present version of the task being shorter than those used elsewhere.

In the present thesis, greater effects of caffeine were also obtained using a within-subjects experimental design. There is substantial evidence suggesting that between-subject factors such as differences in: personality (Gilliland 1980), age (Blanchard & Sawyers 1983), levels of habitual caffeine consumption (Smit & Rogers 2000) and pharmacokinetics (Lader et al. 1996; Magkos & Kavouras 2005) may influence caffeine effects. However, because in this thesis a screening procedure to identify caffeine responders was implemented at the same time as the switch to a within-subjects design, it is impossible to say whether the within-subjects design alone promoted stronger effects of caffeine.

Although the post-session data from Experiment 5 indicated that withdrawal may be associated with positive mood and cognitive effects of caffeine, the results of the screening procedure (Experiment 7) found an effect of habitual consumption that did not seem to be due to tolerance or withdrawal. High consumers were more likely to report positive effects than moderate consumers, who were most likely to report no

effects of caffeine. These findings implied that higher consumers may be more sensitive to the positive effects of caffeine; a factor that may drive their heightened levels of consumption.

12.2.3 Summary

Although significant effects of caffeine were obtained in the later conditioning studies of this thesis (Experiments 5 and 6), these effects were only obtained after methodological changes were implemented to strengthen the weak or null effects of caffeine observed in earlier studies. The most robust effects of caffeine were obtained in a study in which the participants were most likely to correctly identify caffeine and were most likely to report experiencing withdrawal in everyday life. The results of Experiment 7 also suggest that regular caffeine consumers have may have different levels of sensitivity to the effects of caffeine that are not necessarily due differences in tolerance or withdrawal, and these may influence levels of habitual caffeine intake.

12.3 LIMITATIONS AND FUTURE RESEARCH

As a substantial amount of research time was concentrated on establishing reliable effects of caffeine, it was not possible to fulfil the original research aims of this Ph.D. As discussed above, the results obtained in Experiments 5 and 6 are suggestive rather than conclusive and warrant further investigation and replication. It was planned to examine the extent to which a drug-like CR conforms to the principles of classical conditioning, and this still could be examined in future research.

A possible limitation of the findings is that the relatively high level of habitual caffeine intake of the participants who took part in this research is likely to mean that the results may not generalise to lower consumers who have been reported to differ in their responses to caffeine (Chapter 11). Furthermore, the participants in Experiments 5 and 6 were identified as “caffeine responders” in a screening procedure, which may further limit the generalisability of these findings.

In addition, the dose of 400 mg is much higher than is usually consumed in a single cup of tea or coffee. However, there was little evidence to suggest that participants in the present study found this dose aversive, and the use of a vehicle unrelated to caffeine (i.e. capsule as opposed to coffee) may reduce the effect of caffeine due to reduced effects of expectancy (Flaten & Blumenthal 1999). Furthermore, the aim of the present thesis was not to make claims regarding the dose-related effects of caffeine, but to establish reliable drug-induced performance benefits so that these effects could be conditioned to a drug-associated context. There is no reason to believe that similar effects would not be obtained in any situation where a drug reliably enhances performance.

It should also be acknowledged that the sample sizes used in the present series of studies were smaller than would be estimated based on retrospective calculation of effect sizes. This suggests that the null effects may have been due to not testing sufficient numbers of participants. Because the use of large sample sizes in conditioning studies that comprise multiple testing of up to 10 sessions would have been impractical due to time constraints it could be argued that caffeine was not an

appropriate drug to use in the present thesis. However, the sample sizes used here were comparable to those that have been used previously in conditioning (Foltin & Haney 2000; Shapiro & Nathan 1986) and caffeine studies (Durlach 1998; Zwyghuizen-Doorenbos et al. 1990). Furthermore, significant effects of caffeine were obtained in the pilot study and in Experiments 3, 5, 6 and 7 suggesting that the null effects observed were not solely due to small sample sizes. Another psychostimulant drug (e.g. nicotine) could have been tested but methodological problems associated with the use of drugs like nicotine such as difficulties in producing a well-disguised placebo, indicate that there was no obvious superior alternative to caffeine for the present studies.

Mood effects of caffeine were obtained in Experiment 1 but not Experiment 2, despite evidence showing that caffeine significantly benefits mood and that the visual analogue scales employed were sensitive measures of these effects. However, problems associated with these scales as acknowledged by Smit and Rogers (2002) may have attenuated their effectiveness. For example, the meaning of the traits may be construed differently by different individuals and rated differently. To reduce noise in the between-subject data, change from baseline scores were calculated and used in the analysis. However, such problems are not limited to VASs and are common to all subjective mood questionnaires. Smit and Rogers (2002) suggest that repeated testing may result in participants paying less attention to the scales over time. In addition, some participants did not seem to consider the line as a continuous spectrum and restricted their responses to the anchor points on the scale and/or to the middle of the scale. Although written instructions were provided at the top of the

questionnaire, these instructions were reiterated verbally by the experimenter from Experiment 3 onwards, emphasising the use of the whole line and asking participants to think carefully about each scale each time the questionnaire was completed. This appeared to be effective in strengthening the reliability of the scales, as effects of caffeine on mood were obtained on all of the remaining studies (i.e. Experiments 3-7).

Given the changes in arousal which might influence responding over the course of the day, an additional methodological limitation is that stricter control could have been implemented over the time of day at which individual participants were tested. For example, some studies have shown caffeine to be particularly effective shortly after lunch (Smith et al. 1990). However, participants in the present studies were tested at approximately the same time of day (within 1 hour) across sessions and time of day of testing was matched between groups. Furthermore, fluctuations in performance and arousal associated with changes in circadian rhythm and eating episodes (i.e. time since eaten) were considered in the analyses, but no significant effects were observed and therefore the data were not reported. Nevertheless it is possible that some differences in responsiveness to caffeine occurred due to differences in the time of testing which may have contributed to the null effects observed in some of the studies. In future, all participants could be tested at precisely the same time of day.

Some observations from the current series of studies point to potential avenues for future research. Firstly, few studies have examined the testing of multiple doses of caffeine, and the current studies demonstrated substantial intra-individual variation over sessions. For example, on some sessions participants were confident that they

had received caffeine, as was ascertained from the PSQs. They reported subjective feelings of decreased fatigue and increased alertness and intimated confidence that caffeine was the substance that they had received that day. However, on other caffeine sessions, the same participants reported feeling no effect of the capsule and identified the capsule as placebo, despite the relatively high caffeine dose. Why such subjective feelings of caffeine show such inconsistency is not clear and research could examine the factors (such as levels of baseline arousal, mood) that may influence why these subjective effects of caffeine disappear and reappear within individuals on different days.

Secondly, the apparent generalisation effect observed in Experiment 5 could be examined further. Conditioning may have occurred to a discrete stimulus in the CS+ context that was also present in the CS- context (e.g. capsule). Alternatively, the effects of caffeine may have conditioned to the compound context CS+ and the CR generalised to the CS- due to similarities between the contextual stimuli. Future research could explore the role of the two types of cue in the generalisation effect by testing participants in the presence of each cue individually (i.e. in the presence of the discrete cues in a different context, and in the CS+ context without the discrete cues). This would allow the responses elicited by each cue to be viewed individually and thus dissociate their relative importance in the generalisation effect.

Finally, if the effects in Experiment 5 were due to generalisation between similar contexts, it appears that participants constructed a configural representation of the compound stimuli (i.e. room with poster and odour) rather than constructing a detailed

representation (i.e. white room with castle poster and floral odour), which would have resulted in discrimination rather than generalisation. Future research could examine this effect by manipulating the attention paid to the contextual stimulus. For example, the effects of two groups could be examined: one group completes a task noting the features of the room, while another completes an unrelated task. This would draw attention to the details of the context in the former group, and presumably favour discrimination rather than generalisation.

12.4 SUMMARY

The conditioning findings from the present thesis show that returning to a context that has been paired with the ingestion of a performance- and mood-enhancing drug can result in drug-like performance and mood benefits in the absence of the drug. These conditioned effects are a further example of the ability of drug-paired stimuli to acquire the reinforcing effects of drugs, which have previously been implicated in the maintenance of drug use (Stolerman 1993; Carter & Tiffany 1999). Previous research however, has mainly focussed on subjective responses such as feelings of a drug-like “high” (Foltin & Haney 2000), whereas the current research shows that cognitive function may also be improved in an environment in which a stimulant drug has previously been taken.

In addition, a context that shares similar features with a drug-paired environment may also acquire the ability to elicit drug-like responses despite not directly being paired with drug administration. These data have implications for conditioning research and intervention programmes, and support the notion that extinction treatments should

incorporate trials in multiple contexts beyond the original drug environment/stimuli. Moreover, the pattern of generalisation occurred quickly suggesting that learning can occur after only one or two CS-US pairings. Furthermore, these effects occurred without apparent awareness of the relationship between drug and context.

In addition, the present thesis includes data that contributes to the understanding of caffeine effects within a caffeine-consuming population. Caffeine effects were only observed after stringent methodological manipulations, suggesting that the effects of caffeine may be more difficult to observe than perhaps the literature would suggest. Caffeine unquestionably has the potential to facilitate performance and mood, but the conditions under which this occurs are variable and ill-defined. It is further evident that the effects of caffeine are subject to considerable variation depending on the methodology employed and as a result of a variety of between- and within-subjects factors. It is likely that these factors contribute to the inconsistencies in findings that exist in the caffeine literature, and these data suggest substantial consideration should be given to such factors when designing studies using caffeine. In addition, the negligible effects of caffeine cast doubt on the level of performance and/or mood enhancement that should be expected after a single serving of tea and coffee, and challenges the common perception of caffeine as a robust psychostimulant.

In sum, these studies are the first to explicitly demonstrate drug-like conditioned responses on cognitive performance, and suggest that these effects can occur quickly and in the apparent absence of awareness of the context-drug relationship. Further research is needed to examine the extent to which these effects conform to the

conditioning theory in order to substantiate a role of conditioning, as alternative explanations for conditioned effects have been postulated. However, the pattern of effects in Experiment 6, with no evidence of habituation to the CS- context, and the use of an unpaired control, whereby all participants had equal exposure to the US, undermined the popular alternative explanations of anti-habituation and pseudoconditioned effects. Therefore, the best explanation for the present data seems to be that the drug-paired contextual stimuli acquired an ability to directly elicit a drug-related response through the processes of classical conditioning.

REFERENCES

- Abernethy, D. R. & Todd, E. L. (1985). Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *European Journal of Clinical Pharmacology*, 28, 425-428.
- Adams, J. U., Careri, J. M., Efferen, T. R. & Rotrosen, J. (2000). Conditioned locomotor stimulant effects of cocaine in rats do not result from interference with habituation. *Psychopharmacology (Berl)*, 151, 13-18.
- Ahlijanian, M. K. & Takemori, A. E. (1986). Changes in adenosine receptor sensitivity in morphine-tolerant and -dependent mice. *Journal of Pharmacology and Experimental Therapeutics*, 236, 615-620.
- Ahmed, S. H., Cador, M., Le Moal, M. & Stinus L. (1995). Amphetamine-induced conditioned activity in rats: comparison with novelty-induced activity and role of the basolateral amygdale. *Behavioral Neuroscience*, 4, 723-733.
- Ahmed, S. H., Oberling, P., Di, S. G. & Sandner, G. (1996). Amphetamine-induced conditioned activity does not result from a failure of rats to habituate to novelty. *Psychopharmacology (Berl)*, 123, 325-332.
- Akins, C. K. & Domjan, M. (1996). The topography of sexually conditioned behaviour: effects of a trace interval. *Quarterly Journal of Experimental Psychology B*, 49, 346-356.

Amiro, T. W. & Bitterman, M. E. (1980). Second-order appetitive conditioning in goldfish. *Journal of Experimental Psychology: Animal Behavior Processes*, 6, 41-48.

Anagnostaras, S. G. & Robinson, T. E. (1996). Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behavioral Neuroscience*, 110, 1397-1414.

Anderson, K. J. & Revelle, W. (1983). The interactive effects of caffeine, impulsivity and task demands on a visual search task. *Personality and Individual differences*, 4, 127-134.

Andrews, S. E., Blumenthal, T. D. & Flaten, M. A. (1998). Effects of caffeine and caffeine-associated stimuli on the human startle eyeblink reflex. *Pharmacology Biochemistry and Behaviour*, 59, 39-44.

Archer, T., Mohammed, A. K., Danysz, W., Jarbe, T. U. & Jonsson, G. (1986). Attenuation of sensory preconditioning by noradrenaline depletion in the rat. *Behavioural Brain Research*, 20, 47-56.

Arnaud, M. J. (1987). The pharmacology of caffeine. *Progress in Drug Research*, 31, 273-313.

Arnold, M. E., Petros, T. V., Beckwith, B. E., Coons, G. & Gorman, N. (1987). The effects of caffeine, impulsivity, and sex on memory for word lists. *Physiology & Behavior*, 41, 25-30.

- Arvanitogiannis, A., Sullivan, J. & Amir, S. (2000). Time acts as a conditioned stimulus to control behavioral sensitization to amphetamine in rats. *Neuroscience*, 101, 1-3.
- Arwas, S., Rolnick, A. & Lubow, R. E. (1989). Conditioned taste aversion in humans using motion-induced sickness as the US. *Behaviour Research and Therapy*, 27, 295-301.
- Atkinson, J. & Enslen, M. (1976). Self-administration of caffeine by the rat. *Arzneimittelforschung*, 26, 2059-2061.
- Attwood, A. S., Terry, P. & Higgs, S. (2004). Conditioned psychomotor effects of caffeine in humans. *Appetite*, 40, 110.
- Avants, S. K., Margolin, A., Kosten, T. R. & Cooney, N. L. (1995). Differences between responders and nonresponders to cocaine cues in the laboratory. *Addictive Behaviors*, 20, 215-224.
- Babbini, M. & Davis, W. M. (1972). Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. *British Journal of Pharmacology*, 46, 213-224.
- Baddeley, A. D. (1968). A three-minute reasoning test based on grammatical transformation. *Psychonomic Science*, 10, 341-342.
- Badiani, A., Browman, K. E. & Robinson, T. E. (1995). Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of cocaine and amphetamine. *Brain Research*, 674, 291-298.

Badiani, A., Oates, M. M. & Robinson, T. E. (2000). Modulation of morphine sensitization in the rat by contextual stimuli. *Psychopharmacology (Berl)*, 151, 273-282.

Baeyens, F., Eelen, P. & van den Bergh, O. (1990). Contingency awareness in evaluative conditioning: A case for unaware affective-evaluative learning. *Cognition and Emotion*, 4, 3-18

Bailey, C. P. & Connor, M. (2005). Opioids: cellular mechanisms of tolerance and physical dependence. *Current Opinions in Pharmacology*, 5, 60-68.

Baldi, E., Lorenzini, C. A. & Bucherelli, C. (2004). Footshock intensity and generalization in contextual and auditory-cued fear conditioning in the rat. *Neurobiology of Learning and Memory*, 81, 162-166.

Balkenius, C. (2000). Attention, habituation and conditioning: toward a computational model. *Cognitive Science Quarterly*, 1, 171-214.

Barr, G. A., Sharpless, N. S., Cooper, S., Schiff, S. R., Paredes, W. & Bridger, W. H. (1983). Classical conditioning, decay and extinction of cocaine-induced hyperactivity and stereotypy. *Life Sciences*, 33, 1341-1351.

Barrett, J. E., King, M. G. & Pang, G. (2000). Conditioning rhinitis in allergic humans. *Annals of the New York Academy of Sciences*, 917, 853-859.

Battig, K. & Buzzi, R. (1986). Effect of coffee on the speed of subject-paced information processing. *Neuropsychobiology*, 16, 126-130.

Battisti, J. J., Uretsky, N. J. & Wallace, L. J. (2000). Importance of environmental context in the development of amphetamine- or apomorphine-induced stereotyped behavior after single and multiple doses. *Pharmacology Biochemistry and Behaviour*, 66, 671-677.

Bedingfield, J. B., King, D. A. & Holloway, F. A. (1998). Cocaine and caffeine: conditioned place preference, locomotor activity, and additivity. *Pharmacology Biochemistry and Behaviour*, 61, 291-296.

Bennett, C. H., Wills, S. J., Wells, J. O. & Mackintosh, N. J. (1994). Reduced generalization following preexposure: latent inhibition of common elements or a difference in familiarity? *Journal of Experimental Psychology: Animal Behavior Processes*, 20, 232-239.

Bennett, C. H., Tremain, M. & Mackintosh, N. J. (1996). Facilitation and retardation of flavour aversion conditioning following prior exposure to the CS. *Quarterly Journal of Experimental Psychology B*, 49, 220-230.

Benowitz, N. L., Jacob, P., III, Mayan, H. & Denaro, C. (1995). Sympathomimetic effects of paraxanthine and caffeine in humans. *Clinical Pharmacology and Therapeutics*, 58, 684-691.

Blaisdell, A. P., Denniston, J. C. & Miller, R. R. (1998). Temporal encoding as a determinant of overshadowing. *Journal of Experimental Psychology: Animal Behavior Processes*, 24, 72-83.

Blanchard, J. & Sawers, S. J. (1983). Comparative pharmacokinetics of caffeine in young and elderly men. *Journal of Pharmacokinetics and Biopharmaceutics*, 11, 109-126.

Boulenger, J. P., Patel, J., Post, R. M., Parma, A. M. & Marangos, P. J. (1983). Chronic caffeine consumption increases the number of brain adenosine receptors. *Life Sciences*, 32, 1135-1142.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin*, 114, 80-99.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biological Psychiatry*, 52, 976-986.

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning and Memory*, 11, 485-494.

Bouton, M. E. (1983). Contextual control of the extinction of conditioned fear: tests for the associative value of the context. *Journal of Experimental Psychology: Animal Behavior Processes*, 9, 248-265

Bouton, M. E. & Swartzentruber, D. (1986). Analysis of the associative and occasion-setting properties of contexts participating in a Pavlovian discrimination. *Journal of Experimental Psychology: Animal Behavior Processes*, 12, 350.

Bovbjerg, D. H. (2006). The continuing problem of post chemotherapy nausea and vomiting: contributions of classical conditioning. *Autonomic Neuroscience*, 129, 92-98.

Brice, C. F. & Smith, A. P. (2002)a. Effects of caffeine on mood and performance: a study of realistic consumption. *Psychopharmacology (Berl)*, 164, 188-192.

Brice, C. F. & Smith, A. P. (2002)b. Factors associated with caffeine consumption. *International Journal of Food Sciences and Nutrition*, 53, 55-64.

Broadbent, J. & Cunningham, C. L. (1996). Pavlovian conditioning of morphine hyperthermia: assessment of interstimulus interval and CS-US overlap. *Psychopharmacology (Berl)*, 126, 156-164.

Brockwell, N. T., Eikelboom, R. & Beninger, R. J. (1991). Caffeine-induced place and taste conditioning: production of dose-dependent preference and aversion. *Pharmacology Biochemistry and Behaviour*, 38, 513-517.

Brooks, D. C. & Bouton, M. E. (1994) A retrieval cue for extinction attenuates response recovery (renewal) caused by a return to the conditioning context. *Journal of Experimental Psychology: Animal Behavior Processes*, 20, 366-379

Bruce, M., Scott, N., Lader, M. & Marks, V. (1986). The psychopharmacological and electrophysiological effects of single doses of caffeine in healthy human subjects. *British Journal of Clinical Pharmacology*, 22, 81-87.

Bruce, M., Scott, N., Shine, P. & Lader, M. (1992). Anxiogenic effects of caffeine in patients with anxiety disorders. *Archives of General Psychiatry*, 49, 867-869.

Buchel, C. & Dolan, R. J. (2000). Classical fear conditioning in functional neuroimaging. *Current Opinions in Neurobiology*, 10, 219-223.

Bykov, K. M. (1957). *The cerebral cortex and the internal organs*. New York: Chemical Publishing.

CANTABclipse (2006) *The CANTAB Bibliography* (online). Available at www.cantabclipse.com (accessed September 2006).

Carey, R. & Gui, J. (1997). A simple and reliable method for the positive identification of pavlovian conditioned cocaine effects in open-field behavior. *Journal of Neuroscience Methods*, 73, 1-8.

Carey, R. & Gui, J. (1998)a. Cocaine sensitization can accelerate the onset of peak cocaine behavioral effects. *Pharmacology Biochemistry and Behaviour*, 60, 395-405.

Carey, R. & Gui, J. (1998)b. Cocaine conditioning and cocaine sensitization: what is the relationship? *Behavioural Brain Research*, 92, 67-76.

Carter, B. L. & Tiffany, S. T. (1999). Meta-analysis of cue-reactivity in addiction research. *Addiction*, 94, 327-340.

Cepeda-Benito, A. & Tiffany, S. T. (1992). Effect of number of conditioning trials on the development of associative tolerance to morphine. *Psychopharmacology (Berl)*, 109, 172-176.

Cepeda-Benito, A. & Tiffany, S. T. (1995). Role of drug-administration cues in the associative control of morphine tolerance in the rat. *Psychopharmacology (Berl)*, 122, 312-316.

Cepeda-Benito, A., Tiffany, S. T. & Cox, L. S. (1999). Context-specific morphine tolerance on the paw-pressure and tail-shock vocalization tests: evidence of associative tolerance without conditioned compensatory responding.

Psychopharmacology (Berl), 145, 426-432.

Cepeda-Benito, A., Davis, K. W., Reynoso, J. T. & Harraid, J. H. (2005). Associative and behavioral tolerance to the analgesic effects of nicotine in rats: tail-flick and paw-lick assays. *Psychopharmacology (Berl)*, 180, 224-233.

Chandra, S. B. & Singh, S. (2005). Chemosensory processing in the fruit fly, *Drosophila melanogaster*: generalization of a feeding response reveals overlapping odour representations. *Journal of Biosciences.*, 30, 679-688.

Charney, D. S., Galloway, M. P. & Heninger, G. R. (1984). The effects of caffeine on plasma MHPG, subjective anxiety, autonomic symptoms and blood pressure in healthy humans. *Life Sciences*, 35, 135-144.

Childress, A. R., Hole, A. V., Ehrman, R. N., Robbins, S. T., McLellan, A. T. & O'Brien, C. P. (1993). Cue reactivity and cue reactivity interventions in drug dependence. *NIDA Research Monograph*, 137, 73-95.

Childress, A. R., McLellan, A. T. & O'Brien, C. P. (1986). Abstinence opiate abusers exhibit conditioned craving, conditioned withdrawal and reductions in both through extinction. *British Journal of Addiction*, 8, 655-660.

Chotro, M. G. & Alonso, G. (2003). Stimulus preexposure reduces generalization of conditioned taste aversions between alcohol and non-alcohol flavors in infant rats. *Behavioral Neuroscience*, 117, 113-122.

- Chou, D. T., Khan, S., Forde, J. & Hirsh, K. R. (1985). Caffeine tolerance: behavioral, electrophysiological and neurochemical evidence. *Life Sciences*, 36, 2347-2358.
- Christian, K. M. & Thompson, R. F. (2003). Neural substrates of eyeblink conditioning: acquisition and retention. *Learning and Memory*, 10, 427-455.
- Christopher, G., Sutherland, D. & Smith, A. (2005). Effects of caffeine in non-withdrawn volunteers. *Human Psychopharmacology*, 20, 47-53.
- Clark, R. E. & Squire, L. R. (1998). Classical conditioning and brain systems: the role of awareness. *Science*, 280, 77-81.
- Clements, K., Glautier, S., Stolerman, I. P., White, J-A. W. & Taylor C. (1996). Classical conditioning in humans: Nicotine as CS and alcohol as US. *Human Psychopharmacology*, 11, 85-95.
- Cnattingius, S., Signorello, L. B., Anneren, G., Clausson, B., Ekbom, A., Ljunger, E., Blot, W. J., McLaughlin, J. K. Petersson, G., Rane, A. & Granath F. (2000). Caffeine intake and the risk of first-trimester spontaneous abortion. *New England Journal of Medicine*, 343, 1839-1845.
- Cohen, D. H. & Randall, D. C. (1984). Classical conditioning of cardiovascular responses. *Annual Review of Physiology*, 46, 187-197.
- Cohen, L. G., DiBiasio, A., Lisco, S. J. & Hurford, W. E. (1997). Fluconazole serum concentrations and pharmacokinetics in an obese patient. *Pharmacotherapy*, 17, 1023-1026.
- Collier, H. O. (1980). Cellular site of opiate dependence. *Nature*, 283, 625-629.

Collins, R. J., Weeks, J. R., Cooper, M. M., Good, P. I. & Russell, R. R. (1984).

Prediction of abuse liability of drugs using IV self-administration by rats.

Psychopharmacology (Berl), 82, 6-13.

Conklin, C. A. (2006). Environments as cues to smoke: implications for human extinction-based research and treatment. *Experimental and Clinical*

Psychopharmacology, 14, 12-19.

Crombag, H. S., Badiani, A., Chan, J., Dell'Orco, J., Dineen, S. P. & Robinson, T. E.

(2001). The ability of environmental context to facilitate psychomotor sensitization to amphetamine can be dissociated from its effect on acute drug responsiveness and on conditioned responding. *Neuropsychopharmacology*, 24, 680-690.

Crowell, C. R. & Anderson, D. C. (1972). Variations in intensity, interstimulus interval, and interval between preconditioning CS exposures and conditioning with rats. *Journal of Comparative and Physiological Psychology*, 79, 291-298.

Dafters, R. & Anderson, G. (1982). Conditioned tolerance to the tachycardia effect of ethanol in humans. *Psychopharmacology (Berl)*, 78, 365-367.

Dafters, R. & Bach, L. (1985). Absence of environment-specificity in morphine tolerance acquired in non-distinctive environments: habituation or stimulus overshadowing? *Psychopharmacology (Berl)*, 87, 101-106.

Daly, J. W. & Fredholm, B. B. (1998). Caffeine-an atypical drug of dependence. *Drug and Alcohol Dependence*, 51, 199-206.

Damianopoulos, E. N. & Carey, R. J. (1992). Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. *Behavioural Brain Research*, 49, 149-157.

Damianopoulos, E. N. & Carey, R. J. (1994). A new method to assess Pavlovian conditioning of psychostimulant drug effects. *Journal of Neuroscience Methods*, 53, 7-17.

Davey, G. C. L. (1987). An integration of human and animal models of Pavlovian conditioning: associations, cognitions and attributions. In G. C. L. Davey (Ed.), *Cognitive Processes and Pavlovian Conditioning in Humans* (pp 83-114). Chichester, England: Wiley & Sons.

Dawson, M. E. & Biferno, M. A. (1973). Concurrent measurement of awareness and electrodermal classical conditioning. *Journal of Experimental Psychology*, 101, 55-62.

Dawson, M. E. & Schell, A. M. (1985). Information processing and human autonomic classical conditioning. In P. K. Ackles, J. R. Jennings & M. G. Coles (Eds) *Advances in Psychophysiology* (Volume 1, pages 89-165). Greenwich, CT: JAI Press

Delamater, A. R. (2004). Experimental extinction in Pavlovian conditioning: behavioural and neuroscience perspectives. *Quarterly Journal of Experimental Psychology B*, 57, 97-132.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neuroscience*, 13, 281-285.

- Deneau, G., Yanagita, T. & Seevers, M. H. (1969). Self-administration of psychoactive substances by the monkey. *Psychopharmacologia.*, 16, 30-48.
- Devriese, S., Winters, W., Stegen, K., van, Diest, I., Veulemans, H., Nemery, B., Eelen, P., van de Woestijne K. & van den Bergh, O. (2000). Generalization of acquired somatic symptoms in response to odors: a pavlovian perspective on multiple chemical sensitivity. *Psychosomatic Medicine*, 62, 751-759.
- Devriese, S., Winters, W., van, Diest, I. & van den Bergh O. (2004). Contingency awareness in a symptom learning paradigm: necessary but not sufficient? *Consciousness and Cognition*, 13, 439-452.
- Dews, P. B., O'Brien, C. P. & Bergman, J. (2002). Caffeine: behavioral effects of withdrawal and related issues. *Food and Chemical Toxicology.*, 40, 1257-1261.
- di Giusto, E. L. & Bond, N. (1977). Enhancement of pseudoconditioning and retardation of escape by low doses of ethanol. *Pharmacology Biochemistry and Behaviour*, 6, 175-177.
- Dinnerstein, A. J., Lowenthal, M. & Blitz, B. (1966). The interaction of drugs with placebos in the control of pain and anxiety. *Perspectives in Biological Medicine*, 10, 103-117.
- Domjan, M. & Siegel, S. (1971). Conditioned suppression following CS pre-exposure. *Psychonomic Science*, 25, 12.
- Domjan, M. (2005). Pavlovian conditioning: a functional perspective. *Annual Review of Psychology*, 56, 179-206.

- Domjan, M. & Burkhard, B. (1986). *The principles of learning and behavior*. California, USA: Brooks & Cole.
- Drew, K. L. & Glick, S. D. (1988). Environment-dependent sensitization to amphetamine-induced circling behavior. *Pharmacology Biochemistry and Behaviour*, *31*, 705-708.
- Drummond, D. C. (2000). What does cue-reactivity have to offer clinical research? *Addiction*, *95 Suppl 2*, S129-S144.
- Duncan, P. M., Alici, T. & Woodward, J. D. (2000). Conditioned compensatory response to ethanol as indicated by locomotor activity in rats. *Behavioral Pharmacology*, *11*, 395-402.
- Dunwiddie, T. V. & Masino, S. A. (2001). The role and regulation of adenosine in the central nervous system. *Annual Review of Neuroscience*, *24*, 31-55.
- Durkovic, R. G. & Damianopoulos, E. N. (1986). Forward and backward classical conditioning of the flexion reflex in the spinal cat. *Journal of Neuroscience*, *6*, 2921-2925.
- Durlach, P. J. (1998). The effects of a low dose of caffeine on cognitive performance. *Psychopharmacology (Berl)*, *140*, 116-119.
- Ehrman, R., Ternes, J., O'Brien, C. P. & McLellan, A. T. (1992). Conditioned tolerance in human opiate addicts. *Psychopharmacology (Berl)*, *108*, 218-224.

Ehrman, R. N., Robbins, S. J., Childress, A. R. & O'Brien, C. P. (1992). Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology (Berl)*, 107, 523-529.

Eikelboom, R. & Stewart, J. (1981). Temporal and environmental cues in conditioned hypothermia and hyperthermia associated with morphine. *Psychopharmacology*, 72, 147-153

Eikelboom, R. & Stewart, J. (1982). Conditioning of drug-induced physiological responses. *Psychological Review*, 89, 507-528.

Epstein, L. H., Caggiula, A. R. & Stiller, R. (1989). Environment-specific tolerance to nicotine. *Psychopharmacology (Berl)*, 97, 235-237.

Esposito, R. & Kornetsky, C. (1977). Morphine lowering of self-stimulation thresholds: lack of tolerance with long-term administration. *Science*, 195, 189-191.

Evans, S. M. & Griffiths, R. R. (1992). Caffeine tolerance and choice in humans. *Psychopharmacology (Berl)*, 108, 51-59.

Evans, S. M., Critchfield, T. S. & Griffiths, R. R. (1994). Caffeine reinforcement demonstrated in a majority of moderate caffeine users. *Behavioral Pharmacology*, 5, 231-238.

Eysenck, H. J. & Rachman, S. (1965). *The causes and cures of neurosis*. London: Routledge & Kegan Paul.

Fanselow, M. S. (2007). Factors governing one-trial contextual conditioning. *Animal Learning and Behavior*, 18, 264-270.

Farag, N. H., Vincent, A. S., Sung, B. H., Whitsett, T. L., Wilson, M. F. & Lovallo, W. R. (2005)a. Caffeine tolerance is incomplete: persistent blood pressure responses in the ambulatory setting. *American Journal of Hypertension.*, 18, 714-719.

Farag, N. H., Vincent, A. S., McKey, B. S., Whitsett, T. L. & Lovallo, W. R. (2005)b. Hemodynamic mechanisms underlying the incomplete tolerance to caffeine's pressor effects. *American Journal of Cardiology.*, 95, 1389-1392.

Feinstein, A. R., Heinemann, L. A., Dalessio, D., Fox, J. M., Goldstein, J., Haag, G., Ladewig, D. & O'Brien, C. P. (2000). Do caffeine-containing analgesics promote dependence? A review and evaluation. *Clinical Pharmacology and Therapeutics*, 68, 457-467.

Feldman, J. M. (1975). Blocking as a function of added cue intensity. *Animal Learning and Behavior*, 3, 98-102.

Fenu, S., Cauli, O. & Morelli, M. (2000). Cross-sensitization between the motor activating effects of bromocriptine and caffeine: role of adenosine A(2A) receptors. *Behavioural Brain Research*, 114, 97-105.

Ferre, S., Fuxe, K., von, Euler G., Johansson, B. & Fredholm, B. B. (1992). Adenosine-dopamine interactions in the brain. *Neuroscience*, 51, 501-512.

Field, A. P. (2000). Evaluative conditioning is Pavlovian conditioning: issues of definition, measurement, and the theoretical importance of contingency awareness. *Consciousness and Cognition*, 9, 41-49.

- Field, M. & Duka, T. (2001). Smoking expectancy mediates the conditioned responses to arbitrary smoking cues. *Behavioral Pharmacology*, 12, 183-194.
- Field, M. & Duka, T. (2002). Cues paired with a low dose of alcohol acquire conditioned incentive properties in social drinkers. *Psychopharmacology (Berl)*, 159, 325-334.
- File, S. E. (1977). Effects of parachlorophenylalanine and amphetamine on habituation of exploration. *Pharmacology Biochemistry and Behaviour*, 6, 151-156.
- Fillmore, M. & Vogel-Sprott, M. (1992). Expected effect of caffeine on motor performance predicts the type of response to placebo. *Psychopharmacology (Berl)*, 106, 209-214.
- Finkbiner, R. G. & Woodruff-Pak, D. S. (1991). Classical eyeblink conditioning in adulthood: effects of age and interstimulus interval on acquisition in the trace paradigm. *Psychology and Aging*, 6, 109-117.
- Finn, I. B. & Holtzman, S. G. (1986). Tolerance to caffeine-induced stimulation of locomotor activity in rats. *Journal of Pharmacology and Experimental Therapeutics*, 238, 542-546.
- Finn, I. B. & Holtzman, S. G. (1987). Pharmacologic specificity of tolerance to caffeine-induced stimulation of locomotor activity. *Psychopharmacology (Berl)*, 93, 428-434.
- Fisone, G., Borgkvist, A. & Usiello, A. (2004). Caffeine as a psychomotor stimulant: mechanism of action. *Cellular and Molecular Life Sciences*, 61, 857-872.

Flaten, M. A. (1998). Caffeine-induced arousal modulates somatomotor and autonomic differential classical conditioning in humans. *Psychopharmacology (Berl)*, 135, 82-92.

Flaten, M. A. & Blumenthal, T. D. (1999). Caffeine-associated stimuli elicit conditioned responses: an experimental model of the placebo effect. *Psychopharmacology (Berl)*, 145, 105-112.

Flaten, M. A., Aasli, O. & Blumenthal, T. D. (2003). Expectations and placebo responses to caffeine-associated stimuli. *Psychopharmacology (Berl)*, 169, 198-204.

Flaten, M. A., Simonsen, T., Waterloo, K. & Olsen, H. (1997). Pharmacological classical conditioning in humans. *Human Psychopharmacology*, 12, 369-377

Foltin, R. W. & Haney, M. (2000). Conditioned effects of environmental stimuli paired with smoked cocaine in humans. *Psychopharmacology (Berl)*, 149, 24-33.

Franken, I. H. (2003). Drug craving and addiction: integrating psychological and neuropsychopharmacological approaches. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 27, 563-579.

Fredholm, B. B. (1982). Adenosine receptors. *Medical Biology*, 60, 289-293.

Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A. & Zwartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Review*, 51, 83-133.

Freund, H. J. & Hefter, H. (1993). The role of basal ganglia in rhythmic movement. *Advances in Neurology*, 60, 88-92.

- Frewer, L. J. & Lader, M. (1991). The effects of caffeine on two computerised tests of attention and vigilance. *Human Psychopharmacology* 6, 119-128.
- Frey, P. W. & Ross, L. E. (1968). Classical conditioning of the rabbit eyelid response as a function of interstimulus interval. *Journal of Comparative and Physiological Psychology*, 65, 246-250.
- Gallistel, C. R. (1990). *The organization of learning*. MA: MIT Press.
- Garattini, S. (1993). *Caffeine, coffee and health*. New York: Raven Press.
- Garcia, J., Ervin, F. R. & Koelling, R. A. (1966). Learning with prolonged delay of reinforcement. *Psychonomic Science*, 5, 121-122.
- Garrett, B. E. & Holtzman, S. G. (1994). D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. *Pharmacology Biochemistry and Behaviour*, 47, 89-94.
- Garrud, P., Rawlins, J. N., Mackintosh, N. J., Goodall, G., Cotton, M. M. & Feldon, J. (1984). Successful overshadowing and blocking in hippocampectomized rats. *Behavioural Brain Research*, 12, 39-53.
- Gawin, F. H. & Kleber, H. D. (1988). Evolving conceptualizations of cocaine dependence. *Yale Journal of Biological Medicine*, 61, 123-136.
- Giannaris, E. L., Cleland, T. A. & Linster, C. (2002). Intramodal blocking between olfactory stimuli in rats. *Physiology & Behavior*, 75, 717-722.

Gilliland, K. & Bullock, W. (1984). *Caffeine: a potential drug of abuse*. Haworth, New York.

Ginane, C. & Dumont, B. (2006). Generalization of conditioned food aversions in grazing sheep and its implications for food categorization. *Behavioural Processes*, 73, 178-186.

Glautier, S., Drummond, C. & Remington, B. (1994). Alcohol as an unconditioned stimulus in human classical conditioning. *Psychopharmacology (Berl)*, 116, 360-368.

Glautier, S. & Drummond, D. C. (1994). Alcohol dependence and cue reactivity. *Journal of the Studies of Alcohol*, 55, 224-229.

Godoy, A. M. & Delius, J. D. (1999). Sensitization to apomorphine in pigeons is due to conditioning, subject to generalization but resistant to extinction. *Behavioral Pharmacology*, 10, 367-378.

Gold, L. H., Swerdlow, N. R. & Koob, G. F. (1988). The role of mesolimbic dopamine in conditioned locomotion produced by amphetamine. *Behavioral Neuroscience*, 102, 544-552.

Gonzalez, S., Cebeira, M. & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology Biochemistry and Behaviour*, 81, 300-318.

Goodison, T. & Siegel, S. (1995). Tolerance to naloxone-induced suppression of intake: learning and cross-tolerance to cholecystokinin in rats. *Behavioral Neuroscience*, 109, 455-465.

- Gormezano, I., Kehoe, E. & Marshall B., S. (1983). Twenty years of classical conditioning research with the rabbit. In J. M. Prague & A. N. Epstein (Eds.), *Progress in Psychobiology and Physiological Psychology*. New York: Academic Press.
- Grant, D. M., Campbell, M. E., Tang, B. K. & Kalow, W. (1987). Biotransformation of caffeine by microsomes from human liver. Kinetics and inhibition studies. *Biochemical Pharmacology*, 36, 1251-1260.
- Graves, C. A. & Solomon, P. R. (1985). Age-related disruption of trace but not delay classical conditioning of the rabbit's nictitating membrane response. *Behavioral Neuroscience*, 99, 88-96.
- Graybiel, A. M. (1990). The basal ganglia and the initiation of movement. *Revue Neurologie (Paris)*, 146, 570-574.
- Greden, J. F. (1974). Anxiety or caffeinism: a diagnostic dilemma. *Americal Journal of Psychiatry*, 131, 1089-1092.
- Green, L. & Schweitzer, L. (1980). Second-order conditioning of the pigeon's key-peck using an autoshaping procedure. *American Journal of Psychology*, 93, 25-39.
- Griffiths, R. R. & Woodson, P. P. (1988). Caffeine physical dependence: a review of human and laboratory animal studies. *Psychopharmacology (Berl)*, 94, 437-451.
- Griffiths, R. R., Bigelow, G. E. & Liebson, I. A. (1989). Reinforcing effects of caffeine in coffee and capsules. *Journal of the Experimental Analysis of Behavior*, 52, 127-140.

Griffiths, R. R., Evans, S. M., Heishman, S. J., Preston, K. L., Sannerud, C. A., Wolf, B. & Woodson, P. P. (1990). Low-dose caffeine physical dependence in humans.

Journal of Pharmacology and Experimental Therapeutics, 255, 1123-1132.

Gupta, U. (1991). Differential effects of caffeine on free recall after semantic and rhyming tasks in high and low impulsives. *Psychopharmacology (Berl)*, 105, 137-140.

Gupta, U. (1993). Effects of caffeine on recognition. *Pharmacology Biochemistry and Behaviour*, 44, 393-396.

Gutman, N. & Kalish, H. I. (1956). Discriminability and stimulus generalisation.

Journal of Experimental Psychology, 51, 79-88.

Hakan, R. L. & Ksir, C. J. (1988). Nicotine induced locomotor activity in rats: the role of Pavlovian conditioning. *Pharmacology Biochemistry and Behaviour*, 29, 661-665.

Hall, G. & Symonds, M. (2006). Overshadowing and latent inhibition of context aversion conditioning in the rat. *Autonomic Neuroscience*, 129, 42-49.

Hameleers, P. A., Van Boxtel, M. P., Hogervorst, E., Riedel, W. J., Houx, P. J., Buntinx, F. & Jolles, J. (2000). Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. *Human Psychopharmacology*, 15, 573-581.

Hartnoll, R. (1991). Epidemiological approaches to drug misuse in Britain. *Journal of Addictive Diseases*, 11, 33-45.

Hasenfratz, M. & Battig, K. (1994). Acute dose-effect relationships of caffeine and mental performance, EEG, cardiovascular and subjective parameters.

Psychopharmacology (Berl), 114, 281-287.

Haskell, C. F., Kennedy, D. O., Wesnes, K. A. & Scholey, A. B. (2005). Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. *Psychopharmacology (Berl)*, 179, 813-825.

Hayashi, T., Ohashi, K. & Tadokoro, S. (1980). Conditioned drug effects to d-amphetamine- and morphine-induced motor acceleration in mice: experimental approach for placebo effect. *Japanese Journal of Pharmacology*, 30, 93-100.

Hayes, R. L. & Mayer, D. J. (1978). Morphine tolerance: is there evidence for a conditioning model? *Science*, 200, 343-345.

Heatherley, S. V., Hayward, R. C., Seers, H. E. & Rogers, P. J. (2005). Cognitive and psychomotor performance, mood, and pressor effects of caffeine after 4, 6 and 8 h caffeine abstinence. *Psychopharmacology (Berl)*, 178, 461-470.

Hernandez-Avila, M., Colditz, G. A., Stampfer, M. J., Rosner, B., Speizer, F. E. & Willett, W. C. (1991). Caffeine, Moderate Alcohol Intake, and Risk of Fractures of the Hip and Forearm in Middle-Aged Women. *American Journal of Clinical Nutrition*, 54, 157-163.

Herz, R. S. (1999). Caffeine effects on mood and memory. *Behaviour Research and Therapy*, 37, 869-879.

- Hicks, R. A., Hicks, G. J., Reyes, J. R. & Cheers, Y. (1983). Daily caffeine use and the sleep of college students. *Bulletin of the Psychonomic Society*, 21, 24-25.
- Hinson, R. E., Poulos, C. X., Thomas, W. & Cappell, H. (1986). Pavlovian conditioning and addictive behavior: relapse to oral self-administration of morphine. *Behavioral Neuroscience*, 100, 368-375.
- Hofer, I. & Battig, K. (1994). Cardiovascular, behavioral, and subjective effects of caffeine under field conditions. *Pharmacology Biochemistry and Behaviour*, 48, 899-908.
- Hogarth, L. & Duka, T. (2006). Human nicotine conditioning requires explicit contingency knowledge: is addictive behaviour cognitively mediated? *Psychopharmacology (Berl)*, 184, 553-566.
- Hogarth, L., Dickinson, A., Hutton, S. B., Bamborough, H. & Duka, T. (2006). Contingency knowledge is necessary for learned motivated behaviour in humans: relevance for addictive behaviour. *Addiction*, 101, 1153-1166.
- Hogervorst, E., Riedel, W. J., Schmitt, J. A. J. & Jolles, J. (1998). Caffeine improves memory performance during distraction in middle-aged, but not in young or old subjects. *Human Psychopharmacology-Clinical and Experimental*, 13, 277-284.
- Holland, P. C. & Rescorla, R. A. (1975). Second-order conditioning with food unconditioned stimulus. *Journal of Comparative and Physiological Psychology*, 88, 459-467.

Holland, P. C. (1992). Occasion setting in Pavlovian conditioning. In G. Bower (Ed.), *The psychology of learning and motivation* (pp. 69-125). Orlando, FL: Academic Press.

Hughes, J. R., Oliveto, A. H., Helzer, J. E., Higgins, S. T. & Bickel, W. K. (1992). Should caffeine abuse, dependence, or withdrawal be added to DSM-IV and ICD-10? *American Journal of Psychiatry*, 149, 33-40.

Hughes, J. R., Oliveto, A. H., Bickel, W. K., Higgins, S. T. & Badger, G. J. (1993). Caffeine self-administration and withdrawal: incidence, individual differences and interrelationships. *Drug and Alcohol Dependence*, 32, 239-246.

Hull, C. L. (1943). *Principles of behavior*. New York: Appleton-Century Crofts.

Humphreys, M. S. & Revelle, W. (1984). Personality, motivation, and performance: a theory of the relationship between individual differences and information processing. *Psychological Review*, 91, 153-184.

Hunt, D. P. & Daily, R. F. (1974). Differential GSR conditioning accompanied by a two-light prediction task. *Journal of General Psychology*, 90, 165-177.

Izquierdo, I. (1974). Effects of pseudoconditioning of drugs with known central nervous activity. *Psychopharmacologia*, 38, 259-266.

Jacobson, B. H. & Edgley, B. M. (1987). Effects of caffeine on simple reaction time and movement time. *Aviation, Space and Environmental Medicine*, 58, 1153-1156.

Jacobson, B. H., Winter-Roberts, K. & Gemmell, H. A. (1991). Influence of caffeine on selected manual manipulation skills. *Perceptual Motor Skills*, 72, 1175-1181.

- Jacobson, B. H. & Thurman-Lacey, S. R. (1992). Effect of caffeine on motor performance by caffeine-naive and -familiar subjects. *Perceptual Motor Skills*, 74, 151-157.
- James, J. E. (1991). *Caffeine and Health*. London: Academic Press Limited, London.
- James, J. E. (1994). Does caffeine enhance or merely restore degraded psychomotor performance? *Neuropsychobiology*, 30, 124-125.
- James, J. E. (1997). Is habitual caffeine use a preventable cardiovascular risk factor? *Lancet*, 349, 279-281.
- James, J. E. (2005). Hot beverages? Some aren't so hot! *European Health Psychology Society Newsletter*, 6-7.
- James, J. E. & Rogers, P. J. (2005). Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation. *Psychopharmacology (Berl)*, 182, 1-8.
- Jarvis, M. F. & Williams, M. (1989). Direct autoradiographic localization of adenosine A2 receptors in the rat brain using the A2-selective agonist, [3H]CGS 21680. *European Journal of Pharmacology*, 168, 243-246.
- Jarvis, M. J. (1993). Does caffeine intake enhance absolute levels of cognitive performance? *Psychopharmacology (Berl)*, 110, 45-52.
- Jenkins, W. O. & Stanley, J. R. (1950). Partial reinforcement: a review and critique. *Psychological Bulletin*, 47(3), 193-234

- Johnson, L. C., Spinweber, C. L. & Gomez, S. A. (1990). Benzodiazepines and caffeine: effect on daytime sleepiness, performance, and mood. *Psychopharmacology (Berl)*, 101, 160-167.
- Jones, H. E., Herning, R. I., Cadet, J. L. & Griffiths, R. R. (2000). Caffeine withdrawal increases cerebral blood flow velocity and alters quantitative electroencephalography (EEG) activity. *Psychopharmacology (Berl)*, 147, 371-377.
- Jones, S. H., Gray, J. A. & Hemsley, D. R. (1990). The Kamin blocking effect, incidental learning and psychoticism. *British Journal of Psychology*, 81 (Pt. 1), 95-109.
- Juliano, L. M. & Griffiths, R. R. (2001). Is Caffeine a Drug of Dependence? *Psychiatric Times*, 18, 1-7.
- Juliano, L. M. & Griffiths, R. R. (2004). A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology (Berl)*, 176, 1-29.
- Kamimori, G. H., Penetar, D. M., Headley, D. B., Thorne, D. R., Otterstetter, R. & Belenky, G. (2000). Effect of three caffeine doses on plasma catecholamines and alertness during prolonged wakefulness. *European Journal of Clinical Pharmacology*, 56, 537-544.
- Kamin, L. J. (1969). Predictability, surprise, attention, and conditioning. In B.A.Campbell & R. M. Church (Eds.), *Punishment and Aversive Behavior* (New York: Appleton-Century-Crofts.

Kaplan, G. B., Greenblatt, D. J., Ehrenberg, B. L., Goddard, J. E., Cotreau, M. M., Harmatz, J. S. & Shader, R. I. (1997). Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *Journal of Clinical Pharmacology*, 37, 693-703.

Karcz-Kubicha, M., Antoniou, K., Terasmaa, A., Quarta, D., Solinas, M., Justinova, Z., Pezola, A., Reggio, R., Muller, C. E., Fuxe, K., Goldberg, S. R., Popoli, P. & Ferre, F. (2003). Involvement of adenosine A1 and A2A receptors in the motor effects of caffeine after its acute and chronic administration. *Neuropsychopharmacology*, 28, 1281-1291.

Kearns, D. N., Weiss, S. J., Schindler, C. W. & Panlilio, L. V. (2005). Conditioned inhibition of cocaine seeking in rats. *Journal of Experimental Psychology: Animal Behavior Processes*, 31, 247-253.

Kehoe, E. J. (1982). Overshadowing and summation in compound stimulus conditioning of the rabbit's nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes*, 8, 313-328.

Kelemen, W. L. & Creeley, C. E. (2001). Caffeine (4 mg/kg) influences sustained attention and delayed free recall but not memory predictions. *Human Psychopharmacology*, 16, 309-319.

Kenemans, J. L. & Lorist, M. M. (1995). Caffeine and selective visual processing. *Pharmacology Biochemistry and Behaviour*, 52, 461-471.

Kenemans, J. L., Wieleman, J. S., Zeegers, M. & Verbaten, M. N. (1999). Caffeine and stroop interference. *Pharmacology Biochemistry and Behaviour*, 63, 589-598.

- Kerr, J. S., Sherwood, N. & Hindmarch, I. (1991). Separate and combined effects of the social drugs on psychomotor performance. *Psychopharmacology (Berl)*, 104, 113-119.
- Kesner, R. P. & Cook, D. G. (1983). Role of habituation and classical conditioning in the development of morphine tolerance. *Behavioral Neuroscience*, 97, 4-12.
- Kiernan, M. J. & Westbrook, R. F. (1993). Effects of exposure to a to-be-shocked environment upon the rat's freezing response: evidence for facilitation, latent inhibition, and perceptual learning. *Quarterly Journal of Experimental Psychology B*, 46, 271-288.
- Kim, J. A., Siegel, S. & Patenall, V. R. (1999). Drug-onset cues as signals: intraadministration associations and tolerance. *Journal of Experimental Psychology: Animal Behavior Processes*, 25, 491-504.
- Kimmel, H. D. & Bevill, M. J. (1996). Blocking and unconditioned response diminution in human classical autonomic conditioning. *Integrative Physiological and Behavioral Science: The Official Journal of the Pavlovian Society*, 31, 18-43.
- Kintsch, W. & Witte, R. S. (1962). Concurrent conditioning of bar press and salivation responses. *Journal of Comparative and Physiological Psychology*, 55, 963-968.
- Kirsch, I., Lynn, S. J., Vigorito, M. & Miller, R. R. (2004). The role of cognition in classical and operant conditioning. *Journal of Clinical Psychology*, 60, 369-392.

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: Chicago University Press.

Kosowski, A. R. & Liljequist, S. (2005). Behavioural sensitization to nicotine precedes the onset of nicotine-conditioned locomotor stimulation. *Behavioural Brain Research*, 156, 11-17.

Kourtidou-Papadeli, C., Papadelis, C., Louizos, A. L. & Guiba-Tziampiri, O. (2002). Maximum cognitive performance and physiological time trend measurements after caffeine intake. *Brain Research: Cognitive Brain Research*, 13, 407-415.

Kraemer, P. J. & Roberts, W. A. (1984). The influence of flavor preexposure and test interval on conditioned taste aversions in rats. *Learning and Motivation*, 15, 259-278.

Kuznicki, J. T. & Turner, L. S. (1986). The effects of caffeine on caffeine users and non-users. *Physiology & Behavior*, 37, 397-408.

Lader, M., Cardwell, C., Shine, P. & Scott, N. (1996). Caffeine withdrawal symptoms and rate of metabolism. *Journal of Psychopharmacology*, 10, 110-118.

Lambert, J. V. & Whitehouse, W. G. (2002). Conditioned inhibition of cyclophosphamide-induced taste aversion. *Journal of General Psychology*, 129, 68-75.

Lane, J. D. & Manus, D. C. (1989). Persistent cardiovascular effects with repeated caffeine administration. *Psychosomatic Medicine*, 51, 373-380.

- Lane, J. D. (1997). Effects of brief caffeinated-beverage deprivation on mood, symptoms, and psychomotor performance. *Pharmacology Biochemistry and Behaviour*, 58, 203-208.
- Lane, J. D. & Phillips-Bute, B. G. (1998). Caffeine deprivation affects vigilance performance and mood. *Physiology & Behavior*, 65, 171-175.
- Lane, J. D., Pieper, C. F., Phillips-Bute, B. G., Bryant, J. E. & Kuhn, C. M. (2002). Caffeine affects cardiovascular and neuroendocrine activation at work and home. *Psychosomatic Medicine*, 64, 595-603.
- Lazev, A. B., Herzog, T. A. & Brandon, T. H. (1999). Classical conditions of environmental cues to cigarette smoking. *Experimental and Clinical Psychopharmacology*, 7, 56-63.
- Le, A. D., Poulos, C. X. & Cappell, H. (1979). Conditioned tolerance to the hypothermic effect of ethyl alcohol. *Science*, 206, 1109-1110.
- Lieberman, H. R., Wurtman, R. J., Emde, G. G., Roberts, C. & Coviella, I. L. (1987). The effects of low doses of caffeine on human performance and mood. *Psychopharmacology (Berl)*, 92, 308-312.
- Lieberman, H. R., Tharion, W. J., Shukitt-Hale, B., Speckman, K. L. & Tulley, R. (2002). Effects of caffeine, sleep loss, and stress on cognitive performance and mood during U.S. Navy SEAL training. Sea-Air-Land. *Psychopharmacology (Berl)*, 164, 250-261.

- Lieberman, H. R. (2003). Nutrition, brain function and cognitive performance. *Appetite*, 40, 245-254.
- Linster, C. & Smith, B. H. (1999). Generalization between binary odor mixtures and their components in the rat. *Physiology & Behavior*, 66, 701-707.
- Loke, W. H., Hinrichs, J. V. & Ghoneim, M. M. (1985). Caffeine and diazepam: separate and combined effects on mood, memory, and psychomotor performance. *Psychopharmacology (Berl)*, 87, 344-350.
- Lorist, M. M. & Tops, M. (2003). Caffeine, fatigue, and cognition. *Brain and Cognition*, 53, 82-94.
- Lotshaw, S. C., Bradley, J. R. & Brooks, L. R. (1996). Illustrating caffeine's pharmacological and expectancy effects utilizing a balanced placebo design. *Journal of Drug Education*, 26, 13-24.
- Lovibond, P. F., Preston, G. C. & Mackintosh, N. J. (1984). Context specificity of conditioning, extinction, and latent inhibition. *Journal of Experimental Psychology: Animal Behavior Processes*, 10, 360-375.
- Lovibond, P. F. & Shanks, D. R. (2002). The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications. *Journal of Experimental Psychology: Animal Behavior Processes*, 28, 3-26.
- Lubow, R. E. & Moore, A. U. (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology*, 52, 415-419.

Lubow, R. E., Schnur, P. & Rifkin, B. (1976). Latent inhibition and conditioned attention. *Journal of Experimental Psychology: Animal Behavior Processes*, 2, 163-174.

Macfarlane, S. J. & White, J. M. (1989). Acquisition and extinction of an alcohol-opposite conditioned response in humans. *Psychopharmacology (Berl)*, 97, 355-357.

Mackintosh, N. J. (1975). Blocking of conditioned suppression: role of the first compound trial. *Journal of Experimental Psychology: Animal Behavior Processes*, 1, 335-345.

Mackintosh, N. J. (1983). *Conditioning and associative learning*. Oxford University Press.

MacRae, J. R., Scoles, M. T. & Siegel, S. (1987). The contribution of Pavlovian conditioning to drug tolerance and dependence. *British Journal of Addiction*, 82, 371-380.

Maes, J. H. & Vossen, J. M. (2000). Training history affects magnitude of spontaneous recovery from extinction of appetitive conditioned responding. *Behavioural Processes*, 50, 43-57.

Magkos, F. & Kavouras, S. A. (2005). Caffeine use in sports, pharmacokinetics in man, and cellular mechanisms of action. *Critical Reviews in Food Science and Nutrition*, 45, 535-562.

Mahler, S. V. & de Wit, H. (2005). Effects of haloperidol on reactions to smoking cues in humans. *Behavioral Pharmacology*, 16, 123-126.

- Mandel, I. J. & Bridger, W. H. (1973). Cross-modality transfer of differential galvanic skin response conditioning to word stimuli. *Journal of Experimental Psychology*, 99, 157-161.
- Mansfield, J. G. & Cunningham, C. L. (1980). Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. *Journal of Comparative and Physiological Psychology*, 94, 962-969.
- Maren, S. & Holt, W. (2000). The hippocampus and contextual memory retrieval in Pavlovian conditioning. *Behavioural Brain Research*, 110, 97-108.
- Marlin, N. A. (1981). Contextual associations in trace conditioning. *Animal Learning and Behavior*, 9, 519-523.
- Martin-Iverson, M. T. & McManus, D. J. (1990). Stimulant-conditioned locomotion is not affected by blockade of D1 and/or D2 dopamine receptors during conditioning. *Brain Research*, 521, 175-184.
- Martin-Iverson, M. T. & Fawcett, S. L. (1996). Pavlovian conditioning of psychomotor stimulant-induced behaviours: has convenience led us astray? *Behavioral Pharmacology*, 7, 24-41.
- Martin, I. & Levey, A. B. (1988). Classical conditioning in a cognitive era. *Biological Psychology*, 27, 153-166.
- Martin, I. & Levey, A. B. (1991). Blocking observed in human eyelid conditioning. *Integrative Physiological and Behavioral Science: The Official Journal of the Pavlovian Society*, 26, 127-131.

Martin, I. & Levey, A. B. (1978). Evaluative conditioning. *Advances in Behaviour Research and Therapy*, 1, 57-102

Martinez-Mir, M. I., Probst, A. & Palacios, J. M. (1991). Adenosine A2 receptors: selective localization in the human basal ganglia and alterations with disease. *Neuroscience*, 42, 697-706.

Mathew, R. J. & Wilson, W. H. (1985). Caffeine induced changes in cerebral circulation. *Stroke*, 16, 814-817.

Matteson, S., Roscoe, J., Hickok, J. & Morrow, G. R. (2002). The role of behavioral conditioning in the development of nausea. *American journal of Obstetrics and Gynecology*, 186, S239-S243.

McAuliffe, A. E. & Gordon, R. A. (1974). A test of Lindensmith's theory of addiction: the frequency of euphoria among long-term addicts. *American Journal of Sociology*, 79, 795-840.

Meachum, C. L. & Bernstein, I. L. (1990). Conditioned responses to a taste conditioned stimulus paired with lithium chloride administration. *Behavioral Neuroscience*, 104, 711-715.

Meachum, C. L. & Bernstein, I. L. (1992). Behavioral conditioned responses to contextual and odor stimuli paired with LiCl administration. *Physiology & Behavior*, 52, 895-899.

Meliska, C. J., Landrum, R. E. & Landrum, T. A. (1990). Tolerance and sensitization to chronic and subchronic oral caffeine: effects on wheelrunning in rats.

Pharmacology Biochemistry and Behaviour, 35, 477-479.

Mikalsen, A., Bertelsen, B. & Flaten, M. A. (2001). Effects of caffeine, caffeine-associated stimuli, and caffeine-related information on physiological and psychological arousal. *Psychopharmacology (Berl)*, 157, 373-380.

Miller, R. R. & Matute, H. (1996). Biological significance in forward and backward blocking: resolution of a discrepancy between animal conditioning and human causal judgment. *Journal of Experimental Psychology*, 125, 370-386.

Millin, P. M. & Riccio, D. C. (2002). Spontaneous recovery of tolerance to the analgesic effect of morphine. *Physiology & Behavior*, 75, 465-471.

Mitchell, S. H., de, W. H. & Zacny, J. P. (1995). Caffeine withdrawal symptoms and self-administration following caffeine deprivation. *Pharmacology Biochemistry and Behaviour*, 51, 941-945.

Miya, T., Goya, T., Fujii, H., Ohtsu, T., Itoh, K., Igarashi, T., Minami, H. & Sasaki, Y. (2001). Factors affecting the pharmacokinetics of CPT-11: the body mass index, age and sex are independent predictors of pharmacokinetic parameters of CPT-11. *Investigational New Drugs*, 19, 61-67.

Mobini, S., Elliman, T. D. & Yeomans, M. R. (2005). Changes in pleasantness of caffeine-associated flavours consumed at home. *Food Quality and Preference*, 16, 659-666.

- Monk, T. H. (2005). The post-lunch dip in performance. *Clinics in Sports Medicine*, 24, e15-23.
- Montgomery, G. H. & Kirsch, I. (1997). Classical conditioning and the placebo effect. *Pain*, 72, 107-113.
- Morgan, M. J. (1998). Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity. *Neuropsychopharmacology*, 19, 252-264.
- Mucha, R. F., Volkovskis, C. & Kalant, H. (1981). Conditioned increases in locomotor activity produced with morphine as an unconditioned stimulus, and the relation of conditioning to acute morphine effect and tolerance. *Journal of Comparative and Physiological Psychology*, 95, 351-362.
- Mucha, R. F., Pauli, P. & Angrilli, A. (1998). Conditioned responses elicited by experimentally produced cues for smoking. *Canadian Journal of Physiology and Pharmacology*, 76, 259-268.
- Mukhopadhyay, S. & Poddar, M. K. (1998). Is GABA involved in the development of caffeine tolerance? *Neurochemical Research*, 23, 63-68.
- Muller, D., Gerber, B., Hellstern, F., Hammer, M. & Menzel, R. (2000). Sensory preconditioning in honeybees. *Journal of Experimental Biology*, 203, 1351-1364.
- Muntaner, C., Cascella, N. G., Kumor, K. M., Nagoshi, C., Herning, R. & Jaffe, J. (1989). Placebo responses to cocaine administration in humans: effects of prior administrations and verbal instructions. *Psychopharmacology (Berl)*, 99, 282-286.

- Murdock, B. B., Jr. (1951). Forward and backward conditioning of the eyelid with a coordinated voluntary response. *American Journal of Psychology*, 64, 294-298.
- Nehlig, A., Daval, J. L., Boyet, S. & Vert, P. (1986). Comparative effects of acute and chronic administration of caffeine on local cerebral glucose utilization in the conscious rat. *European Journal of Pharmacology*, 129, 93-103.
- Nehlig, A. & Debry, G. (1994). Effects of coffee on the central nervous system. In G. Debry (Ed) *Coffee and Health* (pp. 157-249). Paris: John Libbey.
- Nehlig, A. (1999). Are we dependent upon coffee and caffeine? A review on human and animal data. *Neuroscience and Biobehavioral Reviews*, 23, 563-576.
- Neumann, D. L., Lipp, O. V. & Siddle, D. A. (1997). Conditioned inhibition of autonomic Pavlovian conditioning in humans. *Biological Psychology*, 46, 223-233.
- Newlin, D. B. (1986). Conditioned compensatory response to alcohol placebo in humans. *Psychopharmacology (Berl)*, 88, 247-251.
- Niaura, R. (2000). Cognitive social learning and related perspectives on drug craving. *Addiction*, 95 Supplement 2, S155-S163.
- Norman, C. & Cassaday, H. J. (2004). CER to discrete and contextual stimuli: effects of stimulus modality depend on strain of rat. *Physiology & Behavior*, 82, 611-619.
- O'Brien, C. P. (1976). Experimental analysis of conditioning factors in human narcotic addiction. *Pharmacological Reviews*, 27, 533-543.

- O'Brien, C. P., Childress, A. R., Ehrman, R. & Robbins, S. J. (1998). Conditioning factors in drug abuse: can they explain compulsion? *Journal of Psychopharmacology*, *12*, 15-22.
- Oei, A. & Hartley, L. R. (2005). The effects of caffeine and expectancy on attention and memory. *Human Psychopharmacology*, *20*, 193-202.
- Öhman A. & Soares, J. J. F. (1993). On the automatic nature of phobic fear: conditioned electrodermal responses to masked fear-relevant stimuli. *Journal of Abnormal Psychology*, *102*, 121-132.
- Ohmori, T., Abekawa, T., Ito, K. & Koyama, T. (2000). Context determines the type of sensitized behaviour: a brief review and a hypothesis on the role of environment in behavioural sensitization. *Behavioral Pharmacology*, *11*, 211-221.
- Oliveto, A. H., Bickel, W. K., Hughes, J. R., Shea, P. J., Higgins, S. T. & Fenwick, J. W. (1992). Caffeine drug discrimination in humans: acquisition, specificity and correlation with self-reports. *Journal of Pharmacology and Experimental Therapeutics*, *261*, 885-894.
- Ost, J. W. P. & Lauer, D. W. (1965). Some investigations of classical salivatory conditioning in the dog. In W.F. Prosaky (Ed.), *Classical Conditioning: A Symposium* (pp. 192-207). New York: Appleton-Century-Crofts.
- Pacheco-Lopez, G., Niemi, M. B., Kou, W., Harting, M., Del, R. A., Besedovsky, H. O., Schedlowski, M. (2004). Behavioural endocrine immune-conditioned response is induced by taste and superantigen pairing. *Neuroscience*, *129*, 555-562.

- Paletta, M. S. & Wagner, A. R. (1986). Development of context-specific tolerance to morphine: support for a dual-process interpretation. *Behavioral Neuroscience*, 100, 611-623.
- Parsons, W. D. & Neims, A. H. (1978). Effect of smoking on caffeine clearance. *Clinical Pharmacology and Therapeutics*, 24, 40-45.
- Passmore, A. P., Kondowe, G. B. & Johnston, G. D. (1987). Renal and cardiovascular effects of caffeine: a dose-response study. *Clinical Science (London)*, 72, 749-756.
- Patat, A., Rosenzweig, P., Enslen, M., Trocherie, S., Miget, N., Bozon, M. C., Allain, H. & Gandon, J. M. (2000). Effects of a new slow release formulation of caffeine on EEG, psychomotor and cognitive functions in sleep-deprived subjects. *Human Psychopharmacology*, 15, 153-170.
- Patkina, N. A. & Zvartau, E. E. (1998). Caffeine place conditioning in rats: comparison with cocaine and ethanol. *European Neuropsychopharmacology*, 8, 287-291.
- Patwardhan, R. V., Desmond, P. V., Johnson, R. F. & Schenker, S. (1980). Impaired elimination of caffeine by oral contraceptive steroids. *Journal of Laboratory and Clinical Medicine*, 95, 603-608.
- Pavlov, I. P. (1927). *Conditioned reflexes (translated by G.V. Anrep)*. Oxford: Oxford University Press.

Pearce, J. M. & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review*, 87, 532-552.

Penetar, D., McCann, U., Thorne, D., Kamimori, G., Galinski, C., Sing, H., Thomas, M. & Belenky, G. (1993). Caffeine reversal of sleep deprivation effects on alertness and mood. *Psychopharmacology (Berl)*, 112, 359-365.

Pickens, R. W. & Crowder, W. F. (1967). Effects of CS-US interval on conditioning of drug response, with assessment of speed of conditioning. *Psychopharmacologia*, 11, 88-94.

Post, R. M., Lockfeld, A., Squillace, K. M. & Contel, N. R. (1981). Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization. *Life Sciences*, 28, 755-760.

Poulos, C. X., Hinson, R. E. & Siegel, S. (1981). The role of Pavlovian processes in drug tolerance and dependence: implications for treatment. *Addictive Behaviors*, 6, 205-211.

Poulos, C. X. & Hinson, R. (1982). Pavlovian conditional tolerance to haloperidol catalepsy: evidence of dynamic adaptation in the dopaminergic system. *Science*, 218, 491-492.

Poulos, C. X. & Cappell, H. (1991). Homeostatic theory of drug tolerance: a general model of physiological adaptation. *Psychological Review*, 98, 390-408.

- Quadros, I. M., Souza-Formigoni, M. L., Fornari, R. V., Nobrega, J. N. & Oliveira, M. G. (2003). Is behavioral sensitization to ethanol associated with contextual conditioning in mice? *Behavioral Pharmacology*, 14, 129-136.
- Quinlan, P. T., Lane, J., Moore, K. L., Aspen, J., Rycroft, J. A. & O'Brien, D. C. (2000). The acute physiological and mood effects of tea and coffee: the role of caffeine level. *Pharmacology Biochemistry and Behaviour*, 66, 19-28.
- Rainnie, D. G., Grunze, H. C., McCarley, R. W. & Greene, R. W. (1994). Adenosine inhibition of mesopontine cholinergic neurons: implications for EEG arousal. *Science*, 263, 689-692.
- Rees, K., Allen, D. & Lader, M. (1999). The influences of age and caffeine on psychomotor and cognitive function. *Psychopharmacology (Berl)*, 145, 181-188.
- Reimer, A. R. & Martin-Iverson, M. T. (1994). Nimodipine and haloperidol attenuate behavioural sensitization to cocaine but only nimodipine blocks the establishment of conditioned locomotion induced by cocaine. *Psychopharmacology (Berl)*, 113, 404-410.
- Rescorla, R. A. (1966). Predictability and number of pairings in Pavlovian fear conditioning. *Science*, 4, 383-384.
- Rescorla, R. A. (1968). Probability of shock in the presence and absence of CS in fear conditioning. *Journal of Comparative and Physiological Psychology*, 66, 1-5.

Rescorla, R. A. & Heth, C. D. (1975). Reinstatement of fear to an extinguished conditioned stimulus. *Journal of Experimental Psychology: Animal Behavior Processes*, 1, 88-96.

Rescorla, R. A. (2004). Spontaneous recovery. *Learning and Memory*, 11, 501-509.

Rescorla, R. A. & Wagner, A. R. (2007). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A.H.Black & W. F. Prosaky (Eds.), *Classical conditioning II: Current research and theory* (New York: Appleton-Century-Crofts).

Revelle, W., Humphreys, M. S., Simon, L. & Gilliland, K. (1980). The interactive effect of personality, time of day, and caffeine: a test of the arousal model. *Journal of Experimental Psychology: General*, 109, 1-31.

Ribeiro, J. A., Sebastiao, A. M. & de Mendonca, A. (2003). Adenosine receptors in the nervous system: pathophysiological implications. *Progresses in Neurobiology*, 68, 377-392.

Richardson, N. J., Rogers, P. J., Elliman, N. A. & O'Dell, R. J. (1995). Mood and performance effects of caffeine in relation to acute and chronic caffeine deprivation. *Pharmacology Biochemistry and Behaviour*, 52, 313-320.

Riedel, W., Hogervorst, E., Leboux, R., Verhey, F., van, Praag. H. & Jolles, J. (1995). Caffeine attenuates scopolamine-induced memory impairment in humans. *Psychopharmacology (Berl)*, 122, 158-168.

Rivkees, S. A., Price, S. L. & Zhou, F. C. (1995). Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. *Brain Research*, 677, 193-203.

Rizzo, A. A., Stamps, L. E. & Fehr, L. A. (1988). Effects of caffeine withdrawal on motor performance and heart rate changes. *International Journal of Psychophysiology*, 6, 9-14.

Roache, J. D. & Griffiths, R. R. (1987). Interactions of diazepam and caffeine: behavioral and subjective dose effects in humans. *Pharmacology Biochemistry and Behaviour*, 26, 801-812.

Robbins, S. J. & Ehrman, R. N. (1992). Designing studies of drug conditioning in humans. *Psychopharmacology (Berl)*, 106, 143-153.

Robbins, T. W. (1984). Cortical noradrenaline, attention and arousal. *Psychological Medicine*, 14, 13-21.

Robbins, T. W., Semple, J., Kumar, R., Truman, M. I., Shorter, J., Ferraro, A., Fox, B., McKay, G. & Matthews K. (1997). Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. *Psychopharmacology (Berl)*, 134, 95-106.

Robelin, M. & Rogers, P. J. (1998). Mood and psychomotor performance effects of the first, but not of subsequent, cup-of-coffee equivalent doses of caffeine consumed after overnight caffeine abstinence. *Behavioral Pharmacology*, 9, 611-618.

Robinson, T. E. & Berridge, K. C. (2003). Addiction. *Annual Review of Psychology*, 54, 25-53.

Rogers, P. J., Richardson, N. J. & Elliman, N. A. (1995). Overnight caffeine abstinence and negative reinforcement of preference for caffeine-containing drinks. *Psychopharmacology (Berl)*, 120, 457-462.

Rogers, P. J., Richardson, N. J. & Deroncourt, C. (1995). Caffeine use: is there a net benefit for mood and psychomotor performance? *Neuropsychobiology*, 31, 195-199.

Rogers, P. J. & Deroncourt, C. (1998). Regular caffeine consumption: a balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacology Biochemistry and Behaviour*, 59, 1039-1045.

Rogers, P. J., Martin, J., Smith, C., Heatherley, S. V. & Smit, H. J. (2003). Absence of reinforcing, mood and psychomotor performance effects of caffeine in habitual non-consumers of caffeine. *Psychopharmacology (Berl)*, 167, 54-62.

Rohsenow, D. J., Niaura, R. S., Childress, A. R., Abrams, D. B. & Monti, P. M. (1990). Cue reactivity in addictive behaviors: theoretical and treatment implications. *International Journal of Addiction*, 25, 957-993.

Ross, L. E. & Nelson, M. E. (1973). The role of awareness in differential conditioning. *Psychophysiology*, 10, 91-94.

Rozin, P., Reff, D., Mark, M. & Schull, J. (1984). Conditioned Opponent Responses in Human Tolerance to Caffeine. *Bulletin of the Psychonomic Society*, 22, 117-120.

Ruijter, J., Lorist, M. M., Snel, J. & De Ruiter, M. B. (2000). The influence of caffeine on sustained attention: an ERP study. *Pharmacology Biochemistry and Behaviour*, 66, 29-37.

Rusted, J. (1994). Caffeine and cognitive performance: effects on mood or mental processing? *Pharmacopsychologia*, 7, 49-54.

Sandoz, J. C., Pham-Delegue, M. H., Renou, M. & Wadhams, L. J. (2001). Asymmetrical generalisation between pheromonal and floral odours in appetitive olfactory conditioning of the honey bee (*Apis mellifera* L.). *Journal of Comparative Physiology A*, 187, 559-568.

Sanjuan, M. C., Alonso, G. & Nelson, J. B. (2006). The contribution of latent inhibition to reduced generalization after pre-exposure to the test stimulus. *Behavioural Processes*, 71, 21-28.

Schachtman, T. R., Kaspro, W. J., Chee, M. A. & Miller, R. R. (1985). Blocking but not conditioned inhibition results when an added stimulus is reinforced in compound with multiple pretrained stimuli. *American Journal of Psychology*, 98, 283-295.

Schenk, S. & Partridge, B. (1997). Sensitization and tolerance in psychostimulant self-administration. *Pharmacology Biochemistry and Behaviour*, 57, 543-550.

Scholey, A. B. & Kennedy, D. O. (2004). Cognitive and physiological effects of an "energy drink": an evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions. *Psychopharmacology (Berl)*, 176, 320-330.

- Schuh, K. J. & Griffiths, R. R. (1997). Caffeine reinforcement: the role of withdrawal. *Psychopharmacology (Berl)*, 130, 320-326.
- Schuster, C. R. & Thompson, T. (1969). Self administration of and behavioral dependence on drugs. *Annual Review of Pharmacology*, 9, 483-502.
- Schwarz-Stevens, K. S. & Cunningham, C. L. (1993). Pavlovian conditioning of heart rate and body temperature with morphine: effects of CS duration. *Behavioral Neuroscience*, 107, 1039-1048.
- See, R. E. (2005). Neural substrates of cocaine-cue associations that trigger relapse. *European Journal of Pharmacology*, 526, 140-146.
- Shanks, D. R. (1990). On the cognitive theory of conditioning. *Biological Psychology*, 30, 171-179.
- Shapiro, A. P. & Nathan, P. E. (1986). Human tolerance to alcohol: the role of Pavlovian conditioning processes. *Psychopharmacology (Berl)*, 88, 90-95.
- Shapiro, N. R., Dudek, B. C. & Rosellini, R. A. (1983). The role of associative factors in tolerance to the hypothermic effects of morphine in mice. *Pharmacology Biochemistry and Behaviour*, 19, 327-333.
- Sideroff, S. I. & Jarvik, M. E. (1980). Conditioned responses to a videotape showing heroin-related stimuli. *International Journal of Addiction*, 15, 529-536.
- Siegel, S. (1975)a. Evidence from rats that morphine tolerance is a learned response. *Journal of Comparative and Physiological Psychology*, 89, 498-506.

- Siegel, S. (1975)b. Conditioning insulin effects. *Journal of Comparative and Physiological Psychology*, 89, 89-99.
- Siegel, S. (1976). Morphine analgesic tolerance: its situation specificity supports a Pavlovian conditioning model. *Science*, 193, 323-325.
- Siegel, S. (1978). Tolerance to the hyperthermic effect of morphine in the rat is a learned response. *Journal of Comparative and Physiological Psychology*, 92, 1137-1149.
- Siegel, S. (1999). Drug anticipation and drug addiction. The 1998 II. David Archibald Lecture. *Addiction*, 94, 1113-1124.
- Silverman, K., Evans, S. M., Strain, E. C. & Griffiths, R. R. (1992). Withdrawal syndrome after the double-blind cessation of caffeine consumption. *New England Journal of Medicine*, 327, 1109-1114.
- Smit, H. J. & Rogers, P. J. (2000). Effects of low doses of caffeine on cognitive performance, mood and thirst in low and higher caffeine consumers. *Psychopharmacology (Berl)*, 152, 167-173.
- Smit, H. J. & Rogers, P. J. (2002). Effects of caffeine on mood. *Pharmacopsychologia* 15, 231-257.
- Smit, H. J., Cotton, J. R., Hughes, S. C. & Rogers, P. J. (2004). Mood and cognitive performance effects of "energy" drink constituents: caffeine, glucose and carbonation. *Nutritional Neuroscience*, 7, 127-139.

Smith, A., Maben, A. & Brockman, P. (1994). Effects of evening meals and caffeine on cognitive performance, mood and cardiovascular functioning. *Appetite*, 22, 57-65.

Smith, A. (2002). Effects of caffeine on human behavior. *Food and Chemical Toxicology*, 40, 1243-1255.

Smith, A., Brice, C., Nash, J., Rich, N. & Nutt, D. J. (2003). Caffeine and central noradrenaline: effects on mood, cognitive performance, eye movements and cardiovascular function. *Journal of Psychopharmacology*, 17, 283-292.

Smith, A., Sutherland, D. & Christopher, G. (2005). Effects of repeated doses of caffeine on mood and performance of alert and fatigued volunteers. *Journal of Psychopharmacology*, 19, 620-626.

Smith, A. P., Rusted, J. M., Eaton-Williams, P., Savory, M. & Leathwood, P. (1990). Effects of caffeine given before and after lunch on sustained attention. *Neuropsychobiology*, 23, 160-163.

Smith, A. P., Kendrick, A. M. & Maben, A. L. (1992). Effects of breakfast and caffeine on performance and mood in the late morning and after lunch. *Neuropsychobiology*, 26, 198-204.

Smith, A. P., Whitney, H., Thomas, M., Perry, K. & Brockman P. (1997). Effects of caffeine and noise on mood, performance and cardiovascular functioning. *Human Psychopharmacology*, 12, 27-33.

Smith, B. D., Cranford, D. & Green, L. (2001). Hostility and caffeine: cardiovascular effects during stress and recovery. *Personality and Individual Differences*, 26[1], 1125-1137.

Smits, P., Boekema, P., de, Abreu. R., Thien, T. & van 't, Laar. A. (1987). Evidence for an antagonism between caffeine and adenosine in the human cardiovascular system. *Journal of Cardiovascular Pharmacology*, 10, 136-143.

Snyder, S. H., Katims, J. J., Annau, Z., Bruns, R. F. & Daly, J. W. (1981). Adenosine receptors and behavioral actions of methylxanthines. *Proceedings of the National Academy of Sciences of the United States of America*, 78, 3260-3264.

Soares, J. J. F. & Öhman A. (1993). Backward masking and skin conductance responses after conditioning to nonfeared but fear-relevant stimuli in fearful subjects. *Psychophysiology*, 30, 460-466.

Solinas, M., Ferre, S., You, Z. B., Karcz-Kubicha, M., Popoli, P. & Goldberg, S. R. (2002). Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *Journal of Neuroscience*, 22, 6321-6324.

Stahle, L. (1992). Do autoreceptors mediate dopamine agonist--induced yawning and suppression of exploration? A critical review. *Psychopharmacology (Berl)*, 106, 1-13.

Steinmetz, J. E., Tracy, J. A. & Green, J. T. (2001). Classical eyeblink conditioning: clinical models and applications. *Integrative Physiological and Behavioral Science: The Official Journal of the Pavlovian Society*, 36, 220-238.

- Stewart, J., de Wit, H. & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review*, 91, 251-268.
- Stockhorst, U., Wiener, J. A., Klosterhalfen, S., Klosterhalfen, W., Aul, C. & Steingruber, H. J. (1998). Effects of overshadowing on conditioned nausea in cancer patients: an experimental study. *Physiology & Behavior*, 64, 743-753.
- Stockhorst, U., Gritzmann, E., Klopp, K., Schottenfeld-Naor, Y., Hubinger, A., Berresheim, H. W., Steingruber, H. J. & Gries, F. A. (1999). Classical conditioning of insulin effects in healthy humans. *Psychosomatic Medicine*, 61, 424-435.
- Stockhorst, U., Steingruber, H. J. & Scherbaum, W. A. (2000). Classically conditioned responses following repeated insulin and glucose administration in humans. *Behavioural Brain Research*, 110, 143-159.
- Stockhorst, U., Steingrueber, H. J., Enck, P. & Klosterhalfen, S. (2006). Pavlovian conditioning of nausea and vomiting. *Autonomic Neuroscience*, 129, 50-57.
- Stolerman, I. P. (2007). Components of drug dependence: reinforcement, discrimination and adaptation. *Biochemical Society Symposium*, 59, 1-12.
- Strain, E. C. & Griffiths, R. R. (1995). Caffeine dependence: fact or fiction? *Journal of the Royal Society of Medicine*, 88, 437-440.
- Stewart, J. & Badiani, A. (1993). Tolerance and sensitization to the behavioral effects of drugs. *Behavioral Pharmacology*, 4, 289-312.

- Stewart, J., de Wit, H. & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review*, 91(2), 251-268
- Streufert, S., Pogash, R., Miller, J., Gingrich, D., Landis, R., Lonardi, L., Severs W & Roache, J. D. (1995). Effects of caffeine deprivation on complex human functioning. *Psychopharmacology (Berl)*, 118, 377-384.
- Strickland, T. L., Myers, H. F. & Lahey, B. B. (1989). Cardiovascular reactivity with caffeine and stress in black and white normotensive females. *Psychosomatic Medicine*, 51, 381-389.
- Sunsay, C., Stetson, L. & Bouton, M. E. (2004). Memory priming and trial spacing effects in Pavlovian learning. *Learning and Behavior*, 32, 220-229.
- Svenningsson, P., Nomikos, G. G. & Fredholm, B. B. (1999). The stimulatory action and the development of tolerance to caffeine is associated with alterations in gene expression in specific brain regions. *Journal of Neuroscience*, 19, 4011-4022.
- Tabachnick, B. G. & Fidell, L. S. (2001). *Using Multivariate Statistics (5th Ed.)*. Boston: Allyn & Bacon.
- Teasdale, J. D. (1973). Conditioned abstinence in narcotic addicts. *International Journal of Addiction*, 8, 273-292.
- Thompson, R. & Keene, K. (2005). The pros and cons of caffeine. *The Psychologist* 101, 698-701.
- Thorndike, E. L. (1911). *Animal Intelligence* . New York: Macmillan.

Tiffany, S. T. & Baker, T. B. (1981). Morphine tolerance in rats: congruence with a Pavlovian paradigm. *Journal of Comparative and Physiological Psychology*, 95, 747-762.

Tiffin, P., Ashton, H., Marsh, R. & Kamali, F. (1995). Pharmacokinetic and pharmacodynamic responses to caffeine in poor and normal sleepers. *Psychopharmacology (Berl)*, 121, 494-502.

Tilson, H. A. & Rech, R. H. (1973). Prior drug experience and effects of amphetamine on schedule controlled behavior. *Pharmacology Biochemistry and Behaviour*, 1, 129-132.

Tinley, E. M., Yeomans, M. R. & Durlach, P. J. (2003). Caffeine reinforces flavour preference in caffeine-dependent, but not long-term withdrawn, caffeine consumers. *Psychopharmacology (Berl)*, 166, 416-423.

Tirelli, E. & Terry, P. (1998). Amphetamine-induced conditioned activity and sensitization: the role of habituation to the test context and the involvement of Pavlovian processes. *Behavioral Pharmacology*, 9, 409-419.

Tirelli, E. & Heidbreder, C. (1999). Conditioning of and contextual sensitization to apomorphine-induced climbing in mice: evidence against the habituation hypothesis. *Behavioral Neuroscience*, 113, 368-376.

Tirelli, E., Michel, A. & Brabant, C. (2005). Cocaine-conditioned activity persists for a longer time than cocaine-sensitized activity in mice: implications for the theories using Pavlovian excitatory conditioning to explain the context-specificity of sensitization. *Behavioural Brain Research*, 165, 18-25.

- Tolman, E. C. (1932). *Purposive Behavior in Animals and Men*. New York: Century.
- Tomie, A., Burger, K. M., Di, P. J. & Pohorecky, L. A. (2004). Social opportunity and ethanol drinking in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 28, 1089-1097.
- Tulving, E. & Thompson, D. R. (1973). Encoding specificity and retrieval processes in episodic memory. *Psychological Review*, 80, 352-373.
- Tyson, P. J., Roberts, K. H. & Mortimer, A. M. (2004). Are the cognitive effects of atypical antipsychotics influenced by their affinity to 5HT-2A receptors? *International Journal of Neuroscience.*, 114, 593-611.
- van, Duinen. H., Lorist, M. M. & Zijdwind, I. (2005). The effect of caffeine on cognitive task performance and motor fatigue. *Psychopharmacology (Berl)*, 180, 539-547.
- Varani, K., Portaluppi, F., Merighi, S., Ongini, E., Belardinelli, L. & Borea, P. A. (1999). Caffeine alters A2A adenosine receptors and their function in human platelets. *Circulation*, 99, 2499-2502.
- Varani, K., Gessi, S., Merighi, S., Vincenzi, F., Cattabriga, E., Benini, A., Klotz, K. N., Baraldi, P. G., Tabrizi, M. A., Lennan, S. M., Leung, E. & Borea, P. A. (2005). Pharmacological characterization of novel adenosine ligands in recombinant and native human A2B receptors. *Biochemical Pharmacology.*, 70, 1601-1612.

- Vlachopoulos, C., Hirata, K., Stefanadis, C., Toutouzas, P. & O'Rourke, M. F. (2003). Caffeine increases aortic stiffness in hypertensive patients. *American Journal of Hypertension*, 16, 63-66.
- Wagner, A. R., Mazur, J. E., Donegan, N. H. & Pfautz, P. L. (1980). Evaluation of blocking and conditioned inhibition to a CS signaling a decrease in US intensity. *Journal of Experimental Psychology: Animal Behavior Processes*, 6, 376-385.
- Wajda, I. J., Banay-Schwartz, M. & Lajtha, A. (1989). The effect of caffeine on some mouse brain free amino acid levels. *Neurochemical Research*, 14, 317-320.
- Walter, T. A. & Riccio, D. C. (1983). Overshadowing effects in stimulus control of morphine analgesic tolerance. *Behavioral Neuroscience*, 97(4), 658-662
- Walther, E. & Nagengast, B. (2006). Evaluative conditioning and the awareness issue: assessing contingency awareness with the four-picture recognition test. *Journal of Experimental Psychology: Animal Behavior Processes*, 32, 454-459.
- Warburton, D. M. (1995). Effects of caffeine on cognition and mood without caffeine abstinence. *Psychopharmacology (Berl)*, 119, 66-70.
- Warburton, D. M., Bersellini, E. & Sweeney, E. (2001). An evaluation of a caffeinated taurine drink on mood, memory and information processing in healthy volunteers without caffeine abstinence. *Psychopharmacology (Berl)*, 158, 322-328.
- Warburton, D. M. & Mancuso, G. (1998). Evaluation of the information processing and mood effects of a transdermal nicotine patch. *Psychopharmacology (Berl)*, 135, 305-310.

Wasserman, E. A. (1993). Comparative cognition: beginning the second century of the study of animal intelligence. *Psychological Bulletin*, 113, 211-228.

Wasserman, E. A. & Miller, R. R. (1997). What's elementary about associative learning? *Annual Review of Psychology*, 48, 573-607.

Watson, J., Deary, I. & Kerr, D. (2002). Central and peripheral effects of sustained caffeine use: tolerance is incomplete. *British Journal of Clinical Pharmacology*, 54, 400-406.

Watson, J. M., Lunt, M. J., Morris, S., Weiss, M. J., Hussey, D. & Kerr, D. (2000). Reversal of caffeine withdrawal by ingestion of a soft beverage. *Pharmacology Biochemistry and Behaviour*, 66, 15-18.

Weingarten, H. P. (1983). Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science*, 20, 431-433.

Weiss, S. R., Post, R. M., Pert, A., Woodward, R. & Murman, D. (1989). Context-dependent cocaine sensitization: differential effect of haloperidol on development versus expression. *Pharmacology Biochemistry and Behaviour*, 34, 655-661.

Weissenborn, R. & Duka, T. (2003). Acute alcohol effects on cognitive function in social drinkers: their relationship to drinking habits. *Psychopharmacology (Berl)*, 165, 306-312.

Wenzel, D. G. & Rutledge (1962). Effects of centrally-acting drugs on human motor and psychomotor performance. *Journal of Pharmacological Sciences*, 51, 631-644.

- White, K. & Davey, G. C. (1989). Sensory preconditioning and UCS inflation in human 'fear' conditioning. *Behaviour Research and Therapy*, 27, 161-166.
- Whitsett, T. L., Manion, C. V. & Christensen, H. D. (1984). Cardiovascular effects of coffee and caffeine. *American Journal of Cardiology*, 53, 918-922.
- Withers, N. W., Pulvirenti, L., Koob, G. F. & Gillin, J. C. (1995). Cocaine abuse and dependence. *Journal of Clinical Psychopharmacology*, 15, 63-78.
- Woodruffe-Peacock, C., Turnbull, G. M., Johnson, M. A., Elahi, N. & Preston, G. C. (1998). The Quick Mood Scale: development of a simple mood assessment scale for clinical pharmacological studies. *Human Psychopharmacology*, 13, 53-58.
- Yeo, C. H., Hardiman, M. J., Moore, J. W. & Russell, I. S. (1984). Trace conditioning of the nictitating membrane response in decorticate rabbits. *Behavioural Brain Research*, 11, 85-88.
- Yeomans, M. R., Jackson, A., Lee, M. D., Nesic, J. & Durlach, P. J. (2000). Expression of flavour preferences conditioned by caffeine is dependent on caffeine deprivation state. *Psychopharmacology (Berl)*, 150, 208-215.
- Yeomans, M. R., Ripley, T., Davies, L. H., Rusted, J. M. & Rogers, P. J. (2002). Effects of caffeine on performance and mood depend on the level of caffeine abstinence. *Psychopharmacology (Berl)*, 164, 241-249.
- Yeomans, M. R., Durlach, P. J. & Tinley, E. M. (2005). Flavour liking and preference conditioned by caffeine in humans. *Quarterly Journal of Experimental Psychology B*, 58, 47-58.

- Yeomans, M. R., Ripley, T., Lee, M. D. & Durlach, P. J. (2001). No evidence for latent learning of liking for flavours conditioned by caffeine. *Psychopharmacology (Berl)*, 157, 172-179.
- Yeomans, M. R., Spetch, H. & Rogers, P. J. (1998). Conditioned flavour preference negatively reinforced by caffeine in human volunteers. *Psychopharmacology (Berl)*, 137, 401-409.
- Yerkes, R. M. & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology*, 18, 459-482.
- Yin, H., Barnet, R. C. & Miller, R. R. (1994). Trial spacing and trial distribution effects in Pavlovian conditioning: contributions of a comparator mechanism. *Journal of Experimental Psychology: Animal Behavior Processes*, 20, 123-134.
- Zahn, T. P. & Rapoport, J. L. (1987). Autonomic nervous system effects of acute doses of caffeine in caffeine users and abstainers. *International Journal of Psychophysiology*, 5, 33-41.
- Zajonc, R. B. (1968). Attitudinal effects of mere exposure. *Journal of Personality and Social Psychology*, 9, 1-27.
- Zapantis, A. & Leung, S. (2005). Tolerance and withdrawal issues with sedation. *Critical Care Nursing Clinics of North America*, 17, 211-223.
- Zwyghuizen-Doorenbos, A., Roehrs, T. A., Lipschutz, L., Timms, V. & Roth, T. (1990). Effects of caffeine on alertness. *Psychopharmacology (Berl)*, 100, 36-39.

APPENDIX A: PUBLICATIONS AND PRESENTATIONS

This appendix lists the publications and conference presentations based on the findings reported in this thesis.

Publications:

Academic (peer reviewed) journal papers

Attwood A. S., Higgs S. & Terry P. (2007) Differential responsiveness to caffeine and perceived effects of caffeine in moderate and high regular caffeine consumers. *Psychopharmacology (in press)*

Published abstracts

Attwood A. S., Terry P. & Higgs S. (2006) Exploring factors that mediate responsiveness to caffeine. *Appetite* 47(2): 258

Attwood A. S. & Higgs S. (2004) Conditioned psychomotor effects of caffeine in humans, *Appetite* 43(1) 110

Presentations:

Attwood A. S., Terry P. & Higgs S. Conditioning of caffeine's behavioural effects to previously-neutral contextual stimuli in humans. Poster presented at the annual general meeting of the British Association of Psychopharmacology, Oxford, UK, July 2006.

Attwood A. S., Terry P. & Higgs S. Conditioned cognitive and psychomotor effects of caffeine in humans. Poster presented at the annual general meeting of the College of Problems of Drug Dependence, Scottsdale, Arizona, USA, June 2006.

Attwood A. S., Terry P. & Higgs S. Exploring factors that mediate responsiveness to caffeine. Poster presented at the BFDG conference, Birmingham, UK, April 2006.

Attwood, A S, Terry, P. & Higgs, S. Effects of two doses of caffeine on performance of selected tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB). Poster presented at the BFDG conference Wageningen, Netherlands, April 2005.

Attwood, A S Terry, P. & Higgs, S. Conditioned psychomotor effects of caffeine in humans. Paper presented at the British Feeding and Drinking Group (BFDG) conference in Bristol, in April 2004.