The Acute Biobehavioural Effects of Caffeine in Isolation and in Combination with Other Naturally Concomitant Compounds

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Thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy to Northumbria University, Newcastle upon Tyne

The research described within this thesis was undertaken in the Division of Psychology, School of Psychology and Sport Sciences, Northumbria University

Submitted January 2008

Abstract

Caffeine is often described as the most widely consumed psychoactive drug in the world. Despite a substantial amount of research examining the effects of caffeine on mood and cognition, there remain a number of unresolved issues in this field, two of which formed the focus of this thesis. The first pertains to whether caffeine has any behavioural effects beyond a reversal of withdrawal effects purported to exist in habitual consumers following caffeine deprivation. A second relates to the biobehavioural effects of caffeine when consumed in combination with other potentially psychoactive components, as is usually the case in dietary forms of caffeine. This thesis, therefore, firstly compared the cognitive and mood effects of acute administration of caffeine to habitual consumers and habitual non-consumers of caffeine. The effects of combining caffeine with other naturally concomitant compounds were then explored, firstly by examining the impact of combining caffeine with L-theanine (an inhibitory amino acid found in tea) and then by exploring the effects of guaraná (a caffeine-containing whole extract). Finally, following on from these latter studies, an attempt was made to establish the lowest active dose of caffeine. Each experiment followed a placebo-controlled, double-blind, balanced cross-over design. In each study, treatment-related changes in cognitive performance were assessed with computerised assessment tools (the Cognitive Drug Research battery, a sentence verification task and serial subtractions), and mood was assessed using both Bond-Lader and specifically tailored caffeine research visual analogue scales. Where appropriate, salivary caffeine levels and autonomic activity were monitored.

Performance was similarly improved for habitual consumers and habitual non-consumers of caffeine following caffeine administration. The administration of caffeine in combination with L-theanine led to some modulation of the effects of caffeine. This was also demonstrated when examining the effects of guaraná. A direct comparison of caffeine and guaraná with matched caffeine levels revealed differences in the effects of the two treatments. Exploration of the lowest active dose of caffeine revealed (largely impairing) effects of caffeine at doses lower than those found in decaffeinated beverages.

These findings may have important implications for caffeine research. Firstly, they suggest that behavioural effects of caffeine cannot be attributed wholly to withdrawal reversal. Secondly, they demonstrate that other components commonly co-consumed with caffeine are likely to modulate its biobehavioural effects. Finally, they suggest that levels of caffeine hitherto thought to be inactive may have (negative) psychoactive properties.

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Acknowledgements

My warmest thanks go to Professor Andrew Scholey and Dr David Kennedy, who have not only been the best supervisors I could have hoped for but also true friends.

Thanks to Professor Keith Wesnes and Cognitive Drug Research (CDR Ltd.) for their financial support and provision of their assessment battery and hardware, and also to Pharmaton SA who provided the guaraná extract used throughout this thesis.

I would also like to thank everyone in the Human Cognitive Neuroscience Unit for their friendship and support and a special thank you to Jonathon who has never been too busy to spare the time to listen. Thanks also to Anthea Milne for all of her technical support.

Finally, I'd like to thank my family and friends – especially the Leazes posse – who have kept everything in perspective and managed to make me laugh even at the hardest times. I couldn't have done it without you.

Declaration

This work has not been submitted for any other award. In all experimental chapters of this thesis the author had sole responsibility for the data collection, analysis, and interpretation. The writing of this thesis is the sole work of the author.

CHAPTER 1. INTRODUCTION

1.1 General introduction

Caffeine is often described as the most widely consumed drug in the world (Gilbert 1984). It is found naturally in over 60 species of plants, which include varieties of coffee, tea, cocoa, yerba mate, guaraná, and kola nut. These plants, or extracts of them, are regularly consumed in a normal diet, as are other caffeine-containing products, such as soft drinks and also medications. As a result, caffeine is consumed by the majority of the population in one form or another, and in a recent study by Heatherley et al. (2006) it was found that 91 % of a UK sample consumed daily caffeine amounts equivalent to at least one cup of tea.

In line with this prolific use, research into the psychoactive effects of caffeine is extensive. The first investigation of the effects of caffeine on performance was reported in 1912 (Hollingworth 1912) and research continues to the present day. Despite the enduring interest in the psychoactive effects of caffeine, knowledge in this area is far from complete. This is mainly due to methodological issues (discussed further in Section 1.8). More recently it has also been suggested that caffeine may not have any net benefits (see Section 1.9.1), with any effects being attributed to a reversal of deficits induced by caffeine withdrawal, as a result of abstinence prior to a caffeine challenge (James 1994; Rogers et al. 1995). Another deficiency in the field of caffeine research is the tendency to study caffeine in isolation. This is despite the majority of caffeine consumption being derived from natural sources, which contain other potentially psychoactive components (see Section 1.10). The aim of this thesis is firstly to attempt to address the issue of caffeine withdrawal, in order to assess whether caffeine has any net benefits. A second aim is to explore the biobehavioural effects of caffeine-containing products as they are consumed naturally, i.e. in combination with other components rather than in isolation. This is in order to establish the extent to which any behavioural effects of these can be attributed to their caffeine content alone.

1.2 General information

1.2.1 Sources and extent of caffeine consumption

Caffeine levels vary by source, with a standard cup size of 190 ml (as suggested by the Food Standards Agency 2001) providing approximately 75 mg of caffeine in instant coffee, 50 mg in tea, 23 mg in cola and 4 mg in hot chocolate (Gray 1998). Reported rates of caffeine consumption differ between studies and this may either reflect changes over time or may relate to the great variance in consumption

across cultures, with higher consumption in countries such as the UK, where daily caffeine consumption has recently been reported to be around 225 mg per person (Brice and Smith 2002). However, the main sources of caffeine also differ across countries. Fredholm et al. (1999) reported that 96 mg of caffeine from tea is consumed per person per day in the UK, as compared to 12 mg/pers/day from tea in the US. This preference for tea drinking in the UK means that, although consumption of caffeinated drinks is relatively high, actual caffeine consumption remains lower than other European countries such as France, Germany, Scandinavia, and the Netherlands, where the preferred source of caffeine is coffee. It has also been suggested that retrospective reports of caffeine consumption may underestimate caffeine consumption levels and that food diaries represent a more valuable tool in this area. However, Brice and Smith (2002) found little difference between the two data collection techniques.

1.2.2 Pharmacokinetics

Caffeine belongs to a group of plant alkaloids known as methylxanthines. Other members of this group are theophylline (found in tea) and theobromine (present in large amounts in cocoa). These methylxanthines are rapidly digested from the intestinal tract and quickly transported to all organs of the body, including the brain. Plasma and saliva caffeine levels have been shown to correlate very highly with one another (Newton et al. 1981) and, as such, saliva is often used as a tool for monitoring caffeine absorption. Peak saliva concentrations have been shown to occur between 35 and 45 minutes after oral ingestion and the average peak has been shown to range from 4.8 to 17.3 µg/ml (Liguori et al. 1997). Caffeine is metabolised by the liver and the average elimination half-life is around 5 hours. However, this has been shown to range from 2.7 to 9.9 hours in healthy young males (Blanchard and Sawers 1983). The rate of metabolism is also affected by factors such as smoking, which can reduce the half-life by 50 %, and exercise, which reduces the half-life as well as increasing maximal plasma concentrations (Collomp et al. 1991). According to Callahan et al. (1983), the $t_{1/2}$ is also 20 to 40 % shorter in ovulating women as compared to men. However, this has been shown to fluctuate throughout the menstrual cycle (Lane et al. 1992) and is also affected by taking the contraceptive pill, which in itself can double the half-life (Patwardhan et al. 1980). Pregnancy has also been shown to increase the $t_{1/2}$ with one study showing increases from 3 hours in non-pregnant women to 10.5 hours in the final 4 weeks of pregnancy (Knutti et al. 1982). However, Kaplan et al. (1997) found no differences between men and women in the pharmacokinetics of caffeine after differences in body weight had been accounted for. They also found that the effects of

caffeine were nonlinear with significantly reduced clearance and significantly prolonged elimination rates following 500 mg as compared to 250 mg.

1.2.3 Toxicology

Caffeine is generally regarded as safe. However, toxic effects related to excess caffeine consumption include vomiting, abdominal pain, and central nervous system (CNS) symptoms. There are very few reports of fatalities related to caffeine, but plasma levels of over 100 µg/g are considered lethal and Holmgren et al. (2004) report 4 cases of death by caffeine, where it appears that the lowest lethal dose was 10 grams.

1.2.4 Mechanism of action

A number of different mechanisms of action have been postulated with regard to caffeine. However, a number of these are only apparent at doses that far exceed normal human consumption and, as such, will not be addressed here (see Fredholm et al. 1999 for review). In terms of normal consumption levels it appears most likely that the action of caffeine is the result of antagonism of adenosine receptors, specifically A_1 and A_{2A} sub-receptors (Fredholm et al. 1999). This assertion is supported by studies that have shown the ability of caffeine to counteract the effects of adenosine receptor agonists such as cyclopentyl adenosine (CPA) (e.g. Campos et al. 2005). This blockade of the inhibitory action of adenosine has been linked to increased levels of dopamine, acetylcholine, noradrenaline, serotonin, glutamate, and GABA (Daly 1993).

Dopamine

Caffeine has been related to spontaneous behavioural activity in rodents, and this stimulation is bi-phasic (Thithapandha et al. 1972). According to Powell et al. (2001) current evidence indicates that dopamine-receptor activation is involved in this locomotor stimulant effect of caffeine and Svenningsson et al. (1997) suggest that A_{2A} receptors are the main mediators in this effect. The role of dopamine in the effects of caffeine on movement is highlighted by Fredholm et al.'s (1976) finding that caffeine increases dopamine receptor-mediated increases in rotation behaviour, a finding that was confirmed by Daly (1993).

Dopamine is central to the reward system and, as such, dopamine-mediated effects of caffeine are usually referred to in relation to dependence and caffeine (see Section 1.9.1). However, according to Acquas et al. (2002), dopamine levels in the shell and core of the nucleus accumbens are unaffected by acute caffeine administration at dietary levels. The authors studied the effects of caffeine on

dopamine levels in the rat brain and found that, following caffeine, dopamine concentrations were increased in the medial prefrontal cortex but that no such effects were observed in the nucleus accumbens. This is supported by evidence from Nehlig and Boyet (2000) who used caffeine-mediated increases in cerebral glucose utilisation to observe activation of brain regions in the rat. They found that 1 mg/kg of caffeine was only able to activate the caudate nucleus, which is involved in locomotion, and the raphe nuclei and locus coeruleus, which are related to sleep and mood, whereas 2.5 and 5 mg/kg of caffeine led to activation of other components including the nigrostriatal dopaminergic system. Not until the highest dose (10 mg/kg) was administered was the nucleus accumbens activated, at which point almost all brain regions were affected. Acquas et al. (2002) further explored the effect of caffeine on dopamine levels in the prefrontal cortex and it was found that, following chronic administration of caffeine, dopamine levels in this region were no longer affected. Additionally, tolerance to locomotor stimulating effects developed, which supports the previously stated suggestions of a relationship between caffeine's locomotor effects and dopamine, but also suggests tolerance to these effects with repeated administration. Carter et al. (1995) point out that although a number of researchers have stressed the relationship between caffeine and dopamine, the modulation of sleep-wakefulness behaviour, learning, and memory (see below) cannot be explained by any interaction between dopamine and adenosine receptors.

Acetylcholine

Acquas et al. (2002) studied the effects of caffeine on acetylcholine concentrations and found that, like dopamine, these were increased in the medial prefrontal cortex. Unlike dopamine, these increases were still apparent following chronic administration, suggesting a role for acetylcholine in the effects of caffeine on memory, attention, and vigilance processes. The sleep-related effects of caffeine (see Section 1.4) have also been associated with adenosine-mediated effects of the cholinergic system (Rainnie et al. 1994), and it has been suggested that dose-dependent, caffeine-induced increases in cortical acetylcholine are responsible for the psychostimulant effects of caffeine (Carter et al. 1995). However, Riedel et al. (1995) suggest that, since inhibitory A_1 receptors have been found on cholinergic terminals in the hippocampus and cortex (Briley 1990), then A_1 antagonists, such as caffeine, should enhance cognition rather than simply increasing activation. In order to explore this assertion the authors studied the effects of caffeine when combined with scopolamine (a cholinergic antagonist). The findings showed that caffeine was able to attenuate scopolamine-induced deficits to immediate and delayed recall, accuracy and

speed of word recognition, reading time on the Stroop task, tapping, and perceptual sensitivity in a visual search task. A reversal of the effects of scopolamine on systolic blood pressure was also observed. Other measures not affected were simple and choice reaction time, mood, diastolic blood pressure, and heart rate. These findings suggest that caffeine can act as a cognition enhancer on mnemonic tasks. However, the cholinergic pathway cannot be involved in those tasks not affected using this paradigm and it should be noted that the effects evinced may only be apparent when performance is impaired, in this case due to cholinergic dysfunction produced by scopolamine.

Smith et al. (2003) suggest a role for the cholinergic system in the effects of caffeine on the speed of encoding new information and producing faster performance on a vigilance task. In support of this, Wesnes and Warburton (1984) suggest that speed of processing new information is related to acetylcholine and since mesopontine cholinergic neurones are under the control of endogenous adenosine (Rainnie et al. 1995) it seems logical that caffeine, acting as an adenosine antagonist, will affect speed of processing. Warburton et al. (2001) also hypothesise that caffeine reduces normal fluctuations in arousal that occur in sustained attentional tasks (such as vigilance tasks) and that this is mediated by the ascending cholinergic pathway to the cortex. This assertion regarding fluctuations in arousal is based upon their finding that caffeine was able to reduce the individual differences seen in attentional performance.

Noradrenaline

Caffeine has been shown to activate noradrenergic neurons (Nehlig et al. 1992) and further support for such effects comes from Smith et al. (2003), who explored the effects of caffeine using a clonidine challenge paradigm. Clonidine reduces the turnover of central noradrenaline and, therefore, induces a state of low arousal (Starke 1981); this in turn causes impairment to performance on global measures of accuracy, attention and reaction time rather than memory (Coull et al. 1995). Caffeine was able to attenuate the effects of clonidine by reducing reaction times, number of errors and long responses (lapses in efficiency) on choice reaction time tasks, and by reducing simple reaction time and increasing subjective 'alertness'. Speed of performance on a semantic memory task was also impaired by clonidine and improved by caffeine. Given the role of noradrenaline in arousal it is possible that any modulation of this system has a greater involvement in the effects of caffeine when arousal is low.

Effects were also observed that were independent of clonidine. These were reductions in reaction time on a vigilance task and faster encoding of new information. This suggests that the noradrenergic system is not involved in these effects of caffeine

and further supports the assertion that these effects are related to acetylcholine (see section above).

Summary of mechanism of action

The evidence presented above suggests that dopamine is implicated in the motor effects of caffeine, whilst acetylcholine is related to more accurate and faster cognition, particularly relating to encoding and vigilance. Noradrenaline has also been implicated in the effects of caffeine on semantic memory and speed of attention, such as simple and choice reaction time. The noradrenergic system may be particularly related to these effects of caffeine seen when arousal is low (see Section 1.9). However, it is clear that a number of neurotransmitters are involved in the effects of caffeine and it may not be possible to disentangle these effects. Other neurotransmitters, such as, serotonin, glutamate and GABA, have also been implicated in the mechanism of action of caffeine (Daly 1993). However, the role of these has yet to be explored in detail.

1.3 Physiological effects

1.3.1 Salivary/plasma caffeine levels

Levels of caffeine in saliva and plasma are often recorded at baseline in caffeine challenge studies, in order to confirm that instructions to abstain from caffeine (usually overnight) have been adhered to. Quinlan et al. (2000) found mean baseline salivary caffeine levels of around 2.5 µmol/l (~ 0.5 µg/ml) following 16-hours of caffeine abstention and Childs and de Wit (2006) found baseline levels of 0.1 µg/ml in light, nondependent caffeine consumers. These levels are consistent with plasma baseline levels of 0.1 µg/ml (equivalent to 0.5 µmol/l) of caffeine found in participants following 24-hours of abstention (Kaplan et al. 1997), and 0.2 µg/ml in low caffeine consumers (mean caffeine consumption = 60 mg/day) and 0.5 µg/ml in moderate consumers (mean = 314 mg/day) following 12-hour abstention (Lieberman et al. 1987). Although no significant differences existed between the baseline levels of low and moderate consumers, this difference does represent a statistical trend in the expected direction, with higher caffeine levels in those that report consuming more caffeine.

Measures of salivary and plasma caffeine levels are also often obtained following a caffeine challenge, in order to confirm effective caffeine absorption. Kaplan et al. (1997) found that plasma caffeine levels were increased to 7 and 17.3 µg/ml at peak, following 250 and 500 mg respectively. Lieberman et al. (1987) found that caffeine plasma levels of both low and moderate consumers elevated similarly

following caffeine administration and an increase to only 0.9 µg/ml (following 32 mg of caffeine) was accompanied by performance effects. This was compared to 0.3 µg/ml following placebo. Quinlan et al. (2000) measured salivary caffeine levels 30 minutes following ingestion and found that, 150 mg of caffeine from coffee elevated levels to just over 30 µmol/l (~ 6 µg/ml), and 75 mg of caffeine from coffee led to levels of around 17 µmol/l (~ 3.4 µg/ml). Levels following 75 mg of caffeine from tea were just lower at 14 µmol/l (~ 2.8 µg/ml), and levels following 37.5 mg of caffeine in tea were just under 10 µmol/l (~2 µg/ml). Childs and de Wit (2006) found salivary caffeine levels were raised to 1, 3, and 8 µg/ml, following 50, 150, and 450 mg of caffeine respectively in nondependent consumers. Interestingly, this increase in salivary caffeine level was significantly greater for women as opposed to men following 150 and 450 mg of caffeine. This may reflect the influence of hormonal variations on metabolism, but it is more likely to be indicative of simple differences in weight between men and women (Kaplan et al. 1997). The potential effect of this variable was not considered in the Childs and de Wit (2006) paper.

The findings from Lieberman et al. (1987), of similar elevations in plasma caffeine levels in low and moderate consumers following caffeine, are supportive of the findings from Brice and Smith (2001a), who showed that caffeine consumer status had no effect on salivary caffeine levels following a caffeine challenge, indicating that metabolism of caffeine, is not affected by habitual caffeine consumption. Brice and Smith (2001a) also illustrated that salivary caffeine levels are unaffected by psychosocial factors, personality and health-related behaviours. Caffeine levels were also not related to time of day, a finding which is supported by Hashiquchi et al. (1992), who found no difference in caffeine clearance when administered at 10 am or 10 pm.

The effects that differing caffeine vehicles have on absorption have also been studied, with no significant differences between caffeine administered in coffee, cola, or capsule in terms of mean peak levels, and the only difference in time to peak representing a slower absorption rate from a capsule rather than from either liquid form (Liquori et al. 1997).

The findings of Brice and Smith (2001a) indicate that salivary caffeine levels are a good indicator of the amount of caffeine consumed and are a useful tool in verifying that caffeine abstinence has been adhered to. James et al. (1989) also found that salivary caffeine levels were highly correlated with self-reported caffeine consumption, indicating through objective measures that self-report questionnaires represent a valid tool for assessing habitual caffeine consumption. Findings from Lieberman et al. (1987) indicate that salivary levels as low as 0.9 µg/ml caffeine are able to affect behaviour. Similarly, the results of Landolt et al. (1995) suggest that a salivary caffeine level as

low as 3 μmol/l (~ 0.6 μg/ml) is able to disrupt sleep propensity (see Section 1.4). However, Brice and Smith (2001a) found that salivary caffeine concentrations were not correlated with performance and, as such, individual differences in the metabolism of caffeine were not related to individual differences in the behavioural effects of caffeine.

1.3.2 Electrophysiological

Several studies have considered the effects of caffeine on electroencephalography (EEG) recordings. In one such study Hasenfratz and Battig (1994) found that 3 and 6 mg/kg of caffeine (~ 210 and 420 mg respectively) were capable of increasing the dominant frequency of the α band and 6 mg/kg had the same effect on the β band. They did not find any effects on power bands and event related potentials (ERPs) were similarly unaffected. Conflicting results were found by Kaplan et al. (1997) who showed that 250 and 500 mg of caffeine were capable of reducing β, α, and total amplitude for central and parietal leads with no apparent differences between caffeine doses. However, the authors point out that these effects are small and are dependent upon time, dose, and concentration.

Despite the lack of effects on ERPs in Hasenfratz and Battig's (1994) study, these have recently become the focus of electrophysiological studies of caffeine. Dixit et al. (2006) employed this measure in conjunction with reaction time during an auditory oddball paradigm. They found a significant increase in P3 amplitude following caffeine, which was coupled with faster reaction time on the task 40 minutes after caffeine ingestion. The authors suggest that these effects are indicative of a facilitation of information processing and motor response of the brain. This suggestion is supported by Rao et al. (2005), who examined the effects of an energy drink containing caffeine and glucose on ERPs and performance of a visual vigilance task, and found that both ERPs and performance were modulated. Specifically, with regards EEG, the N1, N2, and P3 components were affected, which could be interpreted as being related to decision-making and response. P1, which relates to visual processing, was also affected. The findings from Deslandes et al. (2005), of reduced α power following administration of 400 mg of caffeine prior to completion of a Stroop task, support the results from Kaplan et al. (1997). In addition, reductions to P3 latency were observed. Similarly, Martin and Garfield (2006) showed that 200 mg of caffeine was able to shorten N2 latency and increase P3 amplitude during a choice reaction time task. These effects were coupled with faster decision time on this task. Ruijter et al. (2000) also found effects on the P3 component as well as P2, which, they suggest, represent a caffeine-induced increase in activity related to information processing. Interestingly,

this effect was seen despite a lack of any effect on a behavioural measure of sustained attention.

Summary of EEG effects

Taken together these findings indicate that caffeine is able to positively modulate attention, specifically information processing, independent of stimulus-specific characteristics. Differences in findings are likely to be related to the behavioural measures employed during or immediately prior to electrophysiological recording. It is also interesting to note that the effects of caffeine on EEG have been demonstrated in rested participants. Barry et al. (2005) found reductions in alpha power following 250 mg of caffeine in participants not engaged in a behavioural task and interpreted this as representing an increase in arousal.

1.3.3 Autonomic system

1.3.3.1 Blood Pressure

A number of studies have considered the effects of caffeine on blood pressure. Quinlan et al. (2000) carried out two studies that examined the effects of low doses of caffeine on mood and measures of heart rate and systolic and diastolic blood pressure. In the first of these studies the effects of tea and coffee, ranging in caffeine dose from 37.5 – 150 mg, were compared to hot water and no drink. Significant increases in systolic and diastolic blood pressure were observed lasting for 60 minutes following all caffeine doses. In the second of these studies the effects of 25, 50, 100, and 200 mg of caffeine, added to decaffeinated tea, were compared to those of decaffeinated tea (containing 5 mg of caffeine), and hot water. It was found that systolic and diastolic blood pressure were again increased, but in this case 100 mg or more was required to increase systolic pressure. Lane and Manus (1989) also found increases to diastolic blood pressure as well as mean arterial pressure following 125 mg of caffeine, despite pre-treatment with 125 mg of caffeine. Similarly, 500 mg of caffeine administered at breakfast led to greater increases in blood pressure, measured over the working day, when compared to the effects of 100 mg administered at the same time of day (Lane et al. 1998). Lane et al. (2002) explored these effects further in a study of habitual caffeine consumers, designed to examine cardiovascular and neuroendocrine effects of caffeine at work and at home. Participants were monitored throughout the day on three separate days. The first day served as a practice day during which participants were allowed *ad libitum* caffeine. The following two days involved substitution with either placebo or a fixed 500 mg dose of caffeine, administered in two capsules of 250

mg, at around 8 am and 4 hours later. The study employed a crossover design with the order of treatments counterbalanced. In line with laboratory-based findings the authors found, on average, a significant increase in systolic blood pressure of 4 mm Hg and a significant increase in diastolic blood pressure of 3 mm Hg, following 500 mg of caffeine. These effects did not differ between the workday and the evening at home.

Childs and de Wit (2006) also found increases in systolic and diastolic blood pressure, when examining the effects of caffeine ranging from 50 to 450 mg in light, nondependent caffeine users. This suggests that the effects of caffeine upon blood pressure are not reliant upon habitual caffeine use. However, Heatherley et al. (2005) found that caffeine was able to increase systolic blood pressure in those participants who had abstained from caffeine for 8 hours but not in those who had only abstained for 4 or 6 hours. This may be indicative of effects of caffeine on this measure only being apparent when consumers are in a state of withdrawal. However, it is also possible that the lack of effects at 4 and 6 hours are due to the residual effects of previous caffeine intake. The issue of caffeine consumer status in relation to blood pressure effects will be discussed further in the following section.

Moderating factors in blood pressure effects

In a review of the literature regarding the chronic effects of coffee on blood pressure, Jee et al. (1999) concluded that coffee use resulted in increases in both diastolic and systolic blood pressure, but that those effects seen on systolic blood pressure were greater. Greater effects were also observed in younger participants, which is indicative of some tolerance to the pressor effects of caffeine. However, these findings suggest that complete tolerance to pressor effects of caffeine does not develop. This assertion, that tolerance is not complete, is in accordance with previous conclusions (Robertson et al. 1981), and other studies have shown incomplete tolerance to the effects of caffeine on blood pressure (e.g. Lovallo et al. 2004; Rachima-Maoz et al. 1998). James (2004) concludes that blood pressure remains reactive to habitual caffeine use but suggests that these effects are modest. However, Lane (1997) found that *ad libitum* consumption of caffeine in moderate habitual consumers led to increases in mean arterial pressure with an effect size of $d = 0.7$.

As well as habitual caffeine intake, other factors have been implicated in the effects of caffeine on blood pressure. Lovallo et al. (2004) discovered individual differences in blood pressure responses, with only half of their participants' responses contributing to the increase in blood pressure. Similarly, Rachima-Maoz et al. (1998) found individual differences and non-responders were more likely to be younger (<44 years). Also, in one study, coffee, but not caffeine, was shown to increase blood

pressure (Sudano et al. 2005), which illustrates the importance of the vehicle that caffeine is administered in, and suggests that the effects of other components within caffeinated beverages should be taken into consideration on this measure (see Section 1.10). In addition, Hodgson and Puddley (2004) found that consumption of a meal was able to negate the increase in blood pressure seen following caffeine.

Additive effects of stress and caffeine have been observed (see Section 1.5) and these are also related to autonomic effects. Shepard et al. (2000) explored the effects of caffeine on blood pressure during a lecture and during an exam. They found that caffeine was able to significantly increase blood pressure in both participant groups who were split on the basis of low or high risk for hypertension, but that these effects were greater when combined with exam stress, such that the high risk group had systolic blood pressure of ≥ 140 mm Hg. These effects were also explored by Pincomb et al. (1988) in a study considering blood pressure effects of caffeine and a mentally demanding task, alone, and in combination. Blood pressure was shown to increase in response to caffeine, as well as to the task, and this effect was greater when the two were combined. However, different mechanisms were highlighted for the effects of caffeine - increases in blood pressure during caffeine at rest were the result of increased vascular resistance, whereas caffeine combined with the task increased cardiac output. This is supported by James (1997) who claimed that the effects of caffeine on blood pressure were the result of vascular resistance. However, Hartley et al. (2004) found that the mechanism involved may be different for men and women. In men, caffeine increased blood pressure at rest, during mental stress and during reading, as a result of increased vascular resistance. However, in women, increases in blood pressure during the same procedures were found to be the result of increased cardiac output and stroke volume, rather than vascular resistance.

1.3.3.2 Heart rate

The effects of caffeine on heart rate are more variable than those on blood pressure. Scholey and Kennedy (2004) measured heart rate before and after completion of a 30 minute test battery, which commenced 30 minutes after drink ingestion of 75 mg of caffeine. They found a trend for a decrease in heart rate at the beginning of the battery, which reached significance when measured following completion of the tasks. Quinlan et al. (2000) also showed significant decreases in heart rate in response to 37.5 – 150 mg of caffeine lasting up to 105 minutes after drink ingestion. Similarly, in a second study, it was found that heart rate significantly decreased following caffeine and that this decline was inversely related to caffeine

dose. The findings in this respect are not unequivocal with Lane et al. (2002) finding that heart rate decreased by 2 bpm on average following 500 mg of caffeine and these effects did not differ between those seen during the work day and those seen at home. Conversely, Lane and Williams (1985) found no effects on heart rate and Lane et al. (1998) found increased heart rate following caffeine. Childs and de Wit (2006) also considered the effects of caffeine on heart rate in light, non-dependent caffeine users and found that, although heart rate decreased, this effect did not reach significance.

Moderating factors in heart rate effects

Lane et al. (2002) showed that, like blood pressure, heart rate is also affected by stress. Heart rate was shown to decrease following caffeine but increase when caffeine was coupled with stress. Similar results were found by Hamer et al. (2006) who showed that habitual coffee use led to increases in heart rate in response to mental stress and that these effects persisted after short-term abstinence from coffee. However, Barry et al. (2005) found no effects of caffeine on heart rate during rest. This illustrates the importance of task demands to the effects of caffeine on this measure. Related to this is the finding that caffeine reduces heart rate in sleep-deprived participants even during stressful periods (Kohler et al. 2006). Lane and Manus' (1989) findings also demonstrate the importance of the length of time after caffeine administration that heart rate recordings are taken. The authors showed that heart rate increased following 125 mg of caffeine in participants pre-treated with 125 mg but that this effect did not become significant until 195 minutes after caffeine administration. This suggests changing effects of caffeine on heart rate over time.

Summary of autonomic effects

The effects of caffeine upon blood pressure are fairly reliable, if modest. These effects are not prone to complete tolerance following habitual caffeine consumption and the effects are greater when caffeine is coupled with a stressor. The effects on heart rate are less reliable and appear to be influenced, to a greater extent than blood pressure, by other factors. This is highlighted by a number of studies that have shown effects of caffeine on blood pressure, with no effects on heart rate (e.g. Ratliff-Crain et al. 1989; Smith et al. 1993; James 1994). These factors relate to arousal level and are affected to the extent that high arousal situations coupled with caffeine lead to increases in heart rate whereas low arousal situations coupled with caffeine lead to decreases in heart rate. This is illustrated by studies measuring effects during stress (e.g. Lane et al. 2002) and those measuring effects in sleep-deprived participants (e.g. Kohler et al. 2006). As with the effects on EEG, it is important to consider the

characteristics of the task being completed during or prior to monitoring of heart rate, as this is likely to modulate the effects of caffeine. The effects of caffeine on the autonomic system are usually attributed to effects upon the noradrenergic system. However, the findings from Riedel et al. (1995) (see Section 1.2.4) suggest that caffeine can affect systolic blood pressure via the cholinergic system and it is, therefore, possible that the varying results on autonomic measures may be indicative of the differing mechanisms underlying the effects.

1.4 Sleep

Support for the arousing effects of caffeine, indicated by both EEG results (see Section 1.3.2) and increases in blood pressure (see Section 1.3.3.1), comes from observation of its effects on sleep. Sleep latency and efficiency have been shown to be disrupted by as little as 200 mg of caffeine administered at 7.10 am prior to a sleep episode at 11 pm (Landolt et al. 1995).

The effects of caffeine on sleep have been shown to be more pronounced on daytime recovery sleep, following deprivation, than on normal nocturnal sleep. In a crossover study of placebo versus 200 mg of caffeine, Carrier et al. (2006) administered two doses of 100 mg of caffeine, one 3 hours before bedtime and another 1 hour before bedtime. Participants were split into two groups, one group went to bed at their habitual circadian time and the other were deprived of one nights sleep and went to bed the following morning. Caffeine was shown to increase sleep latency, increase stage 1 sleep and reduce stage 2 and slow wave sleep in both night and day sleepers. However, the effects of caffeine were greater in the day recovery group in terms of reduced sleep efficiency and decreases in sleep duration and REM sleep were only observed in the day group. This effect has also been shown to be greater in insomniacs, with 200 mg of caffeine administered during a night of sleep deprivation leading to longer sleep latency and less total sleep time in insomniacs, as compared to normal volunteers (Salin-Pascual et al. 2006).

These findings have potential implications for those using caffeine during the night time, such as night-shift workers. However, the benefits of using caffeine to counteract natural circadian rhythms in order to stay awake and vigilant at night (see Section 1.6.3 below) may outweigh the negative impact upon recovery sleep the following day. This point is further validated by a study that explored the effects of caffeine on daytime recovery sleep as well as subsequent performance. LaJambe et al. (2005) compared the effects of repeated doses of 100 mg and 300 mg of caffeine, administered to 20-hour sleep deprived participants at 3.00 am, 5.00 am, 7.00 am, and 10.00 am. An 8-hour sleep session then commenced following a total of 27 hours of

sleep deprivation. The authors found only stage 1 sleep was affected for those in the 100 mg group, whereas the 400 mg group experienced less total sleep time and reduced sleep depth. The effects on slow wave sleep were also greater in habitual low consumers (<100 mg/day) than high consumers (>400 mg/day). Despite these deleterious effects to sleep, no effect of caffeine was found on subsequent performance of a 10-minute psychomotor vigilance task (PVT) upon waking for any caffeine consumption group. The effects on sleep in the 100 mg group who received the equivalent of 400 mg in total are contrary to the findings of Carrier et al. (2006) who found effects on sleep latency following 200 mg.

1.5 Stress

One possible mediator of the effects of caffeine on sleep is the level of stress experienced by the individual. In a study comparing sleep patterns in healthy young participants, split on the basis of high and low scores on a measure of vulnerability to stress-related sleep disturbance, Drake et al. (2006) found significantly increased sleep latency in the high stress group following 3 mg/kg of caffeine administered 1 hour prior to lights-out. This was despite finding no differences between sleeping patterns of the two groups in the absence of caffeine. This finding highlights the importance of individual differences in response to caffeine and the authors also suggest that caffeine could be viewed as a pharmacological stressor.

A number of studies, such as those examining the effects of caffeine on autonomic responses (see Section 1.3.3), have considered the impact of caffeine on the stress response. Measurements of cortisol levels have also been used as an indicator of stress. Lovallo et al. (1996) found that 3.3 mg/kg of caffeine was able to increase plasma cortisol levels 60 – 120 minutes after ingestion, and this was coupled with an increase in adrenocorticotropic hormone (ACTH) at 30 – 180 minutes. It is suggested that this effect of caffeine on cortisol levels is mediated by the release of ACTH from the pituitary. Others have found no effects of caffeine on cortisol. For example, Lane et al. (2002) explored the effects of habitual caffeine consumption on neuroendocrine effects at work and at home. Neuroendocrine activation was measured by urinary-free levels of adrenaline, noradrenaline, and cortisol. Adrenaline and cortisol were found to be higher during the workday, illustrating their receptiveness to stress. However, only noradrenaline was affected by caffeine, with increases following caffeine irrespective of whether at work or at home. These findings all point to a relationship between caffeine and noradrenaline, which is independent of stress. Additionally, no main effect of caffeine on subjective stress ratings was observed during the workday. In a study exploring the effects of 300 mg of caffeine on target detection and rifle

marksmanship, Gillingham et al. (2003) also found no effects of caffeine on plasma cortisol levels and, in fact, found that caffeine was able to alleviate the response to a cold stressor. However, al'Absi et al. (1995) found that caffeine increased cortisol at rest in borderline hypertensive males but not normotensives.

The effects of caffeine on cortisol levels are often more pronounced in stressful situations. Lovallo et al. (2006) found that the impact of mental stress on salivary cortisol was compounded with the addition of 3 repeated doses of 250 mg of caffeine. The effects of 3.5 mg/kg of caffeine on plasma cortisol, adrenaline, noradrenaline, and blood pressure in response to task-induced stress were also studied and it was found that, whilst blood pressure and noradrenaline were elevated by caffeine at rest, cortisol and adrenaline were only elevated when caffeine was combined with stress (Lane et al. 1990). Conversely, Shepard et al. (2000) found that, although stress and caffeine both increased salivary cortisol, there was no interaction between the two on this measure. There was, however, an additive effect of caffeine plus stress on blood pressure (see Section 1.3.3.1).

Possible explanations for discrepancies between studies on this measure are provided by Lovallo et al. (2006) who demonstrated that male and female responses to caffeine differed. Women were observed to produce a lower cortisol response to caffeine when combined with mental stress than men. This effect was not seen with exercise or in the absence of stress. Sex differences in cortisol response to caffeine were also moderated by meal intake, with greater responses to caffeine plus a meal in females than males. Habitual caffeine intake has also been implicated in the effects of caffeine on cortisol. Lovallo et al. (2005) found that five days of 300 or 600 mg of caffeine administration reduced, but did not abolish, the cortisol response to 250 mg of caffeine seen following 5 days of caffeine abstinence. Effects of differing caffeine vehicles have also been observed, with Sudano et al. (2005) reporting stress-induced increases in blood pressure as a consequence of coffee, but not caffeine, in non-habitual coffee drinkers. This suggests that components within coffee other than caffeine are involved in the stress-response to coffee. This is supported by findings of reductions in cortisol levels in response to task-stress following tea compared to a control, despite both treatments containing 72 mg of caffeine (Steptoe et al. 2006). These findings again highlight the importance of other components within caffeinated beverages (see Section 1.10) and suggest a role for components within tea in the reduction of cortisol levels. It appears that caffeine is able to modulate the stress response and it is also possible that stress levels experienced whilst consuming caffeine may impact upon its effects. The experience of these levels of stress is likely

to be mediated by individual differences (see Section 1.9.3) as is the impact of caffeine on the response to stress.

1.6 Cognitive effects

1.6.1 Psychomotor

It has been shown, in rats, that caffeine increases spontaneous activity (Thithapandha et al. 1972), and these effects have been related to dopamine (e.g. Powell et al. 2001). It has been suggested that this caffeine-related increase in motor activity will lead to decreased control over fine movement. However, the evidence in this area is mixed; for example, Richardson et al. (1995) found that hand steadiness, as measured by the accuracy of insertion of a stylus into holes of varying diameter, was decreased in a dose-dependent manner following 70 and 250 mg of caffeine. This finding is supported by results from Heatherley et al. (2005), who found that 1.2 mg/kg of caffeine decreased hand-steadiness on a tapping task in those participants who were 4-hour caffeine abstinent, but not in those who were 6 or 8-hour abstinent. Conversely, Lieberman et al. (1987) failed to find any effects of caffeine doses ranging from 32 to 256 mg on a grooved pegboard test or on fine finger movements. Interestingly, activity as measured by piezoelectrical crystals placed in participants' seats, thereby measuring body movements, has been shown to significantly decrease following 1.5, 3, and 6 mg/kg of caffeine (Hasenfratz and Battig 1994).

Linked to the motor effects of caffeine are its effects on tasks that measure psychomotor ability. Common measures of psychomotor performance are the tapping task and the digit symbol substitution task (DSST – Weschler 1958). Kaplan et al. (1997) found effects on both tapping and DSST of 250 but not 500 mg of caffeine. Improved performance was found to be restricted to plasma caffeine levels between 2.5 and 12.5 µg/ml, with the effect diminishing with concentrations above this level. However, in another study involving a tapping task requiring alternating presses of the 1 and 2 keys of a keyboard, it was found that neither 70 nor 250 mg of caffeine affected performance on this measure (Richardson et al. 1995). Tapping performance was also considered by Lieberman et al. (1987), who found no effects of caffeine on a 1 minute task that required alternate tapping with a metal stylus of two wedge shaped targets separated by 1 cm. In addition, no effects were found on the DSST. Performance of the DSST was also found to be unaffected in nondependent consumers of caffeine following 50, 150, and 450 mg of caffeine. Additionally, no effects were found on the

stop task, which measures behavioural inhibition of motor response (Childs and de Wit 2006). As with heart rate, differences in the findings on these measures are likely to relate to arousal prior to administration of caffeine or prior to measurement of behaviour. The factors that affect this arousal level will be considered in Section 1.9.

1.6.2 Speed of attention

There is a substantial amount of evidence to suggest that caffeine improves reaction time, particularly during simple attention tasks (for example tasks that simply require the pressing of a button in response to a single repeated stimulus). The effects on these measures appear to be enhanced when performance is impaired by other factors, particularly in the case of choice reaction time tasks. However, a number of studies illustrate that improvements on these measures are not limited to reversal of impairment and these are outlined below.

Speed of attention when not impaired

The effects of relatively high doses of caffeine on simple reaction time and movement time were explored in an independent samples study carried out by Jacobson and Edgley (1987). The effects of 300 and 600 mg of caffeine versus placebo were examined and improvements to both measures were found 45 mins after administration of 300 mg, but with no such effects were found following 600 mg. These findings suggest that caffeine at doses far outside those that are normally consumed in a single serving are not as effective as typical dietary doses. Smit and Rogers (2000) examined the cognitive and mood effects of caffeine at lower doses, representative of doses found in a typical serving. They found that 100, 50, and 12.5 mg of caffeine were equally beneficial in producing faster simple reaction time. However, no effect was found with 25 mg. These effects were strongest 45 minutes after ingestion of caffeine capsules with a battery of cognitive tasks that commenced 2 minutes after ingestion and lasted for 95 minutes. Rapid effects of caffeine on choice reaction time were discovered in a study by Durlach et al. (2002), which explored the effects of 60 mg of caffeine administered either in tea or water. The findings showed faster performance in habitual tea drinkers on two choice reaction tasks following caffeine. These effects became apparent as early as 1 minute following administration of the drink with no effect of beverage (tea or water). In a placebo-controlled trial, Richardson et al. (1995) found that 70 mg, but not 250 mg, of caffeine was capable of producing an acute improvement to simple reaction time 45 minutes following administration. Similarly, reaction times on focused attention and categoric search tasks were both faster

following 40 mg of caffeine (Smith et al. 1999b). The findings from a study considering the effects of caffeine and nicotine also showed improvements to choice reaction time following 200 mg of caffeine (Smith et al. 1977). Importantly these effects were evinced on the decision component of the task, which suggests that improvements on this task are not restricted to a motor effect.

In a study exploring the effects of repeated doses of caffeine, Robelin and Rogers (1998) found that 1.2 mg/kg (~84 mg) caffeine, administered in a novel fruit drink, improved performance of a 24-minute simple reaction time task 45 mins after administration. However, subsequent administration of caffeine or placebo, 75, and 165 mins later did not further affect performance of this task. The findings of Hindmarch et al. (2000), described below, are contrary to this finding as they show relative improvements to choice reaction time of caffeine over placebo when a second caffeinated beverage was consumed. The findings from a naturalistic study considering the effects of regular caffeine consumption on efficiency over the course of the working day are also contrary to the findings of Robelin and Rogers (1998). Smith (2005) compared performance of low and high (median split at 220 mg of caffeine per day) caffeine consumers on a simple reaction time task before and after a working day during which their caffeine consumption was recorded and used to ascertain their daily consumption. It was found that those who consumed more caffeine throughout the day had a smaller slowing of simple reaction time over the course of the day than 'low' consumers.

The evidence from van Boxtel et al. (2003) suggests that, not only can an acute caffeine challenge improve reaction time, but habitual consumption can also lead to similar improvement. In a longitudinal study, 1376 participants between the ages of 24 and 81 completed a caffeine intake questionnaire and performed cognitive tasks at baseline. Cognitive performance was then re-assessed 6 years later. After controlling for demographic factors, health status, and baseline performance, higher caffeine intake as calculated at baseline was found to be associated with positive results on the 6-year change in score on a choice reaction task. The results from Jarvis (1993) showing a significant dose-response relationship between habitual caffeine consumption and performance of both simple and choice reaction time tasks support these findings. However, in a rare study of the chronic effects of caffeine, Judelson et al. (2005) employed an independent design to examine the effects of controlled administration of placebo, 3, or 6 mg/kg per day for 5 days in low to moderate habitual consumers. Five days of equilibration with 3 mg/kg per day of caffeine administration in capsule form were followed by 5 days of experimental intervention. When comparing

baseline performance measured on the 6th day with performance on day 11, there were no effects of treatment on a visual four-choice reaction time task.

The majority of these findings suggest that caffeine at varying doses and in varying situations is able to speed simple and choice reaction times. However, as Judelson et al. (2005) illustrate, there are also a number of studies that show no effect of caffeine on these measures and these are summarised below.

Smith et al. (1999b) found no effects of 40 mg of caffeine on a variable fore-period simple reaction time task. Lieberman et al. (1987) failed to find any effects of caffeine on a simple auditory reaction task. Rogers et al. (2003) considered the effects of 100 mg of caffeine administered as a fruit drink on performance of a long-duration simple reaction time task in regular consumers and non-consumers. They found that neither groups' performance was affected by caffeine administration. Similarly, Zahn and Rapoport (1987a) found no effects of 3 mg/kg or 10 mg/kg of caffeine versus placebo on a simple reaction time task in a sample of 20 adult males who were either high or low consumers. However, in a separate paper (Zahn and Rapoport 1987b), improvements were observed utilising the same task and the same doses in a sample of 19 prepubertal boys. This may be indicative of effects of caffeine in suboptimal conditions. For example, the prepubertal boys may have been more prone to distraction or simply less motivated to participate in the task. This assertion is supported by the conclusions from Ruijter et al. (2000) who claim that the effects of caffeine are most pronounced on simple, repetitive tasks where motivation may be an issue due to the low arousal produced by the task itself.

Despite the apparently contradictory nature of these findings it should be noted that there is no substantial evidence to suggest that caffeine has any negative impact upon reaction time. The studies of Zahn and Rapoport (1987a; 1987b) illustrate that differing results can be achieved despite very similar conditions. As indicated, one factor that may have influenced these results is the amount of effort involved and a number of studies have considered the effects of caffeine on reaction time tasks under suboptimal conditions where arousal is low. These are outlined below.

Speed of attention when impaired

Babkoff et al. (2002) investigated the effects of caffeine on nocturnal performance in a simulated shift-work study. Participants were exposed to 200 mg of caffeine and/or bright light during a schedule beginning at 5.30 pm and finishing at 10.00 am. Caffeine was able to reduce choice reaction time in the presence or absence of bright light, but the shortest reaction times were recorded 3 hours and 7 hours after administration of caffeine when participants had been exposed to bright light.

Interestingly, caffeine was also shown to reduce melatonin levels, a hormone that has been suggested as a soporific agent (Cajochen et al. 2003). In a study by Lieberman et al. (2002) administration of 200 and 300 mg of caffeine was shown to speed choice reaction in a dose-dependent manner in a group of 68 Navy trainees who were sleep-deprived and exposed to extreme stress. The effects of caffeine in sleep-deprived participants were also considered by Patat et al. (2000). Twelve healthy young participants received placebo or 600 mg slow release caffeine in capsule form in a crossover study. The findings showed significantly faster choice reaction time in participants who were 12-hour sleep deprived, and these effects were apparent from 3 to 21 hours after dosing. Further support for these findings comes from Kamimori et al. (2000) who administered placebo, 2.1 mg/kg, 4.3 mg/kg, and 8.6 mg/kg of caffeine to participants who were deprived of sleep for 49 hours at dosing. They found significant correlations between serum caffeine levels and choice reaction time throughput (the product of speed-accuracy), indicating a dose-dependent improvement in choice reaction time performance, which was evident from 1 hour and up to 12 hours after dose.

This offsetting of decline is also demonstrated in a study by Azcona et al. (1995) that contrasted the effects of caffeine (400 mg); alcohol (0.8 g kg -1) and a combination of these against a placebo. They found that whilst caffeine speeded response on a simple reaction time task and alcohol slowed it, no effects were seen with the combination, indicating an interaction between caffeine and alcohol effects on this measure. This is supported by the findings from Mackay et al. (2002) showing that although 110 - 120 mg of caffeine had no main effect on a four-choice reaction task, it was able to antagonise the increase in errors on this task resulting from intake of 0.66 mg/kg alcohol when co-administered.

Similarly, Hindmarch et al. (2000) found that caffeine was able to significantly offset the decline in performance over the course of the day seen with placebo. In a study considering the effects of day-long consumption of caffeine, the recognition component of a choice reaction time task was shown to be improved following caffeine, relative to placebo. This effect was only observed following the second of four beverages administered throughout the day. The second drink was administered at 1 pm and may have been more effective than the other beverages due to a post-lunch dip in performance. Interestingly, they also found that 75 mg of caffeine in coffee elicited a significantly faster reaction time than 75 mg of caffeine in tea. This suggests modulation of the effects of caffeine by other components within the two beverages (see Section 1.10).

In line with these findings, van Duinen et al. (2005) assert that the effects of caffeine on cognition are most pronounced in suboptimal conditions. In order to further explore this proposition they adopted a dual-task paradigm known to produce mutual interference between cognitive and motor task performance. The tasks were a motor task consisting of voluntary abductions of the index finger and a cognitive task involving auditory choice reaction time. These were each performed both as single tasks and together as a dual-task. In order to further explore the role of fatigue, single tasks were performed before and after the dual-task. Caffeine did not affect motor performance but did significantly speed reaction time on the choice reaction time task performed after the dual task, as compared to before the dual task, and also speeded reaction time on this measure during the dual-task. These findings support the authors' assertion that the effects of caffeine are more pronounced in suboptimal conditions, in this case when fatigued, as shown by performance after the dual task, and in situations of high demand, as shown by performance of the dual-task. Furthermore, Swift and Tiplady (1988) demonstrated faster choice reaction time following 200 mg of caffeine in elderly participants but the same effect was not observed in young participants. It is suggested that the effect in elderly participants is indicative of an offsetting of cognitive decline associated with ageing.

Findings from Kruk et al. (2001) also highlight the importance of arousal to the effects of caffeine on this measure. In a crossover study exploring the effects of 5 mg/kg of caffeine, exercise, and room temperature on choice reaction time it was shown that caffeine was able to shorten reaction time over that of placebo, but these effects were only observed at 22 °C and not at 4 °C, despite there being no main effects of temperature. Given that arousal has been shown to increase following exposure to low temperatures (van Orden et al. 1999), the lack of effects at 4 °C is suggestive of an interaction between arousal and caffeine, such that the effect is only observed when not in a state of heightened arousal.

1.6.3 Sustained attention

Vigilance

Sustained attention tasks are perhaps the most sensitive measures of the effects of caffeine. In particular, tasks assessing vigilance have shown fairly robust effects of caffeine. Brice and Smith (2001b) considered the effects of caffeine on a visual vigilance task which involved the detection of repeated digits. In a crossover study of decaffeinated coffee versus caffeinated coffee (3 mg/kg of caffeine), participants were presented with a series of 3-digit numbers and their task was to respond whenever the same two 3-digit numbers were presented in succession. The

findings showed a significantly increased hit rate on this measure following caffeine. Childs and de Wit (2006) also found improvements to visual vigilance in terms of increased hit rate and speeded reaction time following 450 mg of caffeine, whereas only hit rate was improved following 150 mg. Smith et al. (1994b) considered the effects of 4 mg/kg of caffeine, administered alone and in conjunction with two different types of breakfast, on a repeated digits task. Despite finding no effect of breakfast on this measure, they did find improved accuracy following caffeine 1 hour after administration and speeded reaction time at 2 hours. Smith et al. (1994a) also considered the effects of caffeine in modulating the performance effects of an evening meal. They found that caffeine improved the hit rate on a repeated digits task 30 minutes after ingestion and also speeded responses at 30 minutes and 120 minutes. However, caffeine did not interact with evening meal effects.

The role of arousal in the effects of caffeine on vigilance has also been explored. Lieberman et al. (1987) found that auditory vigilance as measured by the 1 hour Wilkinson task was significantly improved by 32, 128 and 256 mg of caffeine and, to a lesser but significant extent, by 64 mg. Similarly, reaction time on a four-choice reaction time task was significantly improved by 64 and 256 mg of caffeine and to a lesser extent by 32 and 128 mg. Both tasks are measures of vigilance, but they differ in that the Wilkinson task lasts for 1 hour and requires sustained concentration to detect infrequently-occurring embedded tones, whereas the four-choice reaction task only lasts for 10 minutes and requires continuous responding to easily detected visual stimuli. The authors suggest that the comparative effects of caffeine on these two tasks argue against the suggestion that the effects of caffeine are limited to situations where performance is degraded by factors such as fatigue. Kelemen and Creeley (2001) also referred to this debate and examined performance over the duration of a 12-minute visual vigilance task in order to ascertain whether the effects of caffeine only became apparent towards the end of the task, when the effects of fatigue would be evident. On day 1, participants were required to respond whenever they detected the target letter 'K' amongst a series of letters presented sequentially. On day 2, participants had to respond whenever they saw the letter 'A' followed by the letter 'K'. The findings showed that although overall performance did decline during the task, 4 mg/kg of caffeine was able to reliably improve response latency as compared to placebo as well as improving hit rate on day 1. These benefits of caffeine were apparent throughout the duration of the task and, therefore, support the notion of a consistent benefit following caffeine on this measure. These findings are of relevance to results presented by Lane and Bute-Phillips (1998) who measured vigilance performance of 30 habitual coffee drinkers on a 30-minute visual monitoring task following a morning of either *ad libitum* caffeine

consumption or caffeine deprivation. They found that deprivation led to impaired performance in terms of decreased hit rate and slowed response time. The authors suggest that this result is indicative of the negative effects of caffeine deprivation in habitual caffeine consumers. However, this deterioration in performance on a 30-minute task is likely to represent a fatigue effect, which the *ad libitum* consumption of caffeine is able to offset.

Ruijter et al. (2000) suggest that one of the reasons that caffeine may be able to offset this decline in vigilance or sustained attention performance is its ability to increase arousal. In order to explore this further, participants were administered decaffeinated coffee, either with or without 250 mg of caffeine added, and asked to complete a 10-minute concentration task while ERP measurements were co-monitored. Although the ERP results support the expected arousing effect of caffeine, the behavioural data did not show any effect of caffeine. The authors suggest that the reason for this lack of effect is most likely due to the demands of the task, as unlike the other vigilance tasks described above, the task utilised here required a response to every stimulus, thus making the task more difficult. This suggests an interaction between the arousal level elicited by the task and that produced by caffeine.

The suggestion of a role for task demand in the effects of caffeine is supported by studies that have considered the effects of caffeine on Rapid Information Processing (RIP) tasks. Although these are measures of sustained attention they also involve working memory and are substantially more demanding than the vigilance tasks described above, which generally involve simply pressing a button whenever a repetition of a digit is observed or when two digits match. The effects of caffeine on RIP are fairly reliable but appear to be slightly more fragile than the effects seen on the vigilance measures above. The effects of caffeine on RIP are reviewed below.

Hasenfratz and Battig (1994) considered the effects of caffeine on mental performance, EEG, cardiovascular and subjective parameters in moderate female consumers. In a crossover study of 1.5, 3 and 6 mg/kg of caffeine added to decaffeinated coffee they found that, compared to placebo, all except the middle dose improved speed of response on a 20-minute Rapid Information Processing task (also known as the Bakan task) requiring participants to detect and respond to targets of three consecutive odd or even digits as quickly as possible. The authors suggest that this result is indicative of an inverted-U dose response with individually different optimal doses. This is, however, difficult to reconcile with the U-shaped nature of the published results. In a placebo-controlled crossover study of 75 and 150 mg of caffeine, dissolved in decaffeinated coffee, Warburton (1995) also examined the effects of caffeine on a Rapid Visual Information Processing (RVIP) task with digits presented at a rate of 100

per minute. Unfortunately, no information was given with regards the length of the task. Speed and accuracy of response were found to be significantly improved in a dose-related manner. The doses used by Warburton (1995) equate to around 1 and 2 mg/kg in the average 70 kg adult. Taken together, the results from Warburton (1995) and Hasenfratz and Battig (1994) may suggest that at some point between 2 mg/kg and 3 mg/kg the effects of caffeine on information processing are lost. However, there are differences between the tasks in the two studies that may make this conclusion invalid – the RIP task utilised in Hasenfratz and Battig (1994) not only presented digits at a rate of 90 per minute as opposed to 100 per minute in Warburton (1995), but the rate of presentation was also linked to performance following the first minute so that the inter-digit interval decreased by 33 msec after each correct response and increased by 33 msec after each error i.e. missed target or false alarm. This performance-linked presentation rate serves to eradicate individual differences in performance of the task and maintains an even difficulty level for all participants. It is possible that the lack of effects at the middle dose (3 mg/kg) is indicative of differential effects at high (6 mg/kg) and low (1.5 mg/kg) levels of caffeine related to high and low performance of the task. This proposition would also seem to fit with the authors' suggestion of individually different optimal doses. This difference in task difficulty level may also explain the divergence in results with regard accuracy, i.e. Warburton (1995) reported improvements to speed and accuracy whereas Hasenfratz and Battig (1994) only found effects on speed of response.

Yeomans et al. (2002) also considered the effects of 1 mg/kg of caffeine on RVIP in a placebo-controlled study that explored the effects of preloading with 0, 1, and 2 mg/kg of caffeine. The findings showed that preloading with 1 and 2 mg/kg of caffeine was able to speed performance of this task, in comparison to placebo, 45 minutes following administration. However, the effects of caffeine in a second drink, the test drink, were only observed when the preload had been placebo. Again this was evinced as a speeding of reaction time following caffeine but with an additional increase in the number of correct responses. Smit and Rogers (2000) also found that although low doses of caffeine significantly improved RVIP performance, this effect was only seen in high consumers.

It is clear that, whilst caffeine is able to improve RIP measures, this effect is moderated by other factors, such as dose (which may have an individual optimum level), habitual caffeine consumption, recent caffeine intake, and difficulty of the task. The importance of the difficulty of the task is illustrated in a recent study by Attwood et al. (2007). Whilst the majority of studies show some benefit of caffeine to RIP, Attwood et al. (2007) failed to find any effects on a visual information processing task. This task

was similar to the task used in many other studies of caffeine, which have shown improvements, in that it required the detection of three odd or three even digits presented consecutively in a series of sequentially presented digits. However, in this case the targets were three specific 3-digit number strings, thereby increasing the difficulty of the task quite dramatically. The lack of effects on this task again highlights the importance of arousal levels to the effects of caffeine on behaviour (see Section 1.9).

1.6.4 Working memory

Memory effects of caffeine are less robust than more simple cognitive effects. However, there is some evidence of effects of caffeine on working memory, and it has been suggested that working memory is more susceptible to the effects of caffeine than secondary memory.

One study to date has produced main effects of caffeine on the Sternberg-type memory scanning task. Kerr et al. (1991) found that 300 mg of caffeine was able to produce faster reaction time on a task requiring participants to memorise a new 4-digit string before presentation of each probe. Conversely, Hogervorst et al. (1998) utilised a task that required the identification of 1, 2, or 3 consonants from a list of 48 presented letters. They found that 225 mg of caffeine impaired performance of this task as compared to placebo, but this effect was only evinced in younger participants (26 - 34 years), and not in the two older groups (46 – 54 and 66 – 74 years). Using a lower dose of caffeine (100 mg), Hindmarch et al. (1998) failed to find any effects of caffeine on a Sternberg-type task requiring a judgement as to whether a test probe was contained within a set of digits held in working memory.

Similarly, Warburton (1995) employed a repeated measures design and found that spatial recognition memory and visuo-spatial memory, were unaffected by 75, or 150 mg of caffeine. This finding is supported by Smith et al. (1999a) who found no effect of 200 mg of caffeine on a measure of visuo-spatial memory using an independent groups design, with baseline performance included as a covariate.

The effects of caffeine have frequently been measured using a logical reasoning task. This has produced mixed results with Smith et al. (1992) finding that 4 mg/kg of caffeine was able to increase accuracy as well as reduce response time on this measure. Smith et al. (1994b) also found improvements on this task following 4 mg/kg of caffeine but these effects were only evinced as an increase in speed. Similar improvements have also been observed when participants are tested nocturnally (Smith et al. 1993), and when participants have been exposed to minimal caffeine

abstinence (Warburton 1995). However, Smith et al. (1999b) failed to find any effects on this task employing a dose of 40 mg of caffeine. This lack of effect may be related to this low dose of caffeine but two other studies have failed to find effects, using doses previously shown to have positive effects on this task (Smith et al. 1994b and Smith et al. 1997).

1.6.5 Secondary memory

Secondary memory effects as measured by free recall have received considerable attention in the literature. The majority of studies have found no effects of caffeine upon recall. For example, Rogers and Dernoncourt (1998) examined the effects of placebo and 1 or 2 mg/kg of caffeine, administered in a counterbalanced order. They found no effects of caffeine on immediate recall either in young (20 – 35 years), or older (55 – 84 years) participants. Similarly, no main effects of caffeine on this measure have been demonstrated following 1.5 mg/kg (Smith et al. 1997); 3 mg/kg (Smith et al. 1994a); and 4 mg/kg (Smith et al. 1992). Warburton (1995) also failed to find effects on this measure following 75 and 150 mg of caffeine and similar null findings were elicited by 40 mg of caffeine (Smith et al. 1999b). However, dose-related improvements to delayed verbal memory, as measured by written recall of auditorily presented stimuli, were observed following 75 and 150 mg of caffeine (Warburton 1995). An interaction between caffeine and noise on immediate recall has also been observed, whereby caffeine was able to attenuate the decrement in performance observed in the placebo-noise condition. The same effect was also observed on a delayed word recognition task (Smith et al. 1997). A main effect on this task has also been evinced as faster speed of responding following 40 mg of caffeine (Smith et al. 1999b). However, several other studies have failed to replicate this effect using a range of caffeine doses from 1.5 to 4 mg/kg (e.g. Smith et al. 1992; 1994a; 1994b; 1997).

Smith et al. (1999b) found that 40 mg of caffeine led to improved speed of performance of a semantic memory task. This finding was replicated by Smith et al. (1992), and Smith et al. (1993) also found improved speed of responding on this task following 1.5 and 3 mg/kg, during both daytime and nocturnal testing. Further support for these findings on semantic memory comes from Smith et al. (1994b) who found that caffeine led to significantly faster and more accurate completion of a semantic memory task than placebo. This suggests a fairly robust effect of caffeine upon semantic memory. However, Smith et al. (1997) failed to find evidence of an effect on this task following 1.5 mg/kg of caffeine, either alone or in conjunction with noise.

Summary of cognitive effects

The effects of caffeine on cognition have been highlighted as being greatest when the task requires behavioural routine and speed (Battig et al. 1984). This proposition is supported by findings of reliable improvements on selective attention tasks that involve a time pressure. Effects on tasks that require the processing of information from multiple sources and working memory tasks have been shown to be less susceptible. These differential effects of caffeine on different tasks have been related to arousal. Simple tasks that do not elicit a great arousal response are particularly susceptible to the stimulant properties of caffeine, whereas those tasks that themselves induce increased arousal are often unaffected or even impaired by caffeine due to over-stimulation. This assertion is supported by Humphreys and Revelle's (1984) suggestion that arousal facilitates attention but hinders short-term memory, and Anderson and Revelle's (1983) assertion that the effects of arousal on performance are moderated by task characteristics.

1.7 Mood effects

1.7.1 'Alertness'

Perhaps the most widely accepted effect of caffeine is an increase in 'alertness'. Loke (1988) found that 200 mg and 400 mg of caffeine led to volunteers feeling more 'energetic' and less 'bored'. Quinlan et al. (2000) also found increases in 'energetic arousal' and decreased 'sedation' scores [achieved by combining mean scores for 'tiredness', 'drowsiness', and 'alertness' from Line Analogue Ratings Scale (LARS)] following 37.5, 75, and 150 mg of caffeine. These effects were replicated in a second study presented in the same paper where 'sedation' ratings were decreased by all except a 50 mg dose and 'energetic arousal' was increased following all doses (25, 50, 100, and 200 mg of caffeine). These effects showed a U-shaped response with the lowest and highest doses resulting in the greatest increases. A similar result was found by Hasenfratz and Battig (1994) who showed that 3 and 6 mg/kg of caffeine were capable of improving subjective ratings related to 'wakefulness' whereas 1.5 mg/kg (equivalent to around 90 mg in the average 60 kg female) was not. Herz (1999) considered the effects of caffeine on the Mood Grid (Eich and Metcalfe 1989) and found that 5 mg/kg of caffeine led to more 'aroused' ratings. The Profile of Mood States (POMS – McNair et al. 1971) also showed increased 'vigour' ratings and decreased 'fatigue' ratings as a consequence of caffeine. This was coupled with increased ratings on a 16-item self-report questionnaire targeting affective and somatic symptoms related to caffeine use (Rush et al. 1995).

Richardson et al. (1995) found decreases in 'tired' ratings following 70 and 250 mg of caffeine irrespective of caffeine consumer status. Similarly, Smith et al. (1999b) found increased 'alertness' ratings following 40 mg of caffeine. Smit and Rogers (2000) found that although 100 mg of caffeine was capable of increasing 'energetic arousal' no consistent effects were found with lower doses. However, all doses (12.5, 25, 50, and 100 mg) attenuated an increase in 'bored' ratings seen in the placebo condition. A similar effect was found by Childs and de Wit (2006) who reported that in nondependent caffeine users 'arousal' levels, as measured by the POMS, were significantly increased by 50, 150 and 450 mg of caffeine, 'fatigue' ratings were only decreased by 150 and 450 mg, and only the 450 mg dose increased 'vigour' ratings.

This effect has also been demonstrated in children. In a double-blind placebo-controlled crossover study of 9 - 11 year old children, Heatherley et al. (2006) found that 50 mg of caffeine administered in a fruit drink was capable of increasing self-rated 'alertness' in 17 non/low caffeine consumers and 9 consumers irrespective of caffeine habit. No other mood effects were evinced.

1.7.2 'Tension'

As well as increasing 'alertness' ratings, increases to 'tension' ratings have also been observed following a caffeine challenge. Kaplan et al. (1997) found that 250 and 500 mg increased 'nervousness' but only 500 mg increased 'anxiety', 'irritated' and 'tense' ratings. The intensity of these ratings was found to be related to plasma caffeine levels. Hasenfratz and Battig (1994) showed similar results, in that 3 and 6 mg/kg increased 'state anxiety' (as measured by the State-Trait Anxiety Inventory), compared to placebo and 1.5 mg/kg of caffeine.

However, other studies have found no effects on this dimension. In two studies considering the effects of different doses of caffeine in different vehicles Quinlan et al. (2000) found no effects on 'tense arousal' ratings using UWIST mood scales (Matthews et al. 1990). Similarly, Herz (1999) found no effects of a fairly high dose of caffeine (5 mg/kg) on the 'tension/anxiety' scale of the POMS.

Moreover, in a study comparing 1.5, 3 and 6 mg/kg of caffeine with placebo, Hasenfratz and Battig (1994) found that 1.5 mg/kg of caffeine actually reduced 'nervousness', an effect not seen with the higher caffeine doses or with placebo, which affected ratings of 'nervousness' in a similar way to that of higher doses of caffeine. A similar effect was found in a study by Warburton (1995) in which increased 'calm' and decreased 'tense' ratings were evinced following caffeine. Liquori et al. (1997) also

found that 17 mg of caffeine administered in cola decreased 'tense arousal' and 'irritability'.

The studies in this area seem to suggest that, at moderate doses, caffeine increases 'alertness' but that at higher doses this effect begins to manifest itself as increases in 'tension'. However, large interindividual responses to caffeine have been reported. This was explored by Alsene et al. (2003) with regards to gene polymorphisms, specifically a polymorphism of adenosine receptor genes. The adenosine A_{2a} receptor gene polymorphism 1976T>C, but not the A_1 receptor gene polymorphism 716T>G, has been related to panic disorder and, given that these receptors are involved in the effects of caffeine (Fredholm et al. 1999), it was hypothesised that A_{2a} receptor polymorphisms and possibly A_1 polymorphisms may be related to the anxiogenic effects of caffeine. Following administration of 150 mg of caffeine, subjective ratings of 'anxiety' were shown to be significantly positively associated with two linked polymorphisms on the A_{2a} receptor gene, the 1976C>T and 2592C>Tins polymorphisms, with no differential effects on other measures such as autonomic or cognitive response. These findings suggest that genetic variations can lead to a particular susceptibility to anxiogenic effects of caffeine. However, the volunteers were also divided into three groups based on their genotype at the A_{2a} 263C>T and the A_1 716T>G loci and no significant differences in their responses to caffeine were observed. These findings suggest that susceptibility to the anxiogenic properties of caffeine may be specifically related to the 1976T/T and the 2592 Tins/Tins variants in the A_{2a} adenosine receptor gene.

1.7.3 Other aspects of mood

Improvements to 'hedonic tone' have been reported in some studies - 40 mg of caffeine increased ratings in high and low consumers (Smith et al. 1999b) and Warburton (1995) found increases in 'friendliness', 'content' and 'happiness' ratings (as measured by Bond-Lader mood scales) following 75 mg. However, these effects appear to be less robust than 'arousal' effects. In two studies examining various caffeine doses, Quinlan et al. (2000) found conflicting effects on 'hedonic tone' ratings, with a first study showing significant improvements following all doses (37.5, 75 and 150 mg) and a second study showing no effect following similar doses (25, 50, 100 and 200 mg). Kaplan et al. (1997) found increases in 'elation' following both 250 and 500 mg of caffeine but 500 mg led to decreases in 'pleasant' ratings.

1.8 Possible explanations of differing effects of caffeine

Many studies examining the effects of caffeine have been criticised on the basis of their methodological characteristics. Early experiments suffered from a lack of baseline data and some lacked either a placebo condition or double-blinding. It is possible that the effects of caffeine may not be apparent in certain studies because of a lack of task sensitivity or an inappropriate dose selection. As Hasenfratz and Battig (1994) suggest, different parameters may have different ranges in which they are sensitive to caffeine. This would explain why some studies might find positive effects on a particular measure whereas another may find no effect and, occasionally, even a negative effect. Individual differences have also been highlighted with regard to the effects of caffeine (see Section 1.9.3). This would suggest that small sample sizes also pose a problem, as variance due to individual differences will be more pronounced with small participant numbers. As well as increasing sample size, adopting a crossover design may go some way to combating problems associated with individual differences, particularly in cases of dose-response studies. Individual differences may also have played a greater role in earlier studies due to the use of very high caffeine doses. This is partly illustrated in a more recent study by Kaplan et al. (1997), which examined the effects of 250 mg and 500 mg of caffeine. The higher dose used here is equivalent to over 5 cups of coffee in a single serving, and as such led to somatic symptoms such as nausea, perspiration, palpitations, paresthesias, restlessness and tremor, which were substantially greater than those found with the lower caffeine dose or placebo. More recently the influence that caffeine withdrawal and regular caffeine consumption patterns play in the effects of caffeine have also been highlighted and these will be considered in more detail in the next section.

1.9 Factors affecting the effects of caffeine

1.9.1 Habitual caffeine intake

1.9.1.1 Withdrawal

It has been suggested that caffeine has no net benefits and is only able to reverse deficits seen in habitual caffeine consumers as a result of withdrawal (James 1994; Rogers and Dernoncourt 1998). This suggests that caffeine is merely able to restore normal functioning in the suboptimal situation of caffeine withdrawal. In order to assess the contribution of withdrawal alleviation to the effects of caffeine, it is first necessary to consider the evidence with regards the existence of caffeine withdrawal, this is reviewed below.

The effects of acute caffeine deprivation in habitual caffeine consumers were explored in a series of crossover studies, whereby participants were tested around midday having abstained from caffeine since the previous evening, or, following *ad libitum* consumption of caffeine (confirmed via caffeine diaries). Lane (1997) found that mean arterial blood pressure was decreased following deprivation. Deprivation also led to decreased 'vigour' and increased 'fatigue' as measured by the POMS. Impaired items on a withdrawal scale related to 'arousal', 'well-being', 'sociability', 'motivation to work', 'ability to concentrate' and 'headache'. There were no effects on psychomotor performance as measured by tapping, serial memory, choice reaction, digit symbol substitution, and logical reasoning. The authors propose that these effects demonstrate deleterious effects of caffeine deprivation. Expectancy effects are likely to have affected this study because participants were not blind to the condition that they were in each day and this could be involved in the observation of subjective effects but not objective effects. Nevertheless, these results demonstrate fairly naturalistically the effects of missing morning caffeine, particularly with the inclusion of expectancy effects, as it is unlikely that in everyday life people will be unaware of caffeine deprivation. In a similar experiment Phillips-Bute and Lane (1998) attempted to further explore the effects of deprivation on mood, withdrawal, and psychomotor performance, although slightly different psychomotor tasks were used. In order to overcome potential problems with differences in *ad libitum* consumption in the previous study and to eradicate problems with expectancy associated with *ad libitum* consumption, they pre-dosed participants 4 hours prior to testing with a fixed dose of 250 mg of caffeine or placebo using a double-blind design. They found similar but different effects to Lane (1997), possibly representing the effects of administering a single high dose of caffeine versus naturalistic consumption. Systolic and diastolic blood pressure were lower following deprivation. 'Vigour', as measured by the POMS, was decreased and 'fatigue' was increased. The only withdrawal measures affected were' sleepiness' and yawning. It appears that by administering a single high dose, double-blind, the blood pressure effects of caffeine have increased but subjective effects have decreased. This reduction in effects on subjective measures may be representative of the switch from a naturalistic design to a double-blind one. A number of subjective effects of deprivation were presented in Lane (1997), which were not replicated in Phillips-Bute and Lane (1998). In particular, no increases in headache were observed in the latter study, suggesting a role for expectancy in this effect. Both studies failed to find any effects on psychomotor measures.

Lane and Phillips-Bute (1998) reverted to the naturalistic design employed in Lane (1997) but psychomotor tasks were replaced by a vigilance task and

cardiovascular measures were omitted. They found that deprivation of caffeine led to decreased hits on a 30-minute vigilance task, which required participants to respond whenever the same stimuli were presented twice in succession. They also found slowed response time following deprivation and an interaction between the effects of caffeine and block number in the number of false alarms produced. Caffeine deprivation also led to a decrease in perceived 'success on the task' and an increase in perceived 'difficulty'. Further effects of caffeine deprivation were observed on subjective measures. When deprived of caffeine similar ratings on the withdrawal scale were evinced as in Lane (1997) - where participants were also not blind to condition - with the addition of increased 'irritability' and decreased 'urge to do the task'. Similar effects were also observed on a POMS scale given before the vigilance task. Increased ratings for 'anger', 'depression', 'fatigue' and 'confusion' were coupled with decreased 'vigour' and a trend towards increased 'tension'. There were no effects on change scores on the POMS for post- minus pre-vigilance task scores. However, it is important to note that, although the authors present these effects as evidence of caffeine withdrawal, these results involve a comparison of performance and mood following a morning of caffeine abstention or a morning of caffeine administration. Therefore, these findings are more likely to represent positive effects of caffeine rather than detrimental effects of withdrawal. The finding of effects on a vigilance task with no effects found previously on psychomotor tasks further supports the posit that these effects represent benefits of caffeine administration rather then impairment following caffeine deprivation since vigilance tasks are particularly susceptible to caffeine administration.

However, other studies have considered withdrawal effects in the absence of caffeine administration. Common withdrawal symptoms reported include 'headache', 'drowsiness', and decreased 'concentration' (Griffiths et al. 1990). These symptoms were confirmed in a series of studies that considered the dosing conditions for caffeine withdrawal to occur (Evans and Griffiths 1999). Combined effects across 4 experiments showed that replacement of caffeine with placebo following caffeine maintenance of 300 mg/day led to significant increases in 'headache', 'poor mood', 'tiredness', 'fatigue', 'confusion', and total 'mood disturbance'. 'Activity', 'vigour', and 'friendly' ratings were also decreased. These effects were seen whether caffeine was maintained using a dosing schedule of a single 300 mg dose in the morning each day or using 3 separate 100 mg doses per day. As little as 100 mg/day was shown to be sufficient to produce withdrawal symptoms but the effects were more widespread following 300 mg/day and 600 mg/day. In addition, significant differences existed between 100 mg/day maintenance and 600 mg/day maintenance in terms of the magnitude of withdrawal effects. Substitution of the maintenance dose (300 mg) with 25 mg of caffeine was also

shown to reduce 'activity' ratings and substitution with 25, 50, and 100 mg increased 'tired' ratings. These effects of caffeine withdrawal were shown to develop following as little as 3 days of maintenance with 300 mg of caffeine. Goldstein et al. (1969) also found impairment before and after placebo administration in habitual consumers following overnight abstention, as compared to non-consumers who suffered no such impairments. Prior to administration of placebo, these effects took the form of lower ratings of 'alert', 'content', 'relaxed', 'active', and 'energetic' and higher ratings of 'sleepy', 'drowsy', and 'irritable' in habitual consumers than non-consumers. These effects were less marked following placebo with lower ratings of 'alert' and 'energetic' and higher ratings of 'jittery' and 'irritable' in habitual consumers than non-consumers. Conversely, Bruce et al. (1991) found only a significant impairment to 'physical tiredness' ratings following overnight abstention in habitual consumers as compared to non-consumers, with no significant differences on 'alert', 'content' or 'calm' mood factors (Bond and Lader 1974) and no effects on ratings of headache. However, they found that following placebo consumers had higher ratings of 'physical tiredness' and 'headache' than their non-consumer counterparts.

Although these findings appear to paint a very clear picture of caffeine withdrawal, results from Dews et al. (1999) contradict this. Dews et al. (1999) conducted a population-based telephone survey followed by a controlled study in order to investigate withdrawal effects. Of a sample of 11,112 participants 61 % reported daily caffeine consumption but only 11 % of them reported symptoms upon stopping caffeine. Of those reporting symptoms only 0.9 % of men and 5.5 % of women reported symptoms severe enough to interfere with normal activity. In order to explore this further a subset of daily caffeine consumers who had reported symptoms of caffeine withdrawal were selected to take part in a controlled, blind study of caffeine withdrawal effects. This subset was then split into three groups - those exposed to gradual cessation of caffeine (replacement of coffee with decaffeinated coffee), those exposed to abrupt cessation, and those maintained on caffeine. The findings showed that 37 % of those exposed to abrupt cessation showed some symptom of withdrawal, whereas those exposed to gradual cessation reported minimal if any symptoms. This is despite 100 % of both groups previously reporting symptoms upon cessation.

In a review of the behavioural effects of caffeine withdrawal Dews et al. (2002) points out that there is immense variation in the prevalence of withdrawal symptoms reported ranging from 0 %, in studies not focused upon caffeine withdrawal, to 100 %, when participants knew they were withdrawn. This suggests that expectancy is an issue here, given the high prevalence of symptoms in a non-blind study. Dews et al. (2002) points out that this expectancy appears to extend to laboratories that have

focused upon caffeine withdrawal symptoms, given that studies from Griffiths and colleagues have found prevalence ranging from 82 to 100 %, whereas Dews et al. (1999), where research was not previously linked to caffeine withdrawal, found only 37 %. Dews et al. (1999) suggest that caffeine withdrawal causes a subtle syndrome, which is primarily determined by non-pharmacological factors. In support of this is the finding from Dews et al. (1999) of a great disparity in the reporting of withdrawal symptoms between men and women, with 0.9 % men reporting symptoms compared to 5.5 % women. This disparity is not common, unless in studies related to physical sex characteristics, but has been seen in studies of noxebo effects. It is also clear, as identified from studies that measure caffeine deprivation by comparing caffeine administration with non-administration, that when collecting data on withdrawal symptoms participants may actually be reporting deprivation from the normal enhancement they receive from caffeine rather than a genuine suboptimal situation (Smith et al. 1993). In order to further understand the effects of caffeine withdrawal, a number of studies have compared the behaviour of non-consumers with that of caffeine abstinent consumers and these are reviewed below.

In a study examining the mood effects of chronic caffeine abstinence, Richardson et al. (1995) found that chronic abstinence led to increases in 'headache' as compared to baseline. This effect was apparent at 6 - 7 days abstinence and remained until day 16-17 of abstinence. 'Cheerful' ratings were also significantly reduced but this effect did not become apparent until day 8 - 9 of abstinence and was apparent until the end of the study (day 18 - 19). This study also examined the acute effects of caffeine abstinence by comparing the mood and psychomotor performance of overnight abstainers with the chronic abstainers referred to above as well as 90-minute abstinent consumers and non-consumers (<15 mg/day). They found that overnight abstinence led to significantly increased 'headache' ratings as compared to non-consumers. However, they did not differ significantly from the ratings of chronic abstinent and 90-minute abstinent. 'Clearheaded' ratings were also significantly lower in the overnight group as well as the chronic group as compared to non-consumers and 90-minute abstinent. The 90-minute abstinent group rated themselves as more 'cheerful' and more 'friendly' than the other 3 groups whereas the overnight group were more 'angry', 'dejected', 'tired', and 'drowsy' than the other groups. The study failed to find any significant acute effects of withdrawal on any of the objective measures of simple reaction time, hand steadiness and tap performance. Similarly, in the first of two studies, Rogers et al. (2003) found that overnight abstinent consumers (>200 mg/day) were less 'alert' and more 'tense' than their non-caffeine consuming (~ <50 mg/week) counterparts. However, in a second study Rogers et al. (2003) failed to find this effect

comparing similar populations to the previous study, and also found no evidence of withdrawal effects on a simple reaction time task employed

Several other studies have failed to find any effects of overnight caffeine abstinence when comparing caffeine consumers with low/non-consumers. Smith et al. (2006) compared mood and performance of 25 non-consumers with 25 regular consumers (mean = 195 mg/day) and found only one difference at baseline. The consumers produced fewer false alarms on an RVIP task than non-consumers, but the authors assert that this is likely to be a chance effect. A similar assertion is made regarding the findings from a study comparing non-consumers with high and low consumers. Hewlett and Smith (2006a) compared performance and mood in these three groups following overnight caffeine abstention and found that low consumers had lower 'alertness' scores prior to performance assessment and lower 'hedonic tone' after performance assessment. Low consumers were also more accurate than non-consumers on a semantic memory task, whilst high consumers produced fewer false alarms than non-consumers on a recognition memory task. Attwood et al. (2007) also compared the mood and performance of moderate (<200 mg/per day) and high (>200 mg/day) caffeine consumers and found no differences between the two groups at baseline following 11 hours of abstinence.

It is clear that, despite what appears to be a well-defined syndrome from Evans and Griffiths (1999), studies related to caffeine withdrawal symptoms and negative effects of withdrawal on performance have produced mixed results. Two points raised by Dews et al. (2002) are relevant to the literature in this area - the issue of expectancy of caffeine withdrawal is relevant to studies that impose overnight caffeine abstinence, as consumers clearly are aware that they have abstained from caffeine. This point is supported by findings from two studies using a very similar methodology – Lane (1997) found that unblinded caffeine deprivation led to increased 'headache' ratings, but this effect was not replicated by Phillips-Bute and Lane (1998) when a double-blind procedure was employed. In studies of overnight and chronic abstinence the issue of what is being compared is also of importance because if consumers are comparing their subjective experience in abstinence with that in the presence of caffeine then this could appear to represent impairment. This is the case even when comparing consumers with non-consumers, as consumers may rate themselves as relatively less 'alert', for example, if they are comparing how they feel in the morning following caffeine deprivation with how they normally feel following an acute caffeine dose. The importance of non-pharmacological factors in this area is highlighted by the lack of objective effects of withdrawal. In order to further explore whether caffeine merely

alleviates caffeine withdrawal, several studies have considered the effects of caffeine in non-withdrawn participants and these are reviewed below.

1.9.1.2 Withdrawal alleviation

1.9.1.2.1 Objective effects

A number of studies have compared the effects of an acute caffeine challenge in habitual consumers and low/non-consumers. If non-consumers respond to caffeine in the same way as consumers then this would argue against withdrawal reversal. If non-consumers respond to caffeine but in a different way to consumers then this would also provide evidence against the reversal of withdrawal hypothesis and simply indicate that responses to caffeine may differ as a function of consumption history. However, if no effects of caffeine are found in non-consumers then this would provide support for the alleviation of withdrawal theory.

Smit and Rogers (2000) found that caffeine was equally capable of improving simple reaction time in high caffeine consumers who consumed more than 200 mg per day (mean = 340 mg) as in low consumers who consumed less than 100 mg per day (mean = 84 mg). This result therefore argues against withdrawal alleviation given that, according to Evans and Griffiths (1999), consumption of 100 mg of caffeine per day is sufficient to produce caffeine withdrawal effects. However, Smit and Rogers (2000) also found differences in response to caffeine as a result of consumer status. RVIP performance was significantly improved in high consumers but not low. It should be noted that the high and low consumers in this study differed significantly in age, with high consumers being older than low consumers, and this may account in part for the greater effects seen in the high group (see Section 1.9.5). It is also noteworthy that the high consumers' performance was significantly superior at baseline than the low consumers on this task.

Richardson et al. (1995) also found no significant differences in response to caffeine as a function of group on simple reaction time or hand steadiness in low/non caffeine consumers (<15 mg/day) and habitual consumers (>200 mg <1000 mg/day) who were either 90 minute, overnight, or chronic (7 - 19 days) abstinent. Lieberman et al. (1987) administered various doses of caffeine in capsule form to 20 male participants split in terms of habitual caffeine consumption and found that each dose was capable of improving performance of a modified Wilkinson auditory vigilance task and a four-choice reaction time task, and that low (mean = 60 mg/day) and high (mean = 314 mg/day) habitual consumers were affected similarly. Smith et al. (1999b) found

that 40 mg of caffeine increased speed of focused attention reaction time and categoric search reaction time, increased 'alertness' ratings, 'hedonic tone' and 'anxiety' ratings in high and low consumers and found no interaction between the effects of caffeine and consumer status in the two groups. Hewlett and Smith (2006a) considered the effects of 1 mg/kg of caffeine in low, high and non-consumers of caffeine and found that overall the responses to caffeine were the same for these groups with two exceptions - choice reaction time was significantly faster for consumers following caffeine and significantly slower for non-consumers. There was also a significant difference between low and high consumers on this measure with high consumers receiving a greater benefit. Conversely, focused attention reaction time was significantly faster for both high and non-consumers and significantly slower for low consumers. In addition, the effect was greatest in non-consumers on this measure. These findings seem to suggest few differences in response to caffeine when comparing consumers with non-consumers. James and Rogers (2005) have suggested that comparison of these groups is invalid as they represent two distinct self-selected groups and as such may not be comparable populations. However, Hewlett and Smith (2006b) compared these two groups and found very little difference between them in terms of demographic variables or psychological characteristics. This is supported by findings from Brice and Smith (2001b) which show very little evidence that personality traits influence caffeine consumption levels.

In addition, one study examined the effects of caffeine in light, nondependent users only (<300 mg per/week, mean = 117 mg), with the aim of elucidating any effects of caffeine independent of any withdrawal effects. Childs and de Wit (2006) found that 150 and 450 mg of caffeine increased systolic and diastolic blood pressure and increased hit rate on a visual vigilance task, 450 mg also speeded response time on this task. Impairment was also seen following 450 mg, this took the form of a decrease in the number of digits remembered in a backwards digit span task. The findings of response to caffeine in nondependent consumers would seem to indicate the existence of effects of caffeine outside of withdrawal alleviation.

The role of withdrawal in the effects of caffeine has also been further explored by examining the effects of non-withdrawn caffeine consumers. Warburton (1995) tested the effects of placebo, 75 mg and 150 mg of caffeine in 18 male regular coffee consumers (> 3 cups per day), who were pre-dosed with 75 mg of caffeine 1 hour prior to testing. Improvements were seen to RVIP, semantic verification and delayed recall but no effects were found on two non-verbal memory tasks. Unfortunately, although baseline data is referred to, this data is not presented, and it is clear that baseline performance for each day has not been evaluated. In addition, it has been suggested

that 75 mg may not have been sufficient to alleviate withdrawal in all of the volunteers (Yeomans et al. 2002). In a similar study van Duinen et al. (2005) found effects of 3 mg/kg of caffeine in participants who had been allowed to consume one cup of coffee before 10 am. However, similar criticisms could be applied to this study as those applied to Warburton (1995). In an attempt to overcome this potential problem of insufficient preload, Yeomans et al. (2002) replicated Warburton's (1995) study but contrasted preloads of 1 and 2 mg/kg with placebo in a between subjects design. They also included a baseline assessment each day and attempted to avoid other common methodological problems in caffeine research – high doses; lack of double-blind; use of typically caffeinated beverages resulting in the possibility of increased placebo response; and explicit reference to caffeine resulting in confounding effects of expectancy. Drinks were administered as fruit tea and contained 0, 1, or 2 mg/kg of caffeine when administered as a preload and 0 or 1 mg/kg of caffeine when administered as a challenge. The second drink was administered according to a within subjects design. Results showed that although the caffeine preload speeded RVIP response and increased 'alertness', improvements to RVIP and 'alertness' following the second drink were only seen following caffeine when the preload had been placebo. The authors suggest that these results support the notion of the effects of caffeine only being apparent when consumers are in a state of withdrawal. However, it is also possible that the reason for the lack of effects of a second caffeine dose is due to continuing effects of the caffeine preload. Testing following the second drink takes place only 105 minutes after the preload so it may be the case that caffeine is only capable of improving behaviour to an individual peak and that adding more caffeine will not increase this further. Some support for this notion comes from the rather flat RVIP speed of response curve and 'alertness' curve at 45 and 105 minutes following placebo or caffeine in the 1 mg/kg preload condition. In the 2 mg/kg preload condition RVIP speed begins to slow to baseline levels following both caffeine and placebo suggesting that by adding more caffeine you are actually seeing the equivalent of a high single dose of caffeine, which, as the authors point out, would be less effective and produce more side effects. Further support for this comes from the finding of improved 'alertness' following 1 mg/kg preload but not 2 mg/kg. In a further exploration of the role of withdrawal in the effects of caffeine Christopher et al. (2005) examined the effects of 2 mg/kg of caffeine in regular consumers in the evening following a day of normal caffeine consumption. Consumption of caffeine was verified by analysis of salivary caffeine levels, which were significantly elevated. Caffeine led to increased subjective 'alertness' and faster reaction times on a repeated digits task when compared to placebo despite the lack of deprivation in these participants. The suggestion by

Yeomans et al. (2002) that caffeine following a preload can have no additional benefit also raises the question of why consumers continue to consume caffeine throughout the day (although more so during the morning) since the 1 mg/kg preload utilised equates to around 70 mg, which is less than the average cup of coffee. Consumers should only 'need' their first cup of coffee in order to alleviate withdrawal. This was explored by Heatherley et al. (2005) who considered the effects of caffeine following a pre-load (1.2 mg/kg) given at different intervals prior to the caffeine challenge (1.2 mg/kg). Abstention prior to caffeine challenge was either 4 hours, 6 hours, or 8 hours. The findings showed that only 8 hours abstention resulted in positive effects of caffeine, for example, improvements to focused attention and reasoning as well as increased 'energetic mood' and 'hedonic tone'. Systolic blood pressure was also increased in response to caffeine in the 8 hour abstainers. Interestingly, some effects were evinced in the four hour abstainers. These were impairments to psychomotor performance and increases in perception of demands of the tasks. These results are very interesting in that they are intended to represent the daily pattern of caffeine consumption. The findings do not provide any explanation for the typical pattern of consumption, as no positive effects are observed in response to caffeine until 8 hours after a pre-dose. Furthermore, effects are elicited in the form of impairment 4 hours after the pre-dose. It is not clear why these effects should motivate caffeine consumption as part of a daily routine, nor is there any obvious explanation for why these impairments should occur. Subjective effects have also been considered using the paradigms outlined above, these are described below.

1.9.1.2.2 Subjective effects

Smit and Rogers (2000) found that high and low consumers' ratings of 'energetic arousal' were similarly increased following 100 mg of caffeine. Richardson et al. (1995) also found significant decreases in 'headache' and 'tired' ratings, and increases in 'jittery' ratings following caffeine administration, irrespective of group. The authors suggest that the reason for this lack of effect of consumer status may be that caffeine is capable of increasing 'arousal' when lowered by other factors (see below) and that the monotony and duration (1.5 hours) of the session may explain why non/low consumers were affected by caffeine. However, this finding has also been demonstrated in studies that have not utilised such a long and monotonous task. For instance, Smith et al. (2006) considered the effects of 2 mg/kg of caffeine in non-consumers and overnight withdrawn consumers and found few differences between the two groups' responses in terms of mood and cognition. However, the differences that

were observed all demonstrated a greater response in non-consumers than withdrawn consumers - these effects were increased 'alertness' and 'anxiety', as well as fewer lapses of attention. Increased 'alertness' ratings following 50 mg of caffeine administered in a fruit drink have also been demonstrated in children (17 non/low caffeine consumers and 9 consumers) irrespective of caffeine habit (Heatherley et al. 2006).

In an independent measures study examining the reinforcement effects of caffeine, Rogers et al. (1995) compared preference ratings for caffeinated and decaffeinated fruit drinks in 24 low caffeine consumers (<120 mg; mean = 47 mg), and 25 moderate caffeine consumers (≥ 120 mg; mean = 205 mg). The target drink, which had been ranked fourth out of seven novel flavoured drinks rated for preference in an evaluation session, was administered after breakfast on 10 weekday mornings, and then a preference rating was given on a scale of 0 – 7. A significant 'caffeine use' by 'drink caffeine content' interaction was found with low consumers' drink preference ratings significantly increasing irrespective of caffeine content of the drink, whereas in the moderate consumers, preference ratings increased when given a drink containing 70 mg of caffeine and significantly decreased when given a decaffeinated version. Mood effects 1-hour post caffeine ingestion were also considered. No significant interactions were found for mood ratings but the authors suggest that this was due to small, non-significant effects of caffeine in the low users. As such they carried out separate analyses of the two groups. This produced significant effects of caffeine only in the moderate consumers, with the decaffeinated drink reducing ratings of 'lively', 'clearheaded', 'cheerful' and 'energetic' and increasing 'tired' ratings.

However, Warburton (1995) found effects of caffeine on mood in non-withdrawn consumers who had received a pre-load of 75 mg of caffeine 1 hour prior to caffeine challenge with 75 or 150 mg of caffeine. Caffeine increased 'clearheadedness', 'calmness', and 'happiness' and decreased 'tension' even in those minimally abstinent. These findings are supported by Childs and de Wit (2006) who showed effects of caffeine in non-dependent users (<300 mg/week). Results from the POMS showed increased 'vigour', 'anxiety', and 'arousal' as well as increased 'positive mood' and reduced 'fatigue' ratings. Attwood et al. (2007) also found no significant differences when comparing the mood effects of 400 mg of caffeine in moderate (<200 mg/day) and high (>200 mg/day) regular caffeine consumers. However, a lack of effects was found in moderate consumers on cognitive measures, which the authors state is not attributable to tolerance or alleviation of withdrawal (due to a lack of evidence for withdrawal at baseline). Another difference between the two consumer groups was found in post-session questionnaires – high consumers were more likely to perceive

positive effects of caffeine such as increased 'cheerfulness', 'alertness', and 'well-being'. This finding could be explained by the effects of caffeine on cognition in that group, with improvements in performance leading to more positive perceptions. However, the opposite could also be true, positive perceptions could result in a knock-on effect on performance. It is also possible that the reason these participants are high consumers is because they receive a greater benefit from ingesting caffeine than their moderate consumer counterparts. It is, however, difficult to reconcile these differences in the perceived positivity of the effects of caffeine with a lack of significant differences between the two groups in mood responses to caffeine.

1.9.1.2.3 Summary of withdrawal alleviation

As with studies not related to the question of withdrawal or withdrawal alleviation, there are a number of similar possible explanations of the differing results across studies. These include the sensitivity of tasks, dose-specific effects, and the arousal level of participants. The effects of caffeine appear to be more pronounced in, but not restricted to, low arousal situations. Arousal could be reduced by caffeine withdrawal but the evidence for the existence of withdrawal is equivocal. A number of studies have demonstrated equivalent effects in non-consumers and consumers and the effects of caffeine have also been observed when looking at nondependent users alone. This would suggest that caffeine does have absolute effects on performance and mood. There has been some indication of slight differences in response of consumers and non-consumers to caffeine, which suggests that habitual caffeine intake, may moderate some of the effects seen in response to caffeine. Other factors that have been implicated in moderating the effects of caffeine are outlined below.

1.9.2 Fatigue

It has been suggested that the effects of caffeine are greatest when performance or mood is impaired. This point was explored above in relation to the idea that caffeine only benefits those in a suboptimal state of caffeine withdrawal. A number of factors, such as sleep deprivation, age, and alcohol, were also referred to in Section 1.6.2 in relation to the reaction time effects of caffeine when impaired. This impairment-alleviating effect has also been explored in relation to task-induced fatigue. Of relevance here is the inverted-U hypothesis (Yerkes-Dodson 1908), which states that there is a negative quadratic relationship between arousal and performance, with increasing arousal leading to improved performance until the arousal becomes too high, leading to deficits. Also of relevance is the task difficulty hypothesis (Yerkes-

Dodson 1908) that lower arousal is needed for optimum performance on difficult tasks and higher arousal is needed for optimum performance of simpler tasks. These hypotheses represent the Yerkes-Dodson Law (YDL; Yerkes and Dodson 1908) and caffeine is often used as a test of this law. Essentially, caffeine is viewed as an arousing agent, which, if the dose is too high will have a negative effect upon performance. However, caffeine interacts with other arousing factors, such as the demands of the task, and caffeine administered in conjunction with a low arousal task will result in improved performance, whereas caffeine administered in conjunction with a difficult task will not, and may even result in impairment. Humphreys and Revelle (1984) explain this inverted-U as being the result of a positive relationship between arousal and attentional processes and a negative relationship between arousal and short-term memory processes, which, given that most tasks involve some combination of these two processes, manifests as an inverted-U. This suggests that caffeine as an arousing agent should have a positive correlation with the performance of attentional tasks, and a negative relationship with short-term memory performance, but takes into consideration that the majority of tasks incorporate both processes.

With regards the effects of caffeine during task-induced fatigue, Brice and Smith (2001b) found that 3 mg/kg of caffeine was capable of significantly increasing 'alertness' ratings in habitual caffeine consumers when compared to placebo, and that this was particularly true following a fatiguing driving task. Horne and Reyner (1996) compared the benefits of napping with 150 mg of caffeine in overcoming driver sleepiness elicited by two 1 hour monotonous driving sessions. Caffeine and a 15-minute nap were both shown to reduce driving impairments and subjective 'sleepiness'. These findings received support from a study of the effects of 200 mg of caffeine, a 30-minute nap, or placebo, taken prior to a 200 km drive, showing a reduction in impairment to night-time driving following caffeine or a nap (Philip et al. 2006). These findings are in keeping with government advice regarding tiredness and driving, with the United Kingdom Highway Code recommending naps or coffee to counteract tiredness. Kennedy and Scholey (2004) explored the effects of caffeine combined with glucose utilising a task battery that has been shown to induce increased 'mental fatigue'. They found that caffeine/glucose was able to improve performance on an RVIP task and attenuate the increase in subjective ratings of 'mental fatigue'. The effects of caffeine during fatigue are also illustrated in a study by van Duinen et al. (2005) who found effects on a dual-task involving a motor task and a choice reaction time task. The authors also demonstrated effects of caffeine on the choice reaction time task performed as a single task but this effect was only apparent after completion of the dual-task, suggesting a heightened effect of caffeine when fatigued. Smith et al.

(2005) compared the effects of caffeine in alert and fatigued participants and found improved speed of encoding on a focused attention task, improved accuracy of vigilance and increased 'alertness' in alert participants. Additional effects were found in those participants exposed to fatigue through prolonged testing in the form of faster simple reaction time and fewer long responses on the choice reaction time task. The authors suggest that the effects of caffeine in alert participants are mainly the result of effects on the cholinergic system, whereas effects in fatigued participants reflect the effects of noradrenaline.

1.9.3 Personality traits

It has been suggested that personality traits may modulate the effects of caffeine or at least impact upon consumption patterns. Richardson et al. (1995) compared habitual caffeine consumers with low/non-consumers on dimensions measured by the Eysenck Personality Questionnaire (EPQ) and the Impulsivity Venturesome and Empathy Personality Inventory (IVE). They found consumers scored higher on 'addiction' ratings, 'extraversion', and 'impulsivity' than non-consumers. 'Criminality', 'empathy', 'neuroticism', 'psychoticism' and 'venturesome 'were not related to habitual caffeine intake. This finding relating to 'impulsivity' is supported by evidence from Rogers et al. (1995) who found that those who score high on 'impulsivity' consume greater levels of caffeine. This relationship is indicative of the interaction between caffeine and arousal, i.e. those scoring high on 'impulsivity' have low levels of arousal and are more likely to consume more caffeine in order to increase arousal levels. However, Brice and Smith (2002) failed to find an association between personality traits and caffeine consumption. Similarly, Hewlett and Smith (2006b) found no relationship between caffeine consumption and 'impulsivity', 'extraversion', 'sociability' or trait 'anxiety'. However, Smith et al. (1994a) did find a relationship between 'impulsivity' and response to a caffeine challenge. High 'impulsivity' was associated with poor recall and slowed speed of response on recognition and semantic memory tasks when coupled with caffeine, whereas low 'impulsivity' resulted in impaired performance on a range of tasks when coupled with decaffeinated treatments. Given that highly 'impulsive' individuals tend to have lower arousal levels this is contrary to what would be expected from the inverted-U hypothesis. Evidence from Anderson and Revelle (1983) of an interaction between target size, task sequence, and 'impulsivity' suggests that these effects are moderated by the characteristics of the task. Highly 'impulsive' individuals were shown to be more sensitive to task characteristics than those scoring low on 'impulsivity'. However, Anderson and Revelle

(1994) found that deficits seen in highly 'impulsive' individuals in the morning were reversed when tested in the evening. This represents an interaction between caffeine, 'impulsivity', and time of day. The role of time of day in the effects of caffeine will be considered in the next section.

1.9.4 Time of day

An interaction between caffeine, 'impulsivity', and time of day was referred to in the previous section. This is indicative of changing arousal levels throughout the day. In a study of time of day variations and attention, Kraemer et al. (2000) found that vigilance performance began to increase at 9 am until it reached a peak at 1 pm when it began to decline. Wyatt et al. (2004) considered the effects of caffeine administration on circadian-related performance degradation during extended wakefulness. They found that caffeine attenuated impairment to several cognitive parameters seen as a result of extended wakefulness and that these effects peaked at the nadir of circadian performance. Although time of day effects are often implicated in the effects of caffeine, Smith et al. (1999b) tested the effects of caffeine in the morning between 10 am and 1 pm and in the afternoon between 2 pm and 5 pm and reported no interaction between time of day, personality and the effects of caffeine. It is possible that any effects were missed due to the lateness of the morning session, which actually carried over into the afternoon. With only 1 hour separating the two different times of day it is unlikely that any effects would be captured. This is particularly true in light of Wyatt et al.'s (2004) findings regarding peak vigilance performance at 1 pm. The impact of eating on these effects is also not considered (post-lunch dip) and fluctuating cortisol levels (either in conjunction with food consumption or alone) may also be implicated in time of day effects. The effects on a verbal learning test in adults over 65 following caffeinated or decaffeinated coffee have also been explored on different days in the morning and in the afternoon (Ryan et al. 2002). Free recall and recognition were shown to be affected by time of day, with better performance in the morning than afternoon. Significant interactions between caffeine and time of day were found on these measures demonstrating that caffeine was able to remove the decline in performance seen as a function of time of day. These findings indicate a possible relationship between time of day in relation and age. The effects of age will be considered in the next section.

1.9.5 Age

Although there are no differences in caffeine half-life between the young and the elderly (Blanchard and Sawers 1983), it has been suggested that caffeine will have

greater effects in older adults, perhaps indicating lower arousal in this population. In an epidemiological study, Jarvis (1993) found that increasing caffeine consumption was related to better cognitive performance and that this effect was greater in older adults. In contrast Hameleers et al. (2000) found no difference in sensitivity to caffeine intake between different age groups. The findings from Swift and Tiplady (1988) show differential effects of caffeine as a function of age. In this case the objective effects of caffeine were greater in elderly participants, whereas subjective effects were more pronounced in the young. Improvements to attention, choice reaction time, tapping and body sway were observed in the elderly, whereas only tapping rate was increased in the young. Ratings of 'calm', 'alert', 'interested', and 'steady' were increased in young participants only.

1.10 Effects of caffeine when combined

The behavioural effects of caffeine are often studied in isolation, this is despite the fact that consumption of caffeine in isolation is not commonplace. A number of studies have also studied the effects of caffeine in the form of coffee and compared these effects to the effects of decaffeinated coffee (e.g. Smith et al. 1990 – 1991; Hasenfratz and Battig 1994; Warburton 1995; Ruijter et al. 2000; Brice and Smith 2001b). However, these studies tend to disregard the other components within coffee and attribute any effects solely to caffeine. Although in these studies the only component that has been manipulated is the caffeine level, it remains unclear how caffeine interacts with the other components within coffee in terms of its effects on behaviour. This point is highlighted by the findings from one of very few studies that have considered the behavioural effects of tea. Hindmarch et al. (2000) found that coffee and tea produced significantly different effects on cognition despite a matched caffeine level. The behavioural effects of tea will be explored further in Chapter 3.

Another source of caffeine is energy drinks, which are becoming increasingly popular in the western market place and, consequently, have been the focus of some, limited, research. Red Bull is possibly the best known energy drink in the UK and its psychoactive effects have been considered in a small number of studies. Alford et al. (2001) found improved performance on a five-choice reaction time task in two studies when comparing Red Bull to carbonated water and when comparing Red Bull to carbonated water and to a no drink condition. Similarly, Warburton et al. (2001) carried out two studies examining the effects of Red Bull on RVIP performance, verbal reasoning, and verbal and spatial memory. In the first study the effects of this drink were compared to an inactive placebo and improvements were seen in terms of accuracy and response time on a RVIP task and increased speed of verbal reasoning.

In the second study the effects of this drink were further explored in comparison to a sugar-containing drink, which also contained 22.5 mg of caffeine. Despite the presence of glucose and caffeine within the control drink, the verum drink was again shown to improve RVIP performance in terms of speed and accuracy and to reduce response time on a verbal reasoning task. Improvements to cognition were also demonstrated in another study that explored the effects of Red Bull on daytime recovery sleep as well as subsequent performance (Jay et al. 2006). In a crossover study fifteen participants received Red Bull or placebo during a period of 24.5 hours of extended wakefulness (7.00 am - 7.30 am). The drinks were administered twice, at 1.30 am and 5.30 am. Daytime recovery sleep was subsequently monitored during an 8-hour sleep period and performance on a 10-minute Psychomotor Vigilance Task (PVT) was assessed at 2-hourly intervals for 6 hours upon waking. The findings showed that, although sleep duration and sleep efficiency were significantly reduced by Red Bull there was no subsequent impact of this sleep disruption upon performance of the PVT.

Although 250 ml of Red Bull contains 80 mg of caffeine as well as 1 g taurine, 600 mg glucoronolactone, glucose, and vitamins, the studies described above tend to assign the effects observed to the caffeine contained within the drink. However, Scholey and Kennedy (2004) found that the effects of caffeine and glucose combined are greater than the sum of the two parts. A combination of 37.5 mg glucose and 75 mg of caffeine (with ginkgo and ginseng at flavouring levels) was able to improve secondary memory performance – an effect only evinced as a trend following 75 mg of caffeine alone and with no effects of 37.5 mg glucose on this measure. The combination also produced faster speed of attention and a trend towards faster speed of memory. Neither of these effects was observed with the component parts in isolation. These findings suggest a synergy between caffeine and glucose when combined. Although Warburton et al. (2001) attempt to overcome the problem of a non-matched placebo in the second of their studies by adding glucose and a small amount of caffeine to their placebo, they do not match levels of taurine, glucoronolactone and vitamins - all of which could potentially impact upon cognitive performance, if not in isolation, then possibly in combination with caffeine.

A number of these energy drinks also contain guaraná, which is a natural source of caffeine that also contains othr purine alkaloids and a number of polyphenols. (Espinola et al. 1997). Evidence from rodent studies suggests that guaraná in rodents has behavioural effects. Espinola et al. (1997) demonstrated that chronic (9 months) administration of a lower dose (0.3 mg/ml) of guaraná, but not a higher dose (3.0 mg/ml) of guaraná or 0.1 mg/ml caffeine, improved swimming time in mice, when tested following 100 days and 200 days of treatment. Chronic administration of 0.3

mg/ml guaraná also reversed memory deficits in retention on a passive avoidance task in rats treated with 3.0 mg/kg scopolamine. Similar effects were also demonstrated following acute administration of guaraná. Three mg/kg and 30 mg/kg of guaraná, as well as 1 mg/kg of caffeine, were shown to reverse memory deficits in retention on a passive avoidance task in mice treated with 2 mg/kg of scopolamine. Mattei et al. (1998) also investigated the effects of both acute and chronic administration of guaraná. They found no toxic effects of guaraná and also failed to find any effects on body weight or on modulation of motor activity or pentobarbital-induced sleep parameters. However, the only two studies to date that have examined the behavioural effects of guaraná in humans have failed to find any interpretable effects, either following acute or chronic administration (Galduroz and Carlini 1994; Galduroz and Carlini 1996).

1.11 General conclusions

Reliable effects of caffeine have been shown on measures of sustained attention and 'alertness'. Effects on simple attention tasks such as simple reaction time and particularly choice reaction time appear to be improved by caffeine but the effects on these measures are more pronounced when performance is impaired in some way, such as in situations of low arousal. A number of factors that affect arousal have been highlighted in relation to the effects of caffeine. The findings regarding all of these factors are equivocal and the effects of the majority of these factors appear to be small and interdependent. However, the assertion that caffeine has no absolute benefits and merely reverses impairment produced by withdrawal in habitual consumers is a very important point and is worthy of further exploration.

1.12 Summary of the objectives of the thesis

It is clear that the behavioural effects of caffeine have received considerable attention in the literature. Despite this, the exact nature of the behavioural effects of caffeine could be further delineated. The aim of this thesis is to address some of these gaps in our knowledge in this area. One such issue, which has complicated research into the effects of caffeine, is the suggestion that habitual caffeine consumption may lead to a state of dependency, and that any positive effects of caffeine are merely the result of a reversal of impairment created by caffeine withdrawal. Given the near ubiquitous nature of caffeine, and the consumption of daily caffeine amounts equivalent to at least one cup of tea in 91 % of a UK sample (Heatherley et al. 2006), it is important to ascertain whether caffeine consumption has net benefits to behaviour or, alternatively, if the habitual consumption of caffeine actually has negative effects.

Related to this are the health effects of caffeine. Caffeine has been linked to negative effects on health, such as increased risk of cardiovascular disease (e.g. James 2004), decreased bone density (e.g. Ilich et al. 2002) and lower birth weight in neo-nates exposed to caffeine in utero (e.g. Klebanoff et al. 2002). However, caffeine also has positive effects upon health. These include an offsetting of cognitive decline (e.g. van Gelder et al. 2007), and even possible neuroprotection against Alzheimer's disease (e.g. Maia and de Mendonca 2002) and Parkinson's disease (e.g. Ross et al. 2000). Therefore, if caffeine is shown to have acute net benefits, then, coupled with the known benefits to health, it may be deemed advisable to consume moderate amounts of caffeine regularly. Conversely, if acute effects of caffeine are shown to be merely the result of alleviation of impairment resulting from caffeine withdrawal, then, coupled with the negative effects of caffeine upon health, it would seem more prudent to avoid consumption of caffeine-containing products. In order to study this, Chapter 2 of this thesis will compare the behavioural responses to deprivation from, and administration of, caffeine in habitual consumers and habitual non-consumers of caffeine. Significant differences between the two groups would indicate a role for habitual caffeine consumption in the effects of caffeine. Examination of the responses of consumers and non-consumers separately, will allow further exploration of this and significant improvements in habitual non-consumers of caffeine would provide evidence of net benefits of caffeine.

Another important point relating to caffeine consumption is that it is rarely consumed in isolation, being present in tea, coffee, cocoa (and therefore, chocolate), cola and energy drinks, guaraná, kola nut, maté and over-the-counter medications. However, with the exception of coffee, consumers are often unaware that they are ingesting caffeine when consuming these foodstuffs. In line with this, with the exception of coffee, the effects of caffeine in the forms that it is consumed naturally have been largely neglected in the literature. This would seem to assume that the effects of caffeine tested in isolation can be extrapolated to the effects of caffeinated products as they are consumed in dietary form. This is despite the presence of numerous other components that could interact with, or act upon the same physiological systems as caffeine. Clearly, it is important to understand the effects of caffeine as it is consumed in dietary form, rather than in isolation only. To this end, studies within this thesis will firstly address the effects of combining caffeine with one other concomitant component (L-theanine). This will then be explored further by examining the effects of a plant extract (guaraná) containing caffeine.

Examination of the literature regarding low doses of caffeine reveals that the lower threshold for psychoactive properties of caffeine has not been identified. This is

clearly of fundamental importance when understanding the pharmacology of any substance. Again, this also has important implications for health. In order to address this gap in knowledge, the final experimental chapter of this thesis will explore the effects of doses of caffeine as low as those found in decaffeinated beverages.

The main aims of the thesis are:

1) To systematically assess the cognitive and mood effects of administration of acute doses of caffeine in habitual consumers and habitual non-consumers of caffeine in order to establish whether any net effects of caffeine exist outside of withdrawal alleviation in habitual consumers.

2) To compare the neurocognitive effects of caffeine in isolation with those of caffeine when combined with its naturally concomitant compounds in order to assess the relative contribution of caffeine to the effects seen following caffeinated beverages.

3) To establish a lower dose threshold for any effects of caffeine.

CHAPTER 2. COGNITIVE AND MOOD IMPROVEMENTS OF CAFFEINE IN HABITUAL CONSUMERS AND HABITUAL NON-CONSUMERS OF CAFFEINE

2.1 Introduction

The most commonly reported experimental effects of caffeine are increases in ratings of 'alertness' (Quinlan et al. 2000; Rogers et al. 2003) and improvements in measures of reaction time and vigilance (Lieberman et al. 1987; Richardson et al. 1995; Smit and Rogers 2000). However, even on these measures there is contradictory evidence in the literature, with some studies reporting no effects of caffeine (Loke and Meliska 1984), and others finding positive effects only in certain groups (e.g. the elderly, Swift and Tiplady 1988), or in certain situations (e.g. low arousal, or under the influence of a depressant, Reyner and Horne 2000; Mackay et al. 2002; Smith et al. 2003). There are also reports of positive effects of caffeine on information processing, memory and logical reasoning (Smith et al. 1994b; Smit and Rogers 2000). Whilst there is less support for these latter findings, there is also little evidence to suggest that caffeine produces any impairment to performance, at least at typical everyday levels.

The reason for this lack of consistency in the literature can largely be attributed to methodological issues. For instance, there is large variability between studies in the doses of caffeine administered, with some using single acute doses equivalent to over five-fold the amount found in the average cup of coffee (Kaplan et al. 1997). This should be viewed in the context of reports of positive effects from doses of caffeine as low as 12.5 mg (Smit and Rogers 2000). There are also wide differences in the periods of caffeine abstention prior to testing, with these ranging from one hour (e.g. Warburton 1995) to up to 3 weeks (e.g. Rogers et al. 2005).

One recurrent theme in the literature on the behavioural and subjective effects of caffeine concerns the issue of whether caffeine produces any net benefits, or whether its effects merely represent an alleviation of withdrawal (James 1994; Rogers and Dernoncourt 1998 – see Section 1.9.1.2). The issue of withdrawal in relation to the effects of caffeine stems from the large number of studies that have employed habitual caffeine consumers who have abstained from caffeine overnight for the purposes of the research. The most commonly reported symptoms of caffeine withdrawal include 'headache', 'drowsiness' and 'lethargy', and decreased 'energy' and 'concentration' (Phillips-Bute and Lane 1998). It has also been reported that caffeine withdrawal can lead to impaired performance on vigilance tasks (Lane and Phillips-Bute 1998). These findings have led to the suggestion that participants who have abstained from caffeine

are performing at sub-normal levels and that the administration of caffeine merely restores performance to normal levels.

Several methods have been employed in order to gauge whether improvements in mood and performance following administration of caffeine are evident without the confounding effect of withdrawal. These have included pre-dosing participants with a standard amount of caffeine, allowing *ad libitum* caffeine consumption prior to testing, or withdrawing caffeine consumers from caffeine for a period of a week or more. All three methods present problems. Pre-loading with a standard dose of caffeine does not account for the different doses needed to alleviate withdrawal in different participants. On the other hand *ad libitum* consumption is confounded by individual differences in patterns of daily consumption raising the possibility that participants are at different levels of caffeine withdrawal prior to treatment. "Washing out" caffeine consumers to levels where they no longer show any withdrawal effects presents possibly the best method of assessing positive effects of caffeine. Nevertheless, the method is difficult in practical terms in that it necessitates a high level of compliance (or constant monitoring of dietary habits), including substitution of caffeinated products with decaffeinated equivalents.

An alternative method for measuring the absolute effects of caffeine is to compare its effects in overnight withdrawn consumers with those in habitual non-consumers (who are clearly not withdrawn). This approach is not without its own attendant problems, most notably that non-consumers are rare, and may be considered as self-selecting (possibly due to an inherent caffeine insensitivity or hypersensitivity). Nevertheless, if withdrawn caffeine consumers' performance is significantly lower than that of non-consumers and drinking caffeine simply reverses this deficit, this would offer strong support for a withdrawal alleviation model. If, on the other hand, such differences are not evident in the absence of caffeine, and both groups benefit to a similar extent from a caffeine dose, this would provide evidence in favour of caffeine imparting absolute benefits on performance.

Comparisons of non-consumers' and consumers' baseline scores have previously shown detrimental effects of caffeine withdrawal on consumers' mood but not performance (Rogers et al. 2003). It is possible that these differences between consumers and non-consumers reflect expectancy rather than genuine effects in this case, as the consumers were asked to abstain from caffeine prior to testing. Previous comparisons of the above two groups' responses to caffeine have produced significant effects on mood. For instance, Richardson et al. (1995) found that caffeine influenced ratings of 'headache', 'tiredness', and 'jitteriness', irrespective of group. Rogers et al. (2003) also found that caffeine significantly increased ratings of 'alertness' in both

consumers and non-consumers of caffeine. Using comparisons of habitual consumers and non-consumers has, therefore, generated equivocal results regarding caffeine's mood effects. Moreover, these studies have been limited as to the memory and cognitive measures employed.

The aim of the present study was therefore to conduct a systematic assessment both of the behavioural effects of two doses of caffeine and the contribution to these effects of caffeine withdrawal. This placebo-controlled, double-blind, balanced crossover study examined the effects of caffeine on mood and on the performance of a comprehensive range of tasks from the Cognitive Drug Research (CDR) computerised test battery. A number of the tasks measure aspects of behaviour that have previously been shown to be sensitive to caffeine. However, a subset of tasks that measure aspects of behaviour with no known sensitivity to caffeine were also included. This was in order to produce a cognitive profile for caffeine, thus allowing meaningful comparison with other drugs. Mood and performance were assessed in acutely (overnight) withdrawn habitual consumers and habitual non-consumers of caffeine. As outlined above, individuals who completely forgo caffeine may do so because of abnormal sensitivity to the drug. For this reason, in the present study individuals were selected who consumed very low levels of caffeine and who did not consume tea or coffee on a daily basis. Additionally, the behavioural effects of caffeine administered at typical everyday doses were assessed.

2.2 Materials and Methods

2.2.1 Design

A placebo-controlled, double-blind, balanced-crossover design was employed with two independent groups of participants derived on the basis of caffeine consumer status (see Section 2.2.2). The use of a crossover design was employed throughout this thesis. It was deemed appropriate with studies involving a dietary intervention, which participants may or not consume in their diet, to compare behaviour within participants. Given the individual response to caffeine, which was highlighted in Chapter 1, particularly relating to the personality trait 'impulsivity', this design seemed particularly appropriate and, as the washout for caffeine is known to be less than 48-hours, drop-out or confound due to previous intervention was not an issue. The counterbalanced order of treatments also prevented the problem of order effects often highlighted with this design, and measurement of baseline behaviour on each day allowed appropriate comparison of changes in behaviour on each day in response to treatment.

2.2.2 Initial screening

Participants were initially recruited from a database of potential volunteers on the basis of their self-reported caffeine consumption. Potential volunteers were then asked to complete a questionnaire that assessed average caffeine consumption on the basis of their responses to questions regarding daily consumption of tea, coffee, cocoa and caffeinated soft drinks (see Appendix I). The levels of caffeine content for this questionnaire were taken from Gray (1998) and correspond to 75 mg per 190 ml cup of instant coffee; 50 mg per 190 ml cup of tea; 40 mg per 330 ml can of cola/other caffeinated soft drink. For the purposes of the study 'habitual non-consumers' were defined as those who refrained from drinking tea or coffee and who consumed less than 50 mg per day of caffeine from other sources (primarily soft drinks). The eventual mean consumption for this group was 20 mg of caffeine per day (range 0 mg - 47 mg). Consumers were defined as those who consumed tea and/or coffee and consumed more than 50 mg of caffeine per day (mean consumption 217 mg/day, range 60 mg - 800 mg). Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. Only those participants who reported being in good health and who were taking no medication, other than the contraceptive pill, were included in the study. (Discussion pertaining to this inclusion criterion can be found in Chapter 8, Section 8.8, page 197). Habitual smokers were excluded from the

study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix III).

2.2.3 Participants

Twenty-nine males and 19 females (mean age 23.4 years, SEM 0.80, range 18 - 46 years) took part in the study, which was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki. The participants comprised two groups: 24 habitual consumers (7 female and 17 male, mean age 23.8 years, SEM 1.28, range 19 - 46 years) and 24 habitual non-consumers of caffeine (12 female and 12 male, mean age 22.9 years, SEM 0.99, range 18 - 33 years). The groups did not differ in terms of age ($t(46)=0.59$, $p=0.56$) nor gender composition (χ^2 (df 1)$=2.18$, $p=0.14$).

2.2.4 Cognitive and mood measures

2.2.4.1 CDR assessment battery

A tailored version of the Cognitive Drug Research battery (CDR Ltd, Goring-on-Thames, UK) was employed. This system has been in use since 1984 and has been utilised in several hundred national and international clinical trials assessing over 100 novel compounds. It has been used to detect impairment from drugs such as benzodiazepines and classical antipsychotics as well as improvements from compounds such as anticholinesterase drugs. The CDR computerised assessment battery has also been shown to be sensitive to acute cognitive improvement as well as impairment with a wide variety of non-drug substances (e.g. Moss et al. 1998; Scholey et al. 1999; Kennedy et al. 2002; 2003a; Scholey and Kennedy 2004). The tasks selected from the battery used throughout this thesis measured a range of behaviours including speed and accuracy of attention, and secondary and working memory. The fairly extensive range of tasks allowed systematic assessment of the behavioural effects of the components tested and allowed a cognitive profile to be produced for them thus facilitating meaningful comparison with other drugs.

The selection of computer-controlled tasks from this system was administered with parallel forms of the tests being presented at each testing session. Presentation was via laptop computers. All responses were recorded via two-button (YES/NO)

response boxes with the exception of the written word recall and digit symbol substitution (DSST) tasks, and the tracking task, which involved use of a joystick. Tests were administered in the following order:

2.2.4.1.1 Word presentation: Fifteen words, matched for frequency and concreteness, were presented in sequence on the screen for the participant to remember. Stimulus duration was 1 second, as was the inter-stimulus interval.

2.2.4.1.2 Immediate word recall: The participant was allowed 60 seconds to write down as many of the words as possible. The number of words correctly recalled was scored as a percentage of the total possible.

2.2.4.1.3 Picture presentation: A series of 20 photographic images of everyday objects and scenes were presented on the screen at the rate of 1 every 3 seconds, with stimulus duration of 1 second, for the participant to remember.

2.2.4.1.4 Simple reaction time: The participant was instructed to press the 'YES' response button as quickly as possible every time the word 'YES' was presented on the screen. Fifty stimuli were presented with an inter-stimulus interval that varied randomly between 1 and 3.5 seconds. Reaction times were recorded in msec.

2.2.4.1.5 Digit vigilance task: A target digit was randomly selected and constantly displayed to the right of the screen. A series of digits were presented in the centre of the screen at the rate of 80 per minute and the participant was required to press the 'YES' button as quickly as possible every time the digit in the series matched the target digit. The task lasted three minutes and there were 45 stimulus-target matches. Task measures were accuracy (%), reaction time (msec) and number of false alarms.

2.2.4.1.6 Choice reaction time: Either the word 'NO' or the word 'YES' was presented on the screen and the participant was required to press the corresponding button as quickly as possible. There were 50 trials, of which the stimulus word was chosen randomly with equal probability, with a randomly varying inter-stimulus interval of between 1 and 3.5 seconds. Reaction times (msec) and accuracy (%) were recorded.

2.2.4.1.7 Rapid Visual Information Processing (RVIP): The participant was required to monitor a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits were presented at the rate of 100 per minute and

the participant responded to the detection of a target string by pressing the 'YES' response button as quickly as possible. The task was continuous and lasted for 4 minutes, with 8 correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections (msec), and number of false alarms.

2.2.4.1.8 Tracking: A box appeared on the screen which participants could move in two dimensions using a joystick. Participants were required to use the joystick to make the box follow a randomly moving cross as closely as they could. Task performance was measured as average distance from target (mm). The task lasted for one minute.

2.2.4.1.9 Spatial working memory: A pictorial representation of a house was presented on the screen with four of its nine windows lit. The participant was instructed to memorise the position of the illuminated windows. In 36 subsequent presentations of the house, one of the windows was illuminated and the participant decided whether or not this matched one of the lighted windows in the original presentation. The participant made their response by pressing the 'YES' or 'NO' response button as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded and scores were converted into a sensitivity index (SI). The SI is a measure of the ability to discriminate original and novel stimuli and ranges from 1 (all novel and original items correctly identified) through zero (random performance) to minus 1 (where every stimulus is incorrectly categorised).

2.2.4.1.10 Logical reasoning: A series of statements referring to the relationships between two letters appeared on the screen one at a time (e.g. "a precedes b: ba"). Participants were required to decide if each statement correctly described the order of the 2 letters that followed it by pressing the 'YES' or the 'NO' button. There were 24 stimuli. Mean reaction times were measured in msec, and accuracy of responses were recorded as percentages.

2.2.4.1.11 Numeric working memory: Five digits were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the 'YES' or 'NO' response button as appropriate as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were measured in msec, and accuracy of responses to both original and novel

(distractor) stimuli were recorded and scores were converted into a sensitivity index (SI) as described above.

2.2.4.1.12 Digit Symbol Substitution Task [(DSST) Weschler 1958]: A timed paper and pencil task in which the participant was given 90 seconds to match the numbers 1 to 9 with a specific symbol. The task was scored as number correct.

2.2.4.1.13 Delayed word recall: The participant was again given 60 seconds to write down as many of the words as possible. The number of words correctly recalled was scored as a percentage of the total possible.

2.2.4.1.14 Delayed word recognition: The original words plus 15 distractor words were presented one at a time in a randomised order. For each word the participant indicated whether or not it was recognised as being included in the original list of words by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded and scores were converted into a sensitivity index (SI).

2.2.4.1.15 Delayed picture recognition: The original pictures plus 20 distractor pictures were presented one at a time in a randomised order. For each picture participants indicated whether or not it was recognised as being from the original series by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded and scores were converted into a sensitivity index (SI).

2.2.4.2 Other cognitive measures

2.2.4.2.1 Sentence verification task

The computerised sentence verification task employed within this thesis measures speed and accuracy of retrieval of information from general knowledge. It was developed by Baddeley (1981) as a measure of semantic memory and has been used in numerous studies of the effects of caffeine (Smith et al. 1992; Smith et al. 1993; Smith et al. 1994b; Smith et al. 1997; Smith et al. 1999b).

Participants were presented with a series of sentences on screen and had to decide whether they were 'true' (e.g. forks are manufactured goods) or 'false' (e.g. dogs have wings). Thirty stimuli were presented and performance was measured as accuracy (%) and reaction time (msec).

2.2.4.2.2 Serial subtraction tasks

The serial subtraction tasks employed throughout this thesis are 2-minute computerised versions of the serial subtraction task developed by Hayman (1942), which has appeared in a number of forms, including as part of the Mini-Mental State Examination for dementia screening (Folstein et al. 1975). The computerised version has been used in a number of studies of the behavioural effects of herbal and food supplements, as well as glucose (Kennedy and Scholey 2000; Scholey 2001; Scholey et al. 2001; Scholey and Kennedy 2002).

2.2.4.2.2.1 Serial threes subtraction task: Participants were required to count backwards in threes from a given number as quickly and as accurately as possible using the number keys to enter each response. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. The task was scored for number of correct responses and number of errors. In the case of incorrect responses subsequent responses were scored as positive if they were scored as correct in relation to the new number.

2.2.4.2.2.2 Serial sevens subtraction task: This was identical to the Serial threes task except that it involved serial subtraction of sevens.

2.2.4.3 Subjective mood measures

2.2.4.3.1 Bond-Lader visual analogue scales:

Originally designed for assessing the effects of anxiolytics, Bond-Lader visual analogue mood scales (Bond and Lader 1974) have subsequently been used in a number of pharmacological, psychopharmacological and medical trials. The reliability and validity of these visual analogue scales has been demonstrated (Ahearn 1997). The scales comprise a total of sixteen 100 mm lines anchored at either end by antonyms (e.g. alert-drowsy, calm-excited) on which participants mark their current subjective position.

Scores from the 16 Bond-Lader visual analogue scales were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

2.2.4.3.2 Caffeine research visual analogue scales (see Appendix II):

The caffeine research visual analogue scales utilised within this thesis comprise seven visual analogue scales ('relaxed', 'alert', 'jittery', 'tired', 'tense', 'headache', 'overall mood') that have previously been used in research into the effects of caffeine (Rogers et al. 2003). 'Alert' and 'tired' ratings were combined [as previously recommended (Rogers et al. 2003)] to give a composite 'alertness' rating and 'tense' and 'relaxed' were combined to give a 'tension' rating, more positive scores denoting greater 'alertness' and more 'tension' respectively. A single 'mental fatigue' visual analogue scale was also included, which has been shown to be sensitive to a caffeine-glucose drink (Kennedy and Scholey 2004).

Participants rated their current subjective status for each of the descriptors by making a mark on a 100 mm line with the end points labelled 'not at all' (left hand end) and 'extremely' (right hand end), with the exception of 'overall mood', which was labelled 'very bad' and 'very good'.

2.2.5 Salivary analysis

Saliva samples were obtained by asking participants to expectorate into a tube. Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence and immediately prior to post-treatment assessment to confirm effective caffeine absorption. The saliva samples were immediately frozen at –20 °C until thawing for batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

2.2.6 Treatments

Participants received three drinks containing 0 mg (placebo), 75 mg, and 150 mg of caffeine hydrochloride BP (Merck, Darmstadt, Germany) on separate occasions. In each case the caffeine was presented in a 150 ml drink containing 30 ml of Robinsons Special R Apple and Blackcurrant Juice Drink with no added sugar (Robinsons Soft Drinks Ltd, Chelmsford, UK). Five minutes was allowed for drink consumption. There were several reasons for this choice of a juice drink over a more naturalistic caffeine vehicle. Firstly, tea and coffee and even hot water alone have been

shown to have behavioural effects irrespective of caffeine content that could interfere with the findings (Quinlan et al. 2000). Secondly, there are obvious expectancy effects attached to tea and coffee, which are likely to influence task outcomes, and may differ between the two groups. Finally, many of the habitual non-consumers of caffeine did not drink coffee or tea because they disliked the taste.

2.2.7 Procedure

Each participant was required to attend a total of four study days that were conducted 48 hours apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the three active days of the study.

The first day involved completion of the test battery four times. This was undertaken in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the three active study days comprised two identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day. This was immediately followed by drink consumption with the second assessment commencing 30 minutes post-drink.

Each testing session lasted approximately 30 minutes and comprised producing a saliva sample, completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales.

2.2.8 Statistics

Salivary caffeine levels were analysed to assess compliance to caffeine abstinence and effective caffeine absorption.

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments. To assess the possibility that caffeine withdrawal leads to mood and performance deficits, one way ANOVAs were conducted to ascertain any group (consumers/non-consumers) effects in the absence of treatment.

Scores on the individual task outcomes were analysed as 'change from baseline' using SPSS.

The data from each measure were initially analysed by two-way ANOVA [group (consumers/non-consumers) X treatment (75 mg/150 mg/placebo)] with repeated measures on the latter factor. In the case of any violation of the assumptions of sphericity a Greenhouse Geisser correction was applied. This analysis was followed by a priori planned comparisons of the effect of treatment, with individual comparisons being made between placebo and each of the two levels of caffeine treatment (75 and 150 mg) utilising t tests with the MSError from an omnibus ANOVA as an error term (Keppel 1991). In the case of significant interaction effects further analysis was conducted utilising a priori planned comparisons. To ensure the overall protection level, only those planned comparisons associated with measures that generated a significant main effect or interaction effect on the initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

The analyses described in the preceding sections are adequate to detect withdrawal effects and to determine whether consumers and non-consumers are similarly susceptible to the effects of caffeine. However, to further explore the possibility of group differences in the responses of consumers and non-consumers, a secondary analysis of each separate consumption groups' scores was undertaken by one-way ANOVA (comparing treatments) with any differences between treatments analysed with a priori planned comparisons, as described above. In addition, in response to the assertion that any effects in non-consumers are modest in comparison to those effects in consumers (James and Rogers 2005), effect sizes (Cohen's *d*) were also calculated for any significant effects found when the two groups were analysed separately. Finally, further exploration of the relationship between habitual caffeine consumption level and behaviour pre- and post-treatment was carried out using Pearson's correlation.

Sex differences in response to treatment were also explored in a separate two-way ANOVA [sex (male/female) X treatment (75 mg/150 mg/placebo)] with repeated measures on the latter factor.

2.3 Results

2.3.1 Salivary caffeine levels

Due to factors outside of the author's control it was only possible to obtain full datasets for 30 participants (15 per group) and only these are included in the analysis. However, it should be emphasised that all 48 participants complied in giving each saliva sample. Baseline salivary caffeine levels confirmed compliance with overnight abstinence. The mean values were 0.50 μg/ml (SD = 1.17) for consumers and 0.36 μg/ml (SD = 0.87) for non-consumers (levels just below 1 μg/ml have been reported for overnight caffeine abstinence – Evans and Griffiths 1999). Analysis of post-treatment salivary caffeine levels reflected effective caffeine absorption in both groups [$F(2,56)=6.93$, $p=0.002$). Planned comparisons revealed significantly higher salivary caffeine levels in both groups following both 75 mg of caffeine [$t(56)=2.48$, $p=0.02$] and 150 mg of caffeine [$t(56)=3.64$, $p=0.001$], see Fig 2.1. There was no significant treatment X group interaction.

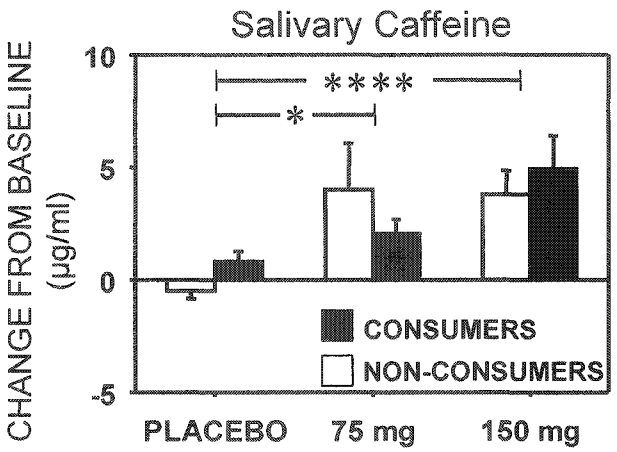

Figure 2.1 Mean ±SEM change from baseline salivary caffeine levels are shown for consumers and non-consumers following placebo and 75 and 150 mg of caffeine. Significant treatment effects compared with placebo are shown (*$p<0.05$, ****$p<0.001$).

2.3.2 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose baseline scores for all three conditions (placebo, 75 mg, 150 mg of caffeine) for each outcome (individual CDR task scores, sentence verification scores, serial subtraction scores and mood scale scores) were subjected to a one-way, repeated-measures, ANOVA. There were no significant differences on any of the measures.

2.3.3 Consumer status effects in the absence of treatment

Mean pre-dose baseline scores for each group (habitual consumers, habitual non-consumers) for each outcome were subjected to a one-way ANOVA to assess any cognitive or mood differences between the groups in the absence of caffeine. There were significant group differences on the serial sevens subtraction task [$F(1,44)=5.96$, $p=0.02$] and the serial threes subtraction task [$F(1,44)=6.99$, $p=0.01$] with habitual consumers performing more correct subtractions than habitual non-consumers. In order to further examine the effects of caffeine withdrawal, controlling for possible 'withdrawal expectancy' effects in consumers, a comparison of placebo change from baseline scores for the two groups was also carried out. There was a significant group effect on ratings of 'jitteriness' [$F(1,44)=7.51$, $p=0.009$] with non-consumers becoming more 'jittery' following placebo than consumers.

2.3.4 Primary analysis

2.3.4.1 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 2.1 along with F values and probabilities for effects of treatment and/or consumer group. Significant differences on cognitive tasks are presented in Figures 2.2 and 2.3. Only significant main effects and/or interactions for each outcome measure are reported below.

2.3.4.1.1 Simple reaction time

Performance of the simple reaction time task was significantly improved irrespective of group following the 75 mg dose of caffeine [$t(92)=2.61$, $p=0.01$]. See Fig 2.2a.

2.3.4.1.2 Digit vigilance reaction time

Digit vigilance reaction time was significantly improved across groups following both the 75 mg [$t(92)=2.09$, $p=0.04$] and the 150 mg dose of caffeine [$t(92)=2.67$, $p=0.009$], see Fig 2.2b.

2.3.4.1.3 Numeric working memory reaction time

Numeric working memory reaction time was significantly faster following 150 mg irrespective of group [$t(92)=2.85$ $p=0.005$], see Fig 2.2c.

Table 2.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition by consumer status. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects, consumer status effects (both pre- and post-treatment) and treatment x status interactions. F and p values are also presented for effects of consumer status at baseline (withdrawal effect). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score		Withdrawal effect	30 min post-dose change from baseline score		Treatment effect	Consumer status effect	Treatment x status interaction
		Consumer	Non-con		Consumer	Non-con			
Immediate word recall accuracy (%)	Placebo	50.7±4.24	42.8±2.98	F(1, 46) =2.95 p=0.09	-3.61±2.82	-0.28±2.49	F(2, 92) =1.25 p>0.1	F(1, 46)<1	F(2, 92)<1
	75 mg	51.8±4.16	43.2±2.58		-8.06±1.85	-3.47±2.72			
	150 mg	48.1±3.00	42.8±3.11		-1.25±2.81	-3.75±2.82			
Simple reaction time (ms)	Placebo	285±11.0	288±6.64	F(1, 46)<1	26.3±5.36	27.3±5.46	**F(2, 92) =4.32 p=0.02**	F(1, 46) =1.17 p>0.1	F(2, 92)<1
	75 mg	284±9.51	282±5.55		9.22±4.57	14.8±4.93			
	150 mg	285±10.2	279±6.66		9.66±8.47	21.7±5.81			
Digit vigilance accuracy (%)	Placebo	94.5±1.71	95.9±0.98	F(1, 46)<1	-0.09±1.47	-2.68±0.85	F(2, 92) =1.38 p>0.1	F(1, 46)<1	F(2, 92)=2.22 p>0.1
	75 mg	95.3±1.44	96.3±0.76		-1.02±0.71	-2.22±1.35			
	150 mg	95.5±1.35	96.1±0.81		-0.83±0.96	0.56±0.62			
Digit vigilance reaction time (ms)	Placebo	425±7.64	427±7.70	F(1, 46)<1	29.8±6.14	31.4±4.87	**F(2, 92) =3.95 p=0.02**	F(1, 46)<1	F(2, 92)=1.25 p>0.1
	75 mg	422±9.76	430±8.12		26.2±6.67	11.5±7.27			
	150 mg	428±8.45	416±7.30		15.9±5.19	15.2±5.25			
Digit vigilance false alarms (number)	Placebo	0.71±0.23	1.08±0.24	F(1, 46)<1	-0.21±0.37	0.21±0.29	F(2, 92) <1	**F(1, 46) =4.59 p=0.04**	F(2, 92)<1
	75 mg	0.83±0.25	0.75±0.24		-0.04±0.30	0.50±0.25			
	150 mg	0.71±0.16	1.04±0.21		-0.04±0.24	0.71±0.29			
Choice reaction time accuracy (%)	Placebo	94.8±0.72	95.1±0.54	F(1, 46)<1	-0.83±0.75	0.33±0.61	F(2, 92) =2.27 p>0.1	F(1, 46)<1	F(2, 92)<1
	75 mg	95.3±0.84	95.6±0.58		1.25±0.99	1.17±0.68			
	150 mg	94.4±0.74	95.5±0.59		0.50±0.65	0.58±0.73			
Choice reaction time (ms)	Placebo	418±13.2	437±10.6	F(1, 46)<1	13.0±9.66	14.7±7.75	F(2, 92) <1	F(1, 46)<1	F(2, 92)<1
	75 mg	424±14.4	436±8.29		15.3±9.97	13.1±6.72			
	150 mg	423±13.4	424±7.90		14.0±10.4	22.0±8.56			
RVIP accuracy (%)	Placebo	65.0±4.90	63.4±4.68	F(1, 45)<1	-2.72±2.63	1.56±2.36	F(2, 90) =2.37 p=0.1	F(1, 45)<1	F(2, 90)=2.38 p=0.1
	75 mg	63.3±4.01	66.0±5.26		7.88±2.53	0.78±2.30			
	150 mg	63.3±4.15	65.9±4.26		4.62±3.41	4.17±2.68			
RVIP reaction time (ms)	Placebo	478±14.9	488±17.7	F(1, 45)<1	14.1±11.5	20.7±11.4	F(2, 90) =2.14 p>0.1	F(1, 45)<1	F(2, 90)<1
	75 mg	488±20.3	479±13.6		-13.4±13.6	0.25±10.6			
	150 mg	480±16.3	485±13.3		3.27±11.9	-8.39±11.1			
RVIP false alarms (number)	Placebo	1.13±0.34	1.42±0.48	F(1, 45)<1	-0.22±0.23	0.46±0.57	F(2, 90) <1	**F(1, 45) =5.64 p=0.02**	F(2, 90)=1.09 p>0.1
	75 mg	1.09±0.29	0.83±0.20		-0.39±0.36	1.00±0.35			
	150 mg	1.39±0.78	1.46±0.30		-0.04±0.15	0.25±0.47			
Tracking (mm)	Placebo	22.7±0.62	25.7±2.58	F(1, 46) =1.30 p>0.1	-0.73±0.55	-2.52±2.63	F(2, 92) <1	F(1, 46)<1	F(2, 92)<1
	75 mg	22.9±0.76	23.0±0.64		-1.12±0.60	-1.45±0.50			
	150 mg	22.8±0.62	24.3±2.01		-1.18±0.62	-2.06±1.92			
Spatial memory (sensitivity index)	Placebo	0.91±0.02	0.94±0.01	F(1, 46) =3.83 p=0.06	0.04±0.02	-0.02±0.02	F(2, 92) <1	**F(1, 46) =6.72 p=0.01**	F(2, 92)=2.64 p=0.08
	75 mg	0.89±0.03	0.96±0.01		0.06±0.03	-0.02±0.02			
	150 mg	0.95±0.01	0.95±0.01		-0.01±0.01	-0.01±0.01			
Spatial memory reaction time (ms)	Placebo	568±31.7	591±33.1	F(1, 46)<1	-52.7±15.1	-55.8±24.8	F(2, 92) =1.31 p>0.1	F(1, 46)<1	F(2, 92)<1
	75 mg	568±28.7	568±19.7		-69.7±9.26	-57.2±9.83			
	150 mg	562±22.9	570±19.8		-41.0±15.1	-38.6±13.9			
Logical reasoning accuracy (%)	Placebo	88.9±2.48	81.9±4.48	F(1, 46) =2.58 p>0.1	-1.21±1.97	-2.43±1.58	F(2, 92) =1.33 p>0.1	F(1, 46) =2.08 p>0.1	F=2.21 p>0.1
	75 mg	90.1±2.65	80.0±4.34		-0.17±1.16	1.04±1.49			
	150 mg	88.9±2.21	82.8±4.18		0.87±1.53	-4.69±1.81			
Logical reasoning reaction time (ms)	Placebo	2969±187	2930±199	F(1, 46)<1	-289±123	117±179	F(2, 92) <1	F(1, 46) =2.64 p>0.1	F(2, 92)<1
	75 mg	2882±183	2937±244		-198±112	-180±164			
	150 mg	2964±217	3031±222		-216±150	-86.1±97.4			
Numeric working memory (sensitivity index)	Placebo	0.91±0.02	0.91±0.00	F(1, 46) =1.49 p>0.1	-0.01±0.02	0.01±0.02	F(2, 92) <1	F(1, 46)<1	F(2, 92)=2.62 p=0.08
	75 mg	0.88±0.02	0.93±0.01		0.03±0.01	0.00±0.01			
	150 mg	0.90±0.02	0.91±0.02		0.00±0.01	0.02±0.01			
Numeric working memory reaction time (ms)	Placebo	583.±22.7	622±23.9	F(1, 46)<1	-23.9±11.0	-2.28±11.3	**F(2, 92) =4.07 p=0.02**	F(1, 46)<1	F(2, 92)<1
	75 mg	591±26.5	623±26.8		-35.7±9.54	-23.7±12.7			
	150 mg	604±27.7	628±26.0		-41.2±11.1	-44.7±11.2			
Digit symbol substitution (number)	Placebo	68.5±2.04	71.5±2.11	F(1, 46)<1	2.50±1.42	0.92±1.64	F(2, 92) <1	F(1, 46)<1 p>0.1	F(2, 92)=2.44 p>0.1
	75 mg	67.7±2.32	70.7±2.32		2.33±0.88	2.79±0.75			
	150 mg	68.8±2.33	70.6±2.22		0.04±1.23	3.08±0.74			
Delayed word recall accuracy (%)	Placebo	29.7±3.68	29.3±3.49	F(1, 46)<1	-6.81±3.18	-10.4±3.52	F(2, 92) <1	F(1, 46)<1	F(2, 92)<1
	75 mg	33.1±3.40	30.0±2.78		-13.1±3.39	-11.9±3.36			
	150 mg	32.4±3.51	32.2±3.49		-7.64±3.67	-13.6±3.49			
Delayed word recognition (sensitivity index)	Placebo	0.61±0.04	0.59±0.05	F(1, 46)<1	-0.06±0.04	-0.02±0.05	F(2, 92) <1	F(1, 46)<1	F(2, 92)<1
	75 mg	0.62±0.04	0.60±0.04		-0.07±0.04	-0.04±0.04			
	150 mg	0.62±0.04	0.65±0.04		-0.04±0.04	-0.05±0.05			
Delayed word recognition reaction time (ms)	Placebo	721±30.2	736±22.6	F(1, 46)<1	-31.0±25.8	18.5±18.6	F(2, 92) <1	F(1, 46) =3.94 p=0.05	F(2, 92)<1
	75 mg	724±33.5	705±17.1		-30.5±25.8	14.0±20.0			
	150 mg	715±18.7	746±24.7		-30.9±13.1	-1.30±21.3			
Delayed picture recognition (sensitivity index)	Placebo	0.64±0.06	0.73±0.04	F(1, 46)<1	0.00±0.04	-0.11±0.05	F(2, 92) <1	F(1, 46)<1	F(2, 92)=1.71 p>0.1
	75 mg	0.68±0.05	0.71±0.04		-0.03±0.04	-0.08±0.05			
	150 mg	0.66±0.05	0.67±0.04		-0.06±0.04	-0.01±0.05			
Delayed picture recognition reaction time (ms)	Placebo	847±39.5	873±25.6	F(1, 46)<1	-51.9±37.3	-32.5±28.3	F(2, 92) =1.50 p>0.1	F(1, 46) =1.10 p>0.1	F(2, 92)=2.79 p=0.07
	75 mg	808±26.6	841±24.5		-2.22±13.0	-29.0±23.1			
	150 mg	866±36.8	831±19.9		-44.1±26.8	39.3±17.9			

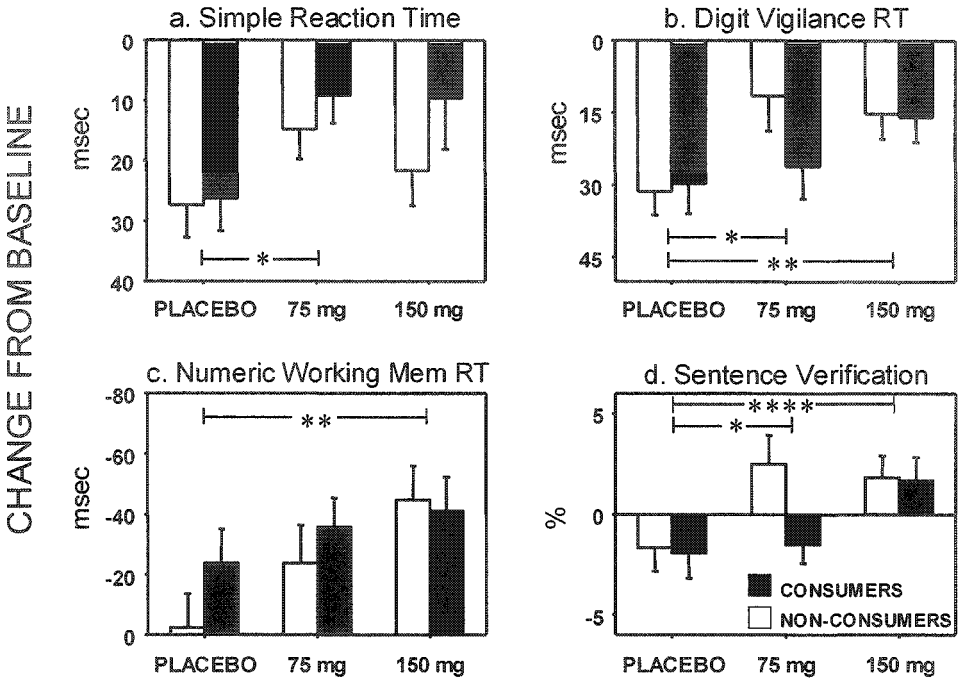

Figure 2.2 Means ±SEM change from baseline scores are shown for consumers and non-consumers following placebo and 75 and 150 mg of caffeine. Significant treatment effects compared with placebo are shown (*p<0.05, **p<0.01, ****p<0.001).

2.3.4.1.4 Digit vigilance false alarms

Whilst there was no effect of treatment overall there was a significant main effect of consumer status on the number of false alarms produced in the digit vigilance task [F(1,46)=4.59, p=0.04], with consumers producing less false alarms than non-consumers. See Fig 2.3a.

2.3.4.1.5 Spatial memory accuracy

There was a significant effect of consumer status on accuracy of the spatial memory task [F(1,46)=6.72, p=0.01]. Consumers outperformed non-consumers on this measure. See Fig 2.3b.

2.3.4.1.6 Rapid Visual Information Processing (RVIP) false alarms

Data capture errors with one dataset resulted in only 47 scores being analysed for this task. There were significant group differences in the number of false alarms generated on the RVIP task [F(1,45)=5.64, p=0.02] with non-consumers producing more false alarms than consumers irrespective of treatment. See Fig 2.3c.

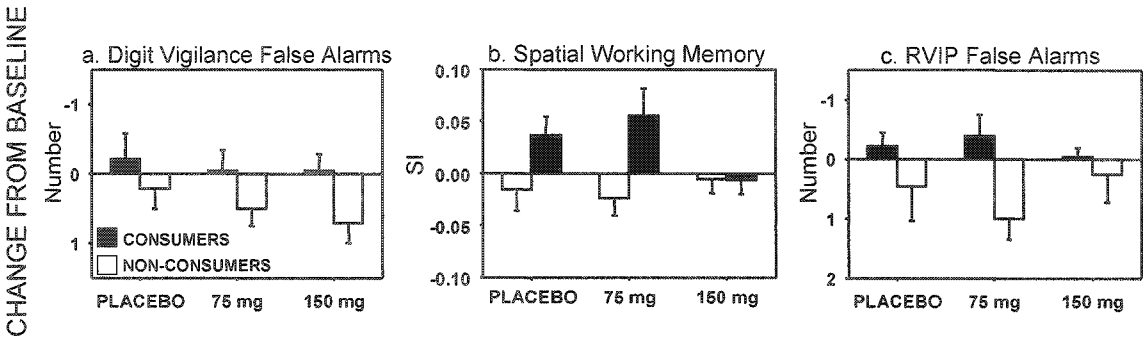

Figure 2.3 Graphic representations of group effects. Means ±SEM change from baseline scores are shown for consumers and non-consumers following placebo and 75 and 150 mg of caffeine.

2.3.4.2 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure along with F values and probabilities for effects of treatment and/or consumer status are presented in Table 2.2 Significant differences are presented in Figure 2.2. Only significant main effects and/or interactions for each outcome measure are reported below.

2.3.4.2.1 Sentence verification accuracy

Accuracy on the sentence verification task was significantly improved across groups following both the 75 mg [t(92)=2.22, p=0.03] and the 150 mg dose of caffeine [t(92)=3.44, p=0.0009], see Fig 2.2d.

Table 2.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition by consumer status. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects, consumer status effects (both pre- and post-treatment) and treatment x status interactions. F and p values are also presented for effects of consumer status at baseline (withdrawal effect). Significant measures are shown in bold.

Measure	Treatment	Baseline		Withdrawal effect	30 min post-dose change from baseline		Treatment effect	Consumer status effect	Treatment x status interaction
		Consumer	Non-con		Consumer	Non-con			
Sentence verification accuracy (%)	Placebo	97.9±0.66	96.4±1.06		-1.94±1.24	-1.67±1.17	**F(2, 92) =6.08 p=0.003**	F(1, 46)=1.59 p>0.1	F(2, 92)=2.29 p>0.1
	75 mg	97.5±0.73	94.4±1.92	F(1, 46)<1	-1.53±0.92	2.50±1.44			
	150 mg	95.6±1.28	94.3±1.43		1.67±1.17	1.81±1.10			
Sentence verification RT (ms)	Placebo	1768±118	1743±100		-273±67.6	-184±57.8		F(1, 46)=1.33 p>0.1	F(2, 92)<1
	75 mg	1884±109	1829±160	F(1, 46)<1	-314±81.6	-311±93.2	F(2, 92)<1		
	150 mg	1851±118	1686±99.8		-305±55.7	-173±68.5			
Serial threes subtraction correct (number)	Placebo	49.0±3.56	35.7±2.21	**F(1, 45) =6.99 p=0.01**	0.87±1.29	1.48±0.97	F(2, 90)<1	F(1, 45)<1	F(2, 90)<1
	75 mg	46.2±2.85	37.0±2.75		4.00±1.48	2.00±1.05			
	150 mg	47.0±3.80	37.9±2.57		3.04±1.84	1.57±1.39			
Serial threes subtraction errors (number)	Placebo	2.48±0.93	3.61±0.48	F(1, 45) =1.14 p>0.1	2.00±1.00	0.26±0.47	F(2, 90)<1	F(1, 45) =1.75 p>0.1	F(2, 90)=1.41 p>0.1
	75 mg	2.30±1.10	3.91±0.69		1.48±0.94	-0.22±0.79			
	150 mg	3.96±1.19	3.35±0.39		0.00±1.22	0.83±0.38			
Serial sevens subtraction correct (number)	Placebo	29.3±2.45	21.1±2.07	**F(1, 45) =5.96 p=0.02**	1.22±1.19	-0.43±1.18	F(2, 90) =1.26 p>0.1	F(1, 45) =1.02 p>0.1	F(2, 90)<1
	75 mg	29.0±2.44	22.0±2.41		1.13±0.91	-0.17±0.95			
	150 mg	28.6±2.81	20.6±1.89		1.70±0.93	2.04±1.09			
Serial sevens subtraction errors (number)	Placebo	3.48±0.57	3.22±0.64		0.35±0.51	0.65±0.58		F(1, 45) =1.09 p>0.1	F(2, 90)<1
	75 mg	3.04±0.41	3.04±0.32	F(1, 45)<1	1.26±0.56	0.13±0.48	F(2, 90)<1		
	150 mg	3.52±0.64	3.09±0.40		0.83±0.56	0.96±0.55			

2.3.4.3 Subjective mood measures

There were no significant effects on the Bond-Lader visual analogue scales. Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 2.3. There were a number of treatment effects on the caffeine research visual analogue scales (presented in Figure 2.4). Due to data capture errors with 2 datasets it is only possible to report data for 46 participants.

Table 2.3 Baseline and change from baseline scores for mood for each treatment condition by consumer status. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects, consumer status effects (both pre- and post-treatment) and treatment x status interactions. F and p values are also presented for effects of consumer status at baseline (withdrawal effect). Significant measures are shown in bold.

Measure		Treatment	Baseline		Withdrawal effect	30 min post-dose change from baseline		Treatment effect	Consumer status effect	Treatment x status interaction
			Consumer	Non-con		Consumer	Non-con			
Bond-Lader factors	Alert	Placebo	54.0±3.75	54.8±4.70	F(1, 46)<1	-4.30±2.01	-7.11±3.98	F(2, 92) =3.04 p=0.05	F(1, 46)<1	F(2, 92)<1
		75 mg	52.8±3.60	53.2±4.05		3.37±2.85	1.21±4.44			
		150 mg	54.3±3.39	55.3±4.28		0.79±4.29	1.28±4.16			
	Content	Placebo	62.7±1.95	65.1±3.56	F(1, 46)<1	-2.27±1.87	-4.82±2.61	F(2, 92) =1.01 p>0.1	F(1, 46) =2.89 p=0.1	F(2, 92)<1
		75 mg	62.6±2.85	64.4±3.99		-1.02±1.34	-4.13±1.87			
		150 mg	62.2±3.36	64.6±3.45		1.54±3.31	-2.58±1.87			
	Calm	Placebo	62.5±3.01	63.3±4.09	F(1, 46)<1	-5.50±2.28	-5.65±3.67	F(2, 92)<1	F(1, 46) =1.05 p>0.1	F(2, 92)<1 p>0.1
		75 mg	65.9±3.02	64.9±3.91		-6.73±2.37	-11.4±3.94			
		150 mg	62.3±2.58	60.6±3.90		-5.38±3.55	-10.3±3.95			
Caffeine research visual analogue scales	Relaxed	Placebo	59.9±3.33	61.3±3.99	F(1, 44)<1	-3.50±3.73	-2.72±4.09	F(2, 88)<1	F(1, 44)<1	F(2, 88)<1
		75 mg	62.3±3.67	62.8±4.97		-3.92±3.47	-9.00±3.19			
		150 mg	56.7±3.88	55.0±4.40		-6.33±6.01	-2.59±4.50			
	Alert	Placebo	55.3±3.77	54.9±4.40	F(1, 44)<1	0.83±4.13	-3.05±5.63	F(2, 88) =1.82 p>0.1	F(1, 44)<1	F(2, 88)<1
		75 mg	52.1±4.54	53.0±4.89		9.42±4.63	5.27±5.89			
		150 mg	48.5±4.66	58.0±4.26		8.38±5.26	3.27±4.39			
	Jittery	Placebo	34.5±3.95	34.7±4.74	F(1, 44)<1	-3.00±3.46	14.6±5.53	F(2, 88)<1	F(1, 44) =1.03 p>0.1	**F(2, 88)=4.83 p=0.01**
		75 mg	29.1±3.79	36.2±4.95		4.46±4.00	8.82±4.52			
		150 mg	30.6±3.78	40.6±5.08		14.2±4.80	4.23±4.96			
	Tired	Placebo	53.0±4.81	49.9±5.79	F(1, 44)<1	1.83±4.30	2.77±5.29	**F(2, 88) =4.56 p=0.02**	F(1, 44) =1.42 p>0.1	F(2, 88)<1
		75 mg	59.3±4.75	52.8±5.33		-12.8±4.68	-3.82±6.34			
		150 mg	58.2±4.92	51.9±4.68		-14.6±6.06	-7.18±3.20			
	Tense	Placebo	39.2±3.53	33.8±4.74	F(1, 44)<1	1.88±3.87	3.09±3.36	F(2, 88)<1	F(1, 44)<1	F(2, 88)<1
		75 mg	36.5±4.69	35.4±4.95		6.13±4.37	4.41±3.84			
		150 mg	35.5±4.89	33.2±5.52		4.96±6.71	5.46±4.07			
	Headache	Placebo	23.8±4.92	26.4±5.05	F(1, 44)<1	3.96±2.51	9.68±3.32	F(2, 88)<1	F(1, 44) =3.27 p=0.08	F(2, 88)<1 p>0.1
		75 mg	24.9±5.34	26.9±5.18		2.25±3.13	3.41±3.66			
		150 mg	27.4±5.54	26.1±5.20		-0.33±4.44	8.59±4.02			
	Overall mood	Placebo	63.0±3.29	66.4±2.97	F(1, 44)<1	0.29±2.24	-3.18±3.08	F(2, 88)<1	F(1, 44) =2.64 p>0.1	F(2, 88)=1.03 p>0.1
		75 mg	61.9±3.32	65.8±3.93		0.79±3.50	-0.73±3.37			
		150 mg	56.5±4.06	66.4±3.10		7.75±3.91	-2.27±3.67			
	Mental fatigue	Placebo	46.3±4.22	46.6±4.72	F(1, 44)<1	1.63±3.96	6.77±5.00	**F(2, 88) =4.27 p=0.02**	F(1, 44) =1.09 p>0.1	F(2, 88)=2.55 p=0.08
		75 mg	45.2±5.04	46.9±4.80		-4.71±5.02	-9.59±5.37			
		150 mg	52.2±4.65	42.9±5.01		-12.6±5.12	1.50±3.45			
Caffeine research visual analogue scales factors	**Alertness**	Placebo	51.2±3.72	52.5±4.40	F(1, 44)<1	-0.50±3.34	-2.91±4.94	**F(2, 88) =3.51 p=0.04**	F(1, 44) =1.55 p>0.1	F(2, 88)<1
		75 mg	46.4±4.24	50.1±4.69		11.1±4.26	4.55±5.56			
		150 mg	45.2±4.61	53.1±4.07		11.5±5.16	5.23±3.45			
	Tension	Placebo	39.7±3.01	36.3±4.07	F(1, 44)<1	2.69±2.77	2.91±3.07	F(2, 88)<1	F(1, 44)<1	F(2, 88)<1
		75 mg	37.1±3.67	36.3±4.68		5.02±3.04	6.71±3.55			
		150 mg	39.4±4.08	39.1±4.17		5.65±5.89	4.02±3.19			

2.3.4.3.1 'Jittery'

There was a significant interaction of group and treatment effects in ratings of 'jitteriness'. Non-consumers were more 'jittery' than consumers in the placebo condition

[t(88)=3.97, p=0.0001] and were less 'jittery' than consumers following 150 mg of caffeine [t(88)=2.25, p=0.03] (Fig 2.4a).

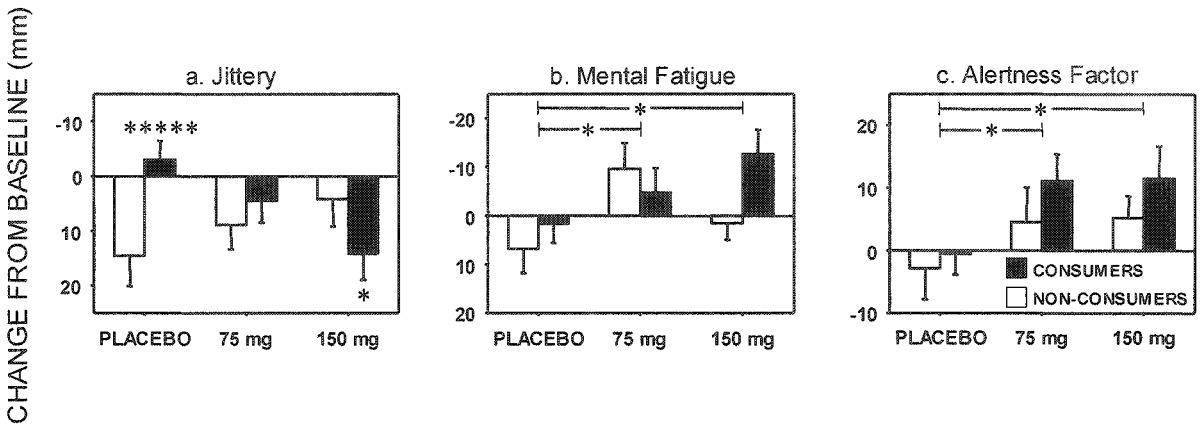

Figure 2.4 Means ±SEM change from baseline mood scores are shown for consumers and non-consumers following placebo and 75 and 150 mg of caffeine. Consumer-non-consumer differences in response to treatment (a) and significant treatment effects, compared with placebo (b, c) are shown (*p<0.05, *****p<0.0005).

2.3.4.3.2 'Mental fatigue'

There was a main effect of treatment on ratings of 'mental fatigue'. 'Mental fatigue' was significantly decreased in both groups following the 75 mg dose [t(88)=2.65, p=0.01] and the 150 mg dose [t(88)=2.37, p=0.02], see Fig 2.4b.

2.3.4.3.3 'Alertness' factor

Subjective ratings of 'alertness' were significantly increased across groups following both 75 mg [t(88)=2.11, p=0.04], and 150 mg of caffeine [t(88)=2.27, p=0.03], see Fig 2.4c. This effect was largely due to effects on ratings of 'tiredness', which decreased following 75 mg [t(88)=2.16, p=0.03] and 150 mg [t(88)=2.67, p=0.009] across both groups.

2.3.5 Secondary analysis

Secondary analyses were carried out separately on the scores from each of the separate consumption groups. This utilised one-way ANOVA (comparing treatments), with any differences between treatments analysed with a priori planned comparisons.

2.3.5.1 Consumers

In consumers RVIP accuracy was significantly affected by treatment [F(2,44)=3.99, p=0.03]. This improvement was apparent following only the 75 mg dose

[t(44)=2.76, p=0.008; effect size *d*=0.9]. There was also a significant main effect of treatment on sentence verification accuracy [F(2,46)=4.86, p=0.01] this was evinced as an improvement following 150 mg [t(46)=2.85, p=0.007; effect size *d*=0.6].

Mood measures were also affected with consumers becoming more 'jittery' [F(2,46)=4.79, p=0.01]. This effect was apparent in response to the 150 mg dose [t(46)=3.09, p=0.003; effect size *d*=0.8]. There was a significant alteration in 'alertness' in response to treatment [F(2,46)=3.87, p=0.03], significant increases followed 75 mg [t(46)=2.37, p=0.02; effect size *d*=0.6] and 150 mg [t(46)=2.45, p=0.02; effect size *d*=0.6]. There was also a significant main effect of treatment on 'mental fatigue' [F(2,46)=3.48, p=0.04]. This was a significant decrease following 150 mg [t(44)=2.63, p=0.01; effect size *d*=0.6].

2.3.5.2 Non-consumers

There was a significant main effect of treatment on digit vigilance accuracy in non-consumers [F(2,46)=4.31, p=0.02]. This was an improvement following the 150 mg dose [t(46)=2.71, p=0.009; effect size *d*=0.9]. Digit vigilance reaction time was also significantly affected by treatment [F(2,46)=4.00, p=0.03]. These improvements followed 75 mg [t(46)=2.66, p=0.01; effect size *d*=0.7] and 150 mg [t(46)=2.16, p=0.04; effect size *d*=0.6]. There was a significant improvement in numeric working memory reaction time [F(2,46)=4.20, p=0.02], this effect was apparent following 150 mg [t(46)=2.90, p=0.006; effect size *d*=0.8]. There was also a significant improvement in sentence verification accuracy [F(46)=3.78, p=0.03]. These improvements were evident following 75 mg [t(46)=2.56, p=0.01; effect size *d*=0.6] and 150 mg [t(46)=2.14, p=0.04; effect size *d*=0.6]. There was a significant main effect of treatment on delayed picture recognition reaction time [F(2,46)=3.58, p=0.04] with detrimental effects following 150 mg [t(46)=2.37, p=0.02; effect size *d*=0.6].

Mood measures were also affected. There was a significant main effect of treatment on subjective ratings of 'mental fatigue' [F(2,42)=3.28, p=0.047]. This was a significant reduction in 'mental fatigue' following 75 mg [t(42)=2.51, p=0.02; effect size *d*=0.7].

Comparing the effect sizes for behavioural effects for the two groups produces a mean effect size (*d*) in consumers of 0.7 with a range of 0.6 to 0.9, the mean effect size in non-consumers was also 0.7, ranging from 0.6 to 0.9.

2.3.6 Consumption correlation

Correlations were carried out between average caffeine consumption levels and performance. Due to a data capture error with 1 dataset it is only possible to report correlations for 47 participants.

2.3.6.1 Baseline scores

Correlations carried out between average baseline performance and average caffeine consumption revealed a positive correlation between consumption and the number of correct responses on the serial threes subtraction task [$r(44)=0.41$, $p=0.005$] and serial sevens [$r(44)=0.35$, $p=0.02$].

2.3.6.2 Post-treatment scores

Following 75 mg of caffeine, higher consumption was correlated with increased accuracy of choice reaction [$r(45)=0.35$, $p=0.02$] and slower choice reaction time [$r(45)=0.32$, $p=0.03$]. Accuracy of numeric working memory [$r(45)=0.31$, $p=0.03$] was also positively correlated with consumption, and DSST performance was negatively correlated [$r(45)=-0.33$, $p=0.02$]. Following 150 mg, DSST performance was again negatively correlated with consumption [$r(45)=-0.46$, $p=0.001$] but higher consumption was correlated with greater accuracy of logical reasoning [$r(45)=0.31$, $p=0.03$], and faster picture recognition [$r(45)=-0.36$, $p=0.01$]. Following placebo, higher consumption was correlated with greater accuracy of picture recognition [$r(45)=0.32$, $p=0.03$] and lower 'jittery' ratings [$r(43)=-0.30$, $p=0.046$].

2.3.7 Sex differences

There were no differences in the responses to caffeine of men and women.

2.4 Discussion

In line with many previous studies these results show that caffeine improves cognitive performance and mood in healthy, young adults in a manner that cannot be explained by withdrawal alleviation. Firstly, there were no baseline differences between the consumer groups that favoured non-consumers and, secondly, positive effects of caffeine were found in both consumers and non-consumers. Irrespective of caffeine habit, caffeine led to faster reaction times on a number of tasks relating to attention and working memory as well as improving sentence verification accuracy. Mood measures relating to arousal were also significantly improved in both groups. The 'alleviation of withdrawal model' suggests that caffeine should only lead to improvements in caffeine consumers who are in a state of withdrawal and should not elicit positive effects in non-consumers who are, by definition, not withdrawn. These findings provide strong support for absolute enhancement of mood and performance following acute caffeine administration.

Analysis of mean baseline scores from the current study also fails to support a withdrawal alleviation model. Only two significant differences in the performance of consumers versus non-consumers were noted, with consumers performing better than non-consumers on the serial sevens and serial threes subtraction tasks. This finding is confirmed by positive correlations between baseline serial subtraction performance and habitual caffeine consumption levels. The withdrawal alleviation model posits that consumers' performance will be impaired as a result of caffeine withdrawal, this was clearly not the case here. The results are consistent with Rogers et al.'s (2003) findings of no significant differences between non-consumers' and acutely withdrawn consumers' performance of a simple reaction time task at baseline. Smit and Rogers (2000) also compared baseline performance in caffeine deprived low and higher caffeine consumers and found that higher consumers performed significantly better on an RVIP task than low consumers. In line with this lack of cognitive deficit due to habitual consumer status, the present study also failed to support previous reports suggesting that consumers' subjective mood state is significantly impaired during caffeine withdrawal (Goldstein et al. 1969; Richardson et al. 1995; Rogers et al. 2003). However, it should be noted that in the second of two reported experiments, Rogers et al. (2003) also failed to replicate the finding of reduced baseline 'alertness' in consumers. This suggests that these effects are fragile and may be influenced by non-pharmacological processes such as expectancy (since presumably consumers are aware that they are caffeine deprived). Such effects will be less marked when examining placebo change from baseline scores rather than baseline scores when assessing withdrawal effects (again we found no evidence for caffeine withdrawal using

such an analysis). Studies comparing the effects of caffeine delivered in both novel vehicles and in coffee beverages may help to disentangle these pharmacological, expectancy and conditioning effects.

Turning to the primary analysis examining treatment X group effects, the results here support previous demonstrations of absolute improvements following caffeine. In two experiments Rogers et al. (2003) found that caffeine was capable of improving 'alertness' in consumers and non-consumers alike. Smit and Rogers (2000) also demonstrated that caffeine doses as low as 12.5 mg were equally capable of producing improvements in simple reaction time in low and higher caffeine consumers. Similarly, Childs and de Wit (2006) demonstrated improvements to vigilance and 'arousal' in light, non-dependent caffeine users. Warburton (1995) also found that caffeine was able to improve performance in those participants who had been pre-dosed with 75 mg of caffeine.

Whilst not wishing to over-interpret the pattern of enhancement found here, the data do support previous reports suggesting that caffeine preferentially improves performance on tasks assessing attention and vigilance [as reflected by improvements on both simple reaction time and digit vigilance reaction time for both doses and for both consumer groups (see Fig 2.2)]. It has been suggested that such effects may be the result of adenosine blockade by caffeine causing acute upregulation of activity within the ascending cholinergic activating system (Warburton et al. 2001). It has previously been argued that cholinergic modulation is unlikely to be the exclusive target for the effects of caffeine (Scholey and Kennedy 2004). Indeed one might expect to see more widespread effects on attentional measures if this were the case. The only other cognitive measure that was robustly affected across groups by both doses was sentence verification (Fig 2.2). The neurochemical substrates underpinning this task are not known. However, the task draws on aspects of cognitive flexibility and retrieval of semantic information. The former modality is also required for logical reasoning, which was unaffected in this study (Table 2.1). It is also worth noting that sentence verification has been shown to be robustly sensitive to caffeine in a series of studies by Smith's group (Nguyen-Van-Tam 2002). While this pattern of results is interesting, it has been argued that caffeine preferentially targets tasks of vigilance performed over a long period. With the possible exception of the 4-min RVIP, the tests used here were of a relatively short period. Even the digit vigilance task was of relatively short duration (3 min) and as such it could be argued that it is not a 'true' test of vigilance. It would be interesting to examine the effects on such tasks using the paradigm employed here.

There were no consistent effects on working memory measures (Figs 2.2 and 2.3). Numeric working memory reaction time was improved by the 150 mg dose alone,

while there were group differences only on spatial working memory sensitivity (with consumers performing better than non-consumers independent of treatment). Interestingly, the latter effect was similar to the results for false alarms during the RVIP (a task with a large working memory component). Further work is necessary in order to draw firm conclusions about the effects of caffeine on working memory, particularly as there was a trend in favour of non-consumers at baseline on spatial memory accuracy, which may have contributed to the post-dose group effects in favour of consumers on this measure.

Turning to the mood measures, the data here confirm that caffeine can improve self-rated 'alertness'. Interestingly, the effect was only found for the caffeine research visual analogue scales and not the Bond-Lader scales, although the effect on this measure did just miss significance with a p-value of 0.05. In the latter measure the 'alert' factor is derived from nine subscales, while in the former the 'alertness' factor is an aggregate of only two measures 'alert' and 'tired'. It is clear that the effects on this scale are largely due to a reduction in rating on the 'tired' subscale (Table 2.3). This contention is supported when examining the effects on the single 'mental fatigue' scale, which was sensitive to both doses of caffeine and across both groups and which showed a pattern of results that was largely a mirror image of the affected 'alertness' scale (Fig 2.4). The separate analyses of consumers and non-consumers suggested that consumers' 'alertness' was more sensitive to the effects of caffeine. This broadly supports the results of Goldstein et al. (1969) who found only consumers were affected on a mood cluster that included measures of 'alert', 'attentive', 'observant', 'able to concentrate'. On the other hand our results show that both consumers and non-consumers self-ratings of 'mental fatigue' were affected (albeit at different doses).

The only interaction between consumer status and treatment effects was found in participants' ratings of 'jitteriness'. Many studies report that caffeine increases 'jitteriness' (Rogers et al. 2003; Richardson et al. 1995), particularly in non-consumers. However, in this case non-consumers rated themselves as more 'jittery' than consumers following placebo and less 'jittery' than consumers following 150 mg of caffeine.

Correlations carried out between average daily caffeine consumption and post-treatment scores failed to produce any clear patterns. Higher caffeine consumption was associated with a speed-accuracy trade-off in choice reaction, improved numeric working memory and impaired DSST following the 75 mg dose. Following 150 mg higher consumption was again correlated with impaired DSST and also improved accuracy of logical reasoning and speed of picture recognition. Higher caffeine consumption was correlated with better picture recognition accuracy and lower 'jittery'

ratings following placebo. However, the only consistent post-caffeine relationship was that between higher caffeine consumption and lower DSST performance. The finding that higher caffeine consumption is only correlated with positive outcomes post-placebo could be said to further suggest a lack of withdrawal effects. In addition, correlations between caffeine consumption and performance at baseline were also only of a positive nature.

The results of the primary analysis do not support withdrawal alleviation models. However, the secondary analyses examining each group's scores separately did reveal some differences (and similarities) between the caffeine responses of habitual consumers and habitual non-consumers of caffeine. Both groups' sentence verification accuracy and 'mental fatigue' were improved by caffeine. Of the five measures that were improved in consumers, three were mood items ('jittery', 'mental fatigue' and 'alertness'), and two were performance outcomes (accuracy of both sentence verification and RVIP). For non-consumers, one measure, delayed picture recognition reaction time, was impaired. Of the five measures that were enhanced by a caffeine challenge only one ('mental fatigue') was a mood item, while the remaining four were performance outcomes (accuracy of sentence verification and digit vigilance and speed of digit vigilance and numeric working memory). The average effect sizes for the two groups were of a similar size and were above the standard medium effect size of d = 0.5. This finding argues against the suggestion that effects in non-consumers are modest (James and Rogers 2005), in fact effect sizes in non-consumers were slightly higher overall.

In its strictest form the withdrawal alleviation model would predict detrimental performance in overnight withdrawn caffeine consumers and no beneficial effects of caffeine in non-consumers. Clearly this interpretation is overly simplistic and these results indicate a more complex picture. The effects of caffeine were positive in both groups and there was no evidence for withdrawal as indicated by the groups' equivalent performance and mood at baseline following overnight caffeine abstinence. Additionally, non-consumers' performance and 'fatigue' levels were improved following a caffeine challenge and effect sizes were similar to those for consumers. On the other hand absolute enhancement by caffeine is not supported unreservedly since the secondary analysis revealed that consumers and non-consumers exhibited slightly different (but not mutually exclusive) responses to caffeine as outlined above. It may be that caffeine use is strongly reinforced through mood benefits and it is these effects that determine whether an individual will become a habitual caffeine consumer or a habitual non-consumer.

Whilst there are problems with relying on self-report caffeine consumption questionnaires in order to determine whether someone is a caffeine consumer or non-consumer, this method is one that is widely used in this area of study and it seems unlikely that people would be deceptive in this matter. However, it may be possible that they were unaware of caffeine consumption in certain products. Future studies of this nature should possibly take salivary samples at recruitment to determine 'typical' caffeine levels. Certainly, compliance to caffeine abstinence was confirmed by analysis of salivary caffeine levels. A further issue is the question of whether the non-consumer group used here can be said to be free of the effects of caffeine withdrawal. It might be that even rather low or intermittent dietary intakes of caffeine cause sufficient adenosine receptor upregulation for significant negative effects to be precipitated on caffeine withdrawal. This issue could only be fully addressed by comparing habitual consumers with both habitual non-consumers and those who abstain from caffeine completely. However, the fact that the two groups were affected differently on some measures does provide some support for the case for them being different populations.

In conclusion, the current study shows that doses of caffeine equivalent to those found in the average cup of coffee (Gray 1998) can improve mood and cognitive performance in caffeine consumers and non-consumers alike. It is not possible to state that either of the doses administered here (75 mg and 150 mg) had a greater impact on performance and mood. Rather it is apparent that response to the different doses varies with regard to task. The main purpose of this study was to examine the effects of realistic everyday doses of caffeine in consumers and non-consumers using sensitive measures of mood and cognitive performance. These findings overwhelmingly support absolute benefits over alleviation of withdrawal.

CHAPTER 3. THE ACUTE BEHAVIOURAL EFFECTS OF CAFFEINE AND L-THEANINE IN ISOLATION AND IN COMBINATION

3.1 Introduction

The behavioural effects of caffeine, particularly in the form of caffeinated coffee, have received considerable attention in the literature. Numerous studies have highlighted the beneficial effects of caffeine on cognition and mood (see Smith 2002), the most commonly reported being increases in ratings of 'alertness' (Rogers et al. 2003) and improvements on measures of reaction time and vigilance (Lieberman et al. 1987; Richardson et al. 1995). The results from the previous chapter support these findings and show that these effects do not differ significantly across habitual consumers and habitual non-consumers of caffeine.

Having established in Chapter 2 that caffeine is able to assert absolute effects on behaviour, rather than merely reversing withdrawal effects, it is now important to consider how this substance affects behaviour when it is consumed in combination with other potentially psychoactive components, as is often the case in natural consumption. When administered in the form of coffee, the effects of caffeine are often attributed solely to its caffeine content with little regard for any other components, which may be eliciting unique effects of their own, or interacting with the effects of caffeine. In addition, caffeine in the form of tea has received surprisingly little attention despite being, with the exception of water, the most widely consumed beverage in the world (Gilbert 1984).

Studies that have considered the effects of tea have found that, although the majority of the effects can be explained by the presence of caffeine, some differences exist between tea and coffee even when the caffeine level is matched. Quinlan et al. (1997) found that tea containing 100 mg of caffeine had significantly greater effects than coffee, with a matched caffeine level, in raising skin temperature. The authors suggest that this is indicative of a greater vasodilatory response - a trait that has previously been linked to flavonoids present in tea. Hindmarch et al. (2000) also found that tea significantly increased critical flicker fusion threshold when compared with coffee, despite both beverages containing 75 mg of caffeine, whereas coffee significantly speeded recognition reaction time in comparison to tea at the same dose. Steptoe et al. (2006) also considered the effects of chronic tea administration, containing 72 mg of caffeine, on acute stress responses compared to a control matched for caffeine level. They found that tea was able to attenuate the effects of

stress on platelet activation and cortisol levels, as well as increasing 'relaxation' ratings relative to control. The finding of decreased cortisol levels is particularly interesting as caffeine has been shown to increase cortisol levels, especially in response to stress (Lovallo et al. 2006). This must suggest that components within tea were actually able to produce an opposite effect to that seen with caffeine.

Henry and Stephens-Larson (1984) carried out a study examining the effects of decaffeinated tea on behaviour in mice. It was found that hypertension in mice, exposed to 3 to 5 months of psychosocial stress, was significantly reduced by chronic administration of decaffeinated green tea as compared to water, an effect they again suggest is related to flavonoids. Since decaffeination does not eliminate all caffeine, it is possible that these effects are related to low levels of caffeine. However, given the levels of caffeine in this case (1.3 mg/litre), conventional wisdom would suggest that this is unlikely. Another component of tea that is not lost in the decaffeination process and which may be responsible for these findings is L-theanine.

L-theanine (γ-glutamylethylamide) is one of the predominant amino acids found in green tea (*Camellia sinensis*) and is also present in other species of *Camellia* as well as in the edible bay boletes mushroom *Xerocomus badius.* The pharmacology of L-theanine is relatively unknown. However, animal studies have identified some neurochemical effects. These include increases in dopamine concentrations in the striatum (Yokogoshi et al. 2000), inhibition of glutamate reuptake (Sadzuka et al. 2002), increased GABA concentration (Kimura and Murata 1971), decreased global brain serotonin with region specific increases in the striatum, hippocampus and hypothalamus (Yokogoshi et al. 1998), and neuroprotective effects through blockade of NMDA and AMPA receptors (Kakuda 2002).

Animal studies have also revealed some effects of L-theanine on behaviour. The effects of L-theanine on blood pressure were investigated and it was found that high doses (1500 mg and 2000 mg) led to significant reductions in blood pressure in spontaneous hypertensive rats 60 minutes after administration (Yokogoshi et al. 1995). This effect may relate to findings of reduced psychosocial hypertension in mice (Henry and Stephens-Larson 1984). Unfortunately it is not possible to ascertain dose levels given in the latter study. Cognitive performance has also been shown to be sensitive to L-theanine. Four month chronic administration of 180 mg/day L-theanine led to improvements in memory and learning ability in Wistar rats as measured by both passive and active avoidance tests (Juneja et al. 1999). Evidence for relaxant effects of L-theanine comes from findings that show an inhibiting effect of L-theanine on the convulsive action and spontaneous activity caused by caffeine administration (Kimura and Murata 1971). The inhibiting effects of L-theanine on the stimulation elicited by

caffeine evaluated by EEG in the rat have also been examined (Kakuda et al. 2000). The results showed that 50 µmol/kg of L-theanine was sufficient to completely suppress stimulation associated with 5 µmol/kg caffeine in the rat. However, a lower dose of 2 µmol/kg was found to induce an excitatory effect in the absence of caffeine. On the other hand, locomotion, standing, hole-poking and grooming were all unaffected by L-theanine at either dose (Yokogoshi and Terashima 2000).

In line with these findings from animal studies, L-theanine has historically been used as a relaxing agent. However, few studies exist to support this practice. Kobayashi et al. (1998) found evidence of relaxing properties from human EEG activity. Administration of 200 mg of L-theanine, but not 50 mg, led to increased α- waves in the occipital and parietal regions of the brain within 40 minutes of ingestion when administered to resting participants, which the authors suggest is indicative of 'relaxation' without 'drowsiness'. Some support for this assertion regarding relaxation without drowsiness comes from a study that considered the subjective and autonomic effects of L-theanine. Lu et al. (2004) found significant relaxant effects of 200 mg as measured by the 'tranquil-troubled' item of the Bond-Lader visual analogue scales (Bond and Lader 1974) when administered in a rested state. This finding was, however, not replicated when participants were under conditions of increased anxiety. These findings are suggestive of differential responses to L-theanine when rested to when aroused. However, although supportive of relaxant effects of L-theanine, the findings of Kimura et al. (2007) also slightly contradict those of Lu et al. (2004) in that they found reduced heart rate and secretory immunoglobulin A (s-Iga) responses to acute stress following L-theanine. In addition, state 'anxiety', as measured by the state element of the state-trait anxiety inventory, (Spielberger 1977), and subjective perceived stress ratings were also reduced following L-theanine as compared to placebo. However, Gomez-Ramirez et al. (2007) found evidence of a decrease in alpha activity following 250 mg of L-theanine when measured during performance of a highly demanding attention task. This appears to be contradictory to the findings of Kobayashi et al. (1998) but may also be indicative of differing EEG effects of L-theanine when administered during a task as opposed to at rest. This study also represents the only investigation to date of cognitive effects of L-theanine in humans, and this decrease in alpha activity was associated with a slowing of reaction time on an auditory attention task. Although limited, this research in indicative of a broadly relaxant effect of L-theanine. However, although cognitive enhancement has been shown in animals, more work is needed in this area in humans. In addition, only one study to date has considered the effects in humans of co-administration of L-theanine and caffeine despite evidence for an interaction between their effects from animal studies. Rogers et

al. (2008) found that 200 mg of L-theanine led to slower reaction time on a visual probe task, indicative of decreased anxiety, and was able to antagonise the increase in systolic and diastolic blood pressure seen following 200 mg of caffeine. No such effect was seen on the increase in 'alert' and 'jittery' ratings as a result of caffeine administration. Unfortunately, this study did not consider the effects of these treatments on cognition.

As L-theanine and caffeine are often found together in tea drinks it is important to assess any combined effects. The current randomised, placebo-controlled, double-blind, balanced crossover study investigated the cognitive and mood effects of administration of the two agents both alone [L-theanine (250 mg), caffeine (150 mg)] and in combination (250 mg/150 mg). The primary aim was to ascertain the effects of caffeine, in isolation and in combination with L-theanine. Combined effects of naturally concomitant psychoactives are complex and often difficult to predict based on the effects of individual components (Scholey et al. 2005). A starting point for exploring this is to examine the effects of combinations where the effects of one or more components are fairly well established (as here). Chapter 2 investigated the effects of 75 and 150 mg of caffeine using the procedure and assessment battery employed here. In this case 150 mg was selected because, although both doses produced the same number of improvements, those effects seen with 150 mg were more distinct. In this first human investigation of the neurocognitive effects of L-theanine, relatively high, known psychoactive doses were chosen. 250 mg of L-theanine has previously been shown to be bioactive and although this combination is neither at a ratio nor dose found in tea beverages it was felt that combining two known psychoactive doses would naturally allow capture of any opposing, additive, or synergistic effects of the two.

The suite of tests used in the current study has been shown to be sensitive to the psychoactive effects of a range of components of food and beverages (e.g. Scholey and Kennedy 2004) in a number of studies including that presented in the previous chapter, which examined the effects of 150 mg of caffeine. The same battery was used here, firstly because it afforded the opportunity to assess the robustness of the psychoactive profile found in the previous chapter and, secondly, because of its sensitivity to dietary components. Furthermore, the additional tasks included, although not typically sensitive to caffeine, may be affected differently when caffeine is combined with another substance. Finally, as the study aimed to conduct a systematic assessment of the behavioural effects of L-theanine, the extensive range of tasks also allowed a cognitive profile for L-theanine to be produced thus facilitating meaningful comparison with other drugs.

3.2 Materials and Methods

3.2.1 Design

A randomised, placebo-controlled, double-blind, balanced-crossover design was employed.

3.2.2 Initial screening

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix IV).

3.2.3 Participants

Participants were informed that the study was investigating the cognitive and mood effects of a commercially available fruit drink containing active components (one of which may be caffeine). Twenty four participants completed the experiment (9 male and 15 female, mean age 21.3 years, SEM 0.83, range 18 - 34 years). All were undergraduate volunteers. Participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session and throughout the morning until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

3.2.4 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence and immediately prior to both post-treatment assessments to confirm effective caffeine absorption. The saliva samples were immediately frozen at -20 °C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure

caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

3.2.5 Assessment

The tasks employed were identical to those described in Chapter 2 with the exception that tracking and the Digit Symbol Substitution Task were not included due to methodological issues and a lack of sensitivity to caffeine.

3.2.6 Treatments

Participants received four drinks containing: 1) 0 mg of caffeine plus 0 mg of L-theanine (placebo), 2) 150 mg of caffeine, 3) 250 mg of L-theanine, and 4) 150 mg of caffeine plus 250 mg of L-theanine on separate occasions. In each case the treatment was presented in a 250 ml modified Peach Lite Lipton Iced Tea drink (Unilever, Colworth, UK). During initial preparation all tea powder (including L-theanine and caffeine) was removed from the drink and the sweetener levels adjusted to mask the bitter taste of the high dose of added caffeine so that no taste difference could be detected between the drinks. The drink also contained the following: trisodium citrate; peach flavour; peach juice; malic acid; aspartame; acesulfame K; and ascorbic acid. Ten minutes was allowed for drink consumption. These drinks were prepared off-site and assigned a treatment code by a disinterested third party.

3.2.7 Procedure

Each participant was required to attend a total of five study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the four active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the four active study days comprised three identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, the second took place 30 minutes post-drink, and the final session took place 90 minutes post-drink.

Each testing session lasted approximately 30 minutes and comprised producing a saliva sample, completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales.

3.2.8 Statistics

Salivary caffeine levels were analysed to assess compliance to caffeine abstinence and effective caffeine absorption.

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab.

The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising t tests with MSError from an omnibus ANOVA as an error term (Keppel 1991). At each time point (30 minutes and 90 minutes post-treatment) data from the placebo condition was compared to that for each of the three active treatments (caffeine, L-theanine, caffeine/L-theanine). Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for treatment, assessment, treatment x assessment and participant (Kirk 1968), was carried out to identify main effects and interaction effects on change from baseline data for each measure. To ensure the overall Type I error protection level only those planned comparisons associated with measures that generated a significant main effect or interaction effect ($p<0.05$) on this initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated. Where baseline differences were observed baseline scores were entered as a covariate in an ANCOVA, with terms fitted to the model for treatment, assessment, treatment x assessment and participant and this was analysed using SAS/STAT.

3.3 Results

3.3.1 Salivary caffeine levels

Salivary analysis confirmed compliance with overnight abstinence, mean baseline values were 0.42 μg/ml (SD = 0.87). 2 datasets from time points other than baseline were unusable and were excluded from any analyses. Analysis of post-treatment salivary caffeine levels revealed significantly higher salivary caffeine levels following caffeine administration [$t(63)=13.29$, $p<0.0001$; $t(63)=6.84$, $p<0.0001$ at 30 minutes and 90 minutes respectively] and following the combination [$t(63)=11.75$, $p<0.0001$; $t(63)=6.36$, $p<0.0001$ at 30 minutes and 90 minutes respectively], see Fig 3.1.

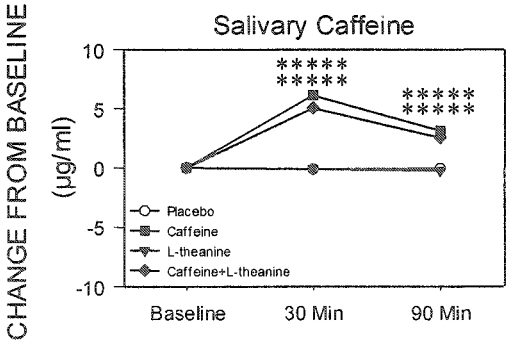

Figure 3.1 Mean change from baseline salivary caffeine levels following placebo, 150 mg of caffeine, 250 mg of L-theanine, and 150 mg of caffeine plus 250 mg of L-theanine. Significant treatment effects compared with placebo are indicated (*****$p<0$ .0005).

3.3.2 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose baseline scores for all four treatments (placebo, 150 mg of caffeine, 250 mg of L-theanine, 150 mg of caffeine plus 250 mg of L-theanine) for each outcome were subjected to a one-way, repeated-measures, ANOVA. There were significant baseline differences in accuracy of spatial memory and 'content' ratings, with significantly poorer spatial memory performance in the caffeine condition ($F(3, 69)=2.82$, $p=0.045$) and significantly lower ratings of 'content' in the L-theanine condition [$F(3, 69)=3.00$, $p=0.036$].

3.3.3 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 3.1. Significant differences are presented in Figure 3.2. Only planned comparisons for those measures that generated significant main effects and/or interactions for each outcome measure are reported below.

Table 3.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score		Main effect of treatment
			30 minutes	90 minutes	
Immediate word recall accuracy (%)	Placebo	51.4±4.00	-3.47±3.95	-5.14±3.05	F(3, 69)<1
	Caffeine	47.6±3.70	-3.06±2.98	-5.56±3.46	
	L-theanine	47.9±4.06	-5.14±2.56	-2.64±2.51	
	Caff+L-thea	47.6±3.28	-3.33±2.90	-0.28±3.69	
Simple reaction time (ms)	Placebo	294±7.57	17.4±7.20	31.0±10.2	**F(3, 69)=5.32 p=0.002**
	Caffeine	296±7.62	4.26±6.18	6.76±9.86	
	L-theanine	296±7.94	8.27±5.98	23.5±8.16	
	Caff+L-thea	296±7.77	-0.98±5.99	-1.12±6.53	
Digit vigilance accuracy (%)	Placebo	92.7±1.42	0.56±0.79	-3.98±1.29	F(3, 69)=1.11 p>0.1
	Caffeine	92.7±1.41	-0.74±1.58	-0.37±1.74	
	L-theanine	93.1±1.43	-2.04±1.91	-3.80±1.61	
	Caff+L-thea	94.8±1.68	-2.50±1.31	-2.69±1.38	
Digit vigilance reaction time (ms)	Placebo	440±6.51	20.0±7.06	21.8±6.97	**F(3, 69)=4.94 p=0.003**
	Caffeine	443±9.73	1.68±5.65	-1.88±8.66	
	L-theanine	433±7.61	19.2±7.15	24.6±6.94	
	Caff+L-thea	436±8.72	2.50±8.35	8.93±6.39	
Digit vigilance false alarms (number)	Placebo	1.25±0.23	-0.17±0.27	0.38±0.31	F(3, 69)=1.13 p>0.1
	Caffeine	1.71±0.34	-0.92±0.38	0.13±0.31	
	L-theanine	1.42±0.25	0.17±0.35	0.04±0.36	
	Caff+L-thea	1.33±0.38	-0.13±0.37	0.25±0.30	
Choice reaction time accuracy (%)	Placebo	94.8±0.79	1.67±0.66	-0.50±0.79	F(3, 69)=1.16 p>0.1
	Caffeine	95.3±0.67	-0.25±0.89	0.33±0.70	
	L-theanine	95.2±0.84	-0.17±0.68	-1.33±0.80	
	Caff+L-thea	95.1±0.82	0.25±0.67	0.17±0.70	
Choice reaction time (ms)	Placebo	434±9.97	20.7±9.19	17.8±9.68	**F(3, 69)=4.05 p=0.008**
	Caffeine	444±15.1	-13.6±11.8	-16.6±9.51	
	L-theanine	442±14.9	-5.43±10.8	-6.12±12.0	
	Caff+L-thea	441±12.6	1.94±11.1	4.76±11.8	
RVIP accuracy (%)	Placebo	59.5±5.05	-6.38±1.81	-6.64±2.50	**F(3, 69)=11.73 p<0.001**
	Caffeine	55.2±4.87	-1.30±2.50	8.20±2.70	
	L-theanine	57.4±4.91	-4.69±2.44	-4.69±2.36	
	Caff+L-thea	56.6±4.27	3.52±2.40	4.82±2.66	
RVIP reaction time (ms)	Placebo	502±18.6	4.57±15.6	7.26±19.1	**F(3, 69)=2.83 p=0.04**
	Caffeine	485±12.4	-17.6±11.3	-10.2±14.7	
	L-theanine	505±17.0	19.2±16.6	-4.95±10.7	
	Caff+L-thea	524±20.5	-36.1±15.9	-25.9±19.2	
RVIP false alarms (number)	Placebo	0.83±0.19	0.38±0.32	0.50±0.32	F(3, 69)=1.18 p>0.1
	Caffeine	1.33±0.37	-0.17±0.29	-0.08±0.46	
	L-theanine	1.00±0.23	0.17±0.21	0.29±0.20	
	Caff+L-thea	1.46±0.32	0.38±0.37	0.33±0.32	
Spatial memory (sensitivity index)	Placebo	0.95±0.01	-0.02±0.01	-0.12±0.04	**F(3, 69)=6.54 p<0.001**
	Caffeine	0.86±0.05	0.09±0.05	0.07±0.05	
	L-theanine	0.95±0.02	-0.05±0.03	-0.05±0.03	
	Caff+L-thea	0.94±0.01	0.00±0.02	-0.09±0.05	
Spatial memory reaction time (ms)	Placebo	551±28.7	-3.18±27.9	15.2±15.1	F(3, 69)=1.26 p>0.1
	Caffeine	540±19.7	-9.75±10.7	-30.1±9.89	
	L-theanine	550±24.8	-23.3±21.4	5.96±29.9	
	Caff+L-thea	555±23.2	-28.0±15.3	-28.7±15.6	
Logical reasoning accuracy (%)	Placebo	74.1±4.36	-4.69±2.32	-1.04±1.96	F(3, 69)=1.17 p>0.1
	Caffeine	73.4±4.60	-1.74±1.50	1.56±1.39	
	L-theanine	71.9±5.24	0.35±1.82	-0.17±1.67	
	Caff+L-thea	73.8±5.20	-1.22±2.12	-2.43±1.84	
Logical reasoning reaction time (ms)	Placebo	2736±196	21.5±155	-184±235	F(3, 69)<1
	Caffeine	2442±153	-16.1±99.4	-126±81.8	
	L-theanine	2557±183	-157±127	-223±111	
	Caff+L-thea	2615±222	-30.4±124	-202±127	
Numeric working memory (sensitivity index)	Placebo	0.90±0.02	-0.01±0.01	-0.02±0.01	F(3, 69)<1
	Caffeine	0.91±0.01	0.00±0.01	-0.02±0.02	
	L-theanine	0.90±0.02	0.00±0.02	0.00±0.02	
	Caff+L-thea	0.92±0.01	0.00±0.01	-0.02±0.02	
Numeric working memory reaction time (ms)	Placebo	571±18.5	-13.7±8.71	3.66±14.0	**F(3, 69)=6.86 p<0.001**
	Caffeine	564±16.6	-36.3±11.9	-27.3±11.3	
	L-theanine	555±18.5	17.1±14.7	8.36±11.0	
	Caff+L-thea	562±15.1	-33.4±7.97	-21.8±7.64	

Delayed word recall accuracy (%)	Placebo	39.2±3.68	-6.67±3.38	-13.3±2.95	F(3, 69)<1
	Caffeine	35.8±3.08	-9.72±3.43	-9.58±3.57	
	L-theanine	35.6±3.08	-8.89±3.29	-10.8±3.36	
	Caff+L-thea	36.5±2.92	-12.8±3.89	-7.08±3.63	
Delayed word recognition (sensitivity index)	Placebo	0.67±0.04	-0.07±0.04	-0.08±0.04	F(3, 69)<1
	Caffeine	0.65±0.05	-0.05±0.05	-0.08±0.04	
	L-theanine	0.64±0.05	-0.12±0.05	-0.03±0.03	
	Caff+L-thea	0.68±0.04	-0.07±0.05	-0.07±0.05	
Delayed word recognition reaction time (ms)	Placebo	720±33.5	4.83±29.0	-11.2±15.1	**F(3, 69)=6.64 p<0.001**
	Caffeine	694±21.5	-1.73±17.4	-14.7±26.2	
	L-theanine	689±22.1	13.9±17.6	31.2±21.6	
	Caff+L-thea	755±34.2	-83.6±37.3	-86.6±39.8	
Delayed picture recognition (sensitivity index)	Placebo	0.64±0.04	0.02±0.04	-0.04±0.04	F(3, 69)<1
	Caffeine	0.64±0.05	-0.04±0.06	0.02±0.04	
	L-theanine	0.64±0.05	-0.03±0.04	-0.07±0.04	
	Caff+L-thea	0.65±0.05	-0.05±0.05	-0.04±0.06	
Delayed picture recognition reaction time (ms)	Placebo	781±26.1	7.32±16.7	38.7±24.2	F(3, 69)=1.78 p>0.1
	Caffeine	788±24.9	-9.84±20.3	-22.8±16.9	
	L-theanine	809±36.2	-13.9±27.2	-21.1±28.3	
	Caff+L-thea	793±23.3	-9.97±19.8	-27.5±20.4	

3.3.3.1 Simple reaction time

Performance of the simple reaction time task was significantly improved following administration of caffeine [t(69)=2.05, p=0.04; t(69)=3.77, p=0.0003 at 30 mins and 90 mins respectively] and following caffeine plus L-theanine [t(69)=2.87, p=0.005; t(69)=5.00, p<0.00001, at 30 mins and 90 mins respectively], see Fig 3.2a.

3.3.3.2 Digit vigilance reaction time

There were significant improvements in digit vigilance reaction time following caffeine at both time-points [t(69)=2.66, p0.0096; t(69)=3.45, p=0.00096, at 30 mins and 90 mins respectively]. In the combination condition these improvements were only apparent at 30 mins post-dose [t(69)=2.54, p=0.02], see Fig 3.2b.

3.3.3.3 Choice reaction time

There were significant improvements in choice reaction time at both time-points following both caffeine [t(69)=4.45, p=0.00003; t(69)=4.46, p=0.00003, at 30 mins and 90 mins respectively] and L-theanine [t(69)=3.39, p=0.001; t(69)=3.10, p=0.003, at 30 mins and 90 mins respectively]. The combination only improved performance at 30 mins post-treatment [t(69)=2.43, p=0.02] see Fig 3.2c.

3.3.3.4 Rapid Visual Information Processing (RVIP)

Accuracy of RVIP was significantly improved at 30 mins [t(69)=2.54, p=0.01] and 90 mins post-dose [t(69)=7.44, p<0.00001] following administration of caffeine and following administration of the combination [t(69)=4.96, p<0.00001; t(69)=5.74, p<0.00001 at 30 mins and 90 mins respectively] see Fig 3.2d. Speed improvements

were also apparent at 30 mins [t(69)=2.68, p=0.009] and 90 mins post-dose [t(69)=2.18, p=0.03] following administration of the combination, see Fig 3.2e.

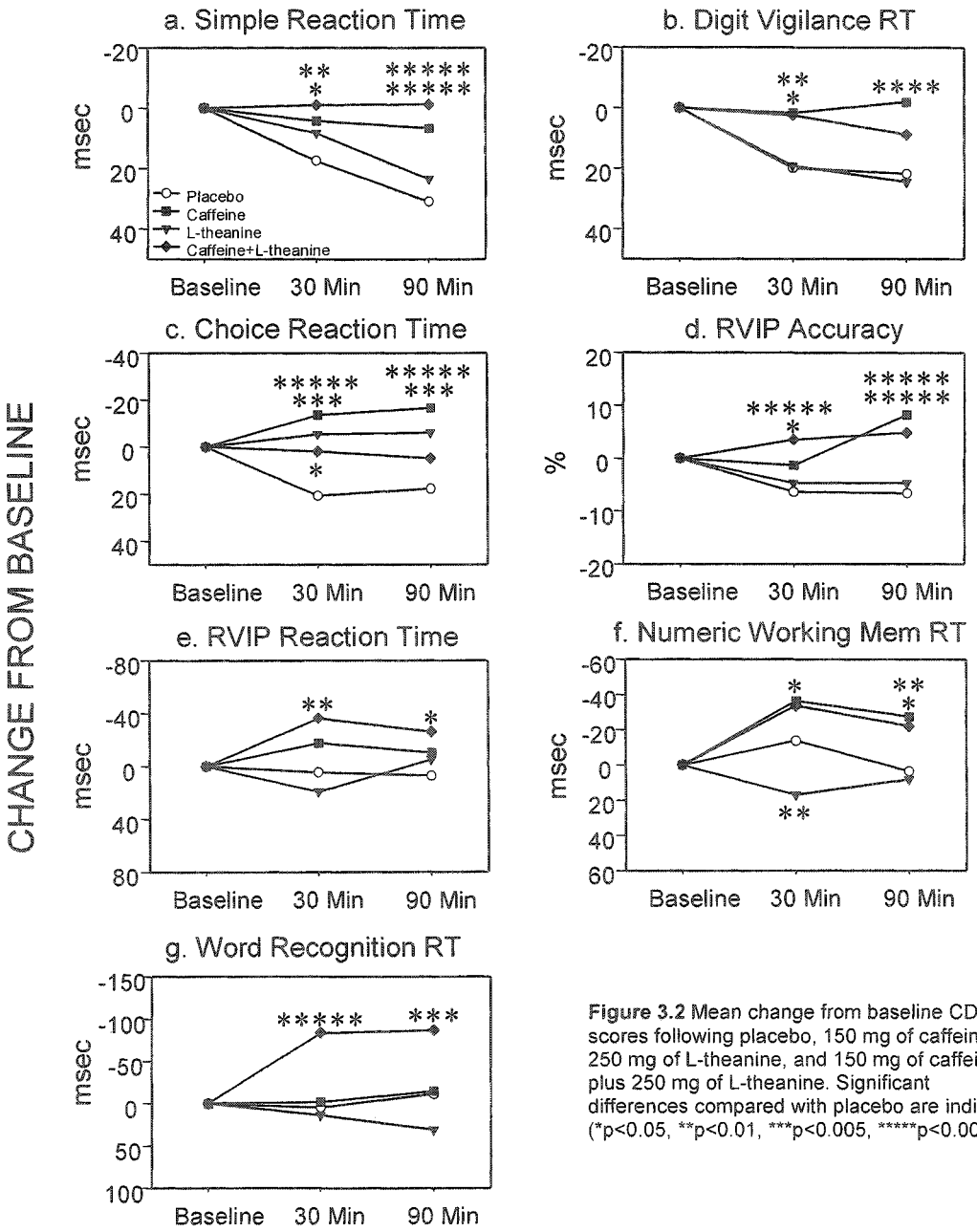

Figure 3.2 Mean change from baseline CDR scores following placebo, 150 mg of caffeine, 250 mg of L-theanine, and 150 mg of caffeine plus 250 mg of L-theanine. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

3.3.3.5 Spatial memory accuracy

Significant differences in accuracy of spatial memory were no longer apparent following ANCOVA with baseline scores included as a covariate.

3.3.3.6 Numeric working memory reaction time

Speed of numeric working memory was significantly improved following caffeine at 30 mins [t(69)=2.02, p=0.047] and 90 mins post-dose [t(69)=2.77, p=0.007], and at 90 mins following caffeine plus L-theanine [t(69)=2.28, p=0.03]. There was a significant impairment at 30 mins following administration of L-theanine [t(69)=2.75, p=0.008], see Fig 3.2f.

3.3.3.7 Delayed word recognition reaction time

Speed on the word recognition task was significantly improved following the combination at 30 minutes [t(69)=3.73, p=0.0004] and at 90 mins [t(69)=3.18, p=0.002] see Fig 3.2g.

3.3.4 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 3.2. Significant differences are presented in Figure 3.3. Only planned comparisons for those measures that generated significant main effects and/or interactions for each outcome measure are reported below. Due to a data capture error with 2 datasets these analyses look at only 22 participants.

Table 3.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score		Main effect of treatment
			30 minutes	90 minutes	
Sentence verification accuracy (%)	Placebo	96.5±1.25	-1.52±1.24	-1.36±1.11	F(3, 63)=2.62 p=0.05
	Caffeine	95.6±1.08	0.30±1.22	0.15±1.06	
	L-theanine	96.4±1.03	0.15±1.15	-0.75±0.97	
	Caff+L-thea	96.1±1.04	-0.15±1.06	2.42±1.03	
Sentence verification reaction time (ms)	Placebo	1345±82.2	-79.7±74.9	-70.8±41.8	F(3, 63)=1.97 p>0.1
	Caffeine	1306±45.4	-57.8±32.8	-84.1±37.4	
	L-theanine	1366±64.9	-179±51.0	-80.0±60.9	
	Caff+L-thea	1267±63.4	-9.55±50.3	-87.0±36.6	
Serial threes subtraction correct (number)	Placebo	38.0±2.34	2.64±1.45	4.05±1.83	**F(3, 63)=2.97 p=0.03**
	Caffeine	39.7±2.56	4.41±1.28	6.36±0.95	
	L-theanine	40.5±2.53	1.64±1.58	4.65±1.68	
	Caff+L-thea	39.8±2.64	5.55±1.39	5.77±1.57	
Serial threes subtraction errors (number)	Placebo	4.18±0.46	0.82±0.54	-0.23±0.36	F(3, 63)=1.25 p>0.1
	Caffeine	4.55±0.75	-0.64±0.72	-0.77±0.61	
	L-theanine	3.82±0.56	0.09±0.51	-0.32±0.73	
	Caff+L-thea	3.77±0.50	-0.32±0.46	-0.59±0.46	
Serial sevens subtraction correct (number)	Placebo	22.1±2.16	0.50±0.91	3.32±1.15	**F(3, 63)=3.36 p=0.02**
	Caffeine	22.6±2.02	1.82±0.90	4.64±1.13	
	L-theanine	22.4±2.08	1.41±1.00	2.81±1.25	
	Caff+L-thea	23.4±2.04	1.41±0.98	2.32±1.23	
Serial sevens subtraction errors (number)	Placebo	2.95±0.41	0.27±0.46	0.32±0.51	F(3, 63)<1
	Caffeine	4.00±0.62	0.59±0.79	-0.14±0.80	
	L-theanine	4.00±0.69	-0.86±0.67	0.36±0.68	
	Caff+L-thea	3.68±0.43	-0.45±0.46	0.05±0.58	

3.3.4.1 Serial threes subtraction task

The number of correct serial threes subtractions significantly increased at 30 minutes following caffeine plus L-theanine [t(63)=2.02, p=0.047], see Fig 3.3a.

3.3.4.2 Serial sevens subtraction task

The number of correct serial sevens subtractions significantly decreased at 90 minutes following L-theanine [t(63)=3.87, p=0.0003], see Fig 3.3b.

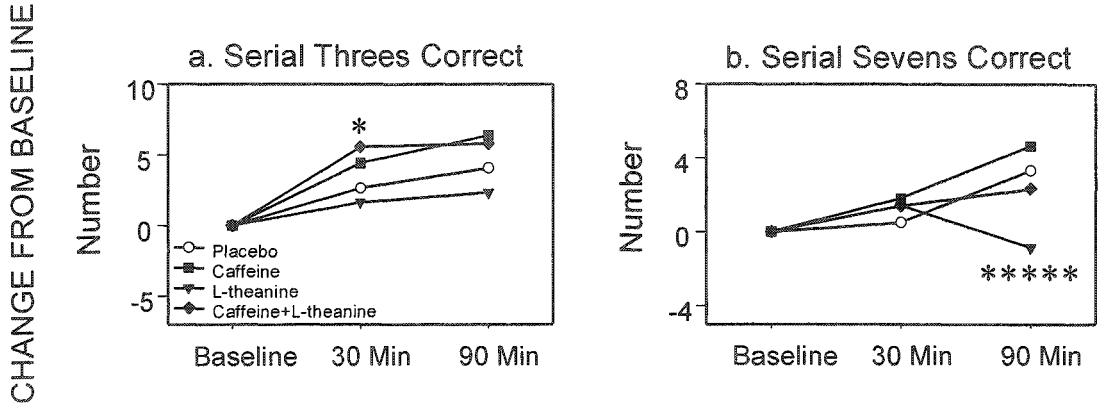

Figure 3.3 Mean change from baseline scores for serial subtraction measures following placebo, 150 mg of caffeine, 250 mg of L-theanine, and 150 mg of caffeine plus 250 mg of L-theanine. Significant differences compared with placebo are indicated (*p<0.05; *****p<0.0005).

3.3.5 Subjective mood measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 3.3. Only planned comparisons for those measures that generated significant main effects for each outcome measure are reported below. Significant differences in mood are presented in Figure 3.4.

3.3.5.1 Bond-Lader 'Alert' factor

Ratings on the Bond-Lader visual analogue scales 'alert' factor were significantly improved following caffeine plus L-theanine at 30 minutes [t(69)=3.40, p=0.001] and at 90 minutes [t(69)=3.04, p=0.003], see Fig 3.4a.

3.3.5.2 Bond-Lader 'Calm' factor

There were significant main effects of treatment on the Bond-Lader visual analogue scales 'calm' factor. At 30 minutes post-dose L-theanine improved 'calm' ratings [t(69)=2.36, p=0.02] whereas the combination reduced them [t(69)=2.64, p=0.01], see Fig 3.4b.

Table 3.3 Baseline and change from baseline scores for mood for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure		Treatment	Pre-dose baseline score	Post-dose change from baseline score		Main effect of treatment
				30 minutes	90 minutes	
Bond-Lader factors	**Alert**	Placebo	50.0±3.72	-2.70±2.78	-2.95±3.82	**F(3, 69)=6.68 p<0.001**
		Caffeine	55.4±2.70	0.11±2.25	-0.07±1.67	
		L-theanine	47.2±2.93	-3.63±3.04	-3.19±2.84	
		Caff+L-thea	53.4±3.47	6.24±1.82	5.03±2.52	
	Content	Placebo	60.5±3.03	-2.45±1.31	-1.06±1.78	F(3, 69)=2.44 p=0.07
		Caffeine	59.5±3.04	-0.22±1.46	0.40±1.38	
		L-theanine	53.1±3.47	1.41±1.65	3.15±2.00	
		Caff+L-thea	62.8±3.27	1.27±2.13	2.74±2.23	
	Calm	Placebo	62.2±3.36	-4.40±2.74	-4.85±2.56	**F(3, 69)=3.25 p=0.02**
		Caffeine	61.5±2.79	-3.58±3.22	-4.96±3.22	
		L-theanine	57.9±3.10	0.81±2.97	-2.44±2.31	
		Caff+L-thea	63.1±2.94	-10.2±3.30	-9.00±2.95	
Caffeine research visual analogue scales	Relaxed	Placebo	62.9±4.17	-6.13±3.52	-10.8±5.26	F(3, 66)=2.21 p=0.09
		Caffeine	60.7±4.36	-6.43±4.60	-6.22±4.96	
		L-theanine	53.6±3.86	7.26±3.28	-5.00±4.77	
		Caff+L-thea	61.0±3.85	-7.35±5.34	-6.57±5.06	
	Alert	Placebo	45.8±4.94	0.91±4.90	8.13±4.87	**F(3, 66)=3.81 p=0.01**
		Caffeine	56.0±4.01	4.09±2.97	6.52±4.86	
		L-theanine	48.5±4.73	-6.17±4.98	0.57±4.36	
		Caff+L-thea	51.3±4.59	10.2±4.66	12.0±4.12	
	Jittery	Placebo	23.7±4.53	4.09±2.90	10.7±4.74	F(3, 66)=1.91 p>0.1
		Caffeine	23.6±3.52	14.2±5.64	11.4±5.80	
		L-theanine	24.6±4.76	4.30±3.57	5.48±4.67	
		Caff+L-thea	27.4±5.30	6.65±4.70	7.43±4.94	
	Tired	Placebo	55.0±5.83	-1.22±5.95	-3.39±6.83	**F(3, 66)=10.28 p<0.001**
		Caffeine	51.7±4.10	-17.7±4.05	-16.1±4.38	
		L-theanine	54.1±5.35	7.22±5.93	-1.13±5.11	
		Caff+L-thea	56.7±4.69	-18.7±4.69	-22.5±3.90	
	Tense	Placebo	28.9±4.81	3.35±4.66	11.1±5.04	F(3, 66)=1.64 p>0.1
		Caffeine	33.7±4.67	5.22±4.54	6.57±4.67	
		L-theanine	38.4±5.44	-4.96±3.39	0.52±3.50	
		Caff+L-thea	30.4±5.06	7.13±5.92	0.57±4.42	
	Headache	Placebo	17.7±4.74	3.09±1.86	2.65±1.97	**F(3, 66)=11.79 p<0.001**
		Caffeine	18.7±4.41	-2.22±1.91	-3.13±2.76	
		L-theanine	19.1±4.86	5.57±1.86	10.0±3.26	
		Caff+L-thea	17.5±4.33	0.00±1.92	-4.04±3.01	
	Overall mood	Placebo	61.9±3.89	-1.09±2.70	2.26±2.60	**F(3, 66)=3.32 p=0.02**
		Caffeine	63.2±4.60	2.13±4.49	1.00±2.90	
		L-theanine	53.4±4.13	-1.35±2.62	1.00±2.61	
		Caff+L-thea	60.4±3.99	6.96±2.96	8.83±2.89	
	Mental fatigue	Placebo	38.8±5.05	11.0±2.93	4.87±4.13	**F(3, 66)=4.29 p=0.006**
		Caffeine	36.7±3.99	-2.74±4.51	-4.17±4.62	
		L-theanine	44.5±4.81	1.65±5.89	5.35±4.64	
		Caff+L-thea	42.1±4.21	-2.87±3.75	-5.22±3.78	
Caffeine research visual analogue scales factors	**Alertness**	Placebo	-9.13±10.4	2.13±10.4	11.5±11.2	**F(3, 66)=8.33 p<0.001**
		Caffeine	4.35±7.28	21.8±5.76	22.6±7.10	
		L-theanine	-5.57±9.08	-13.4±10.3	1.70±8.42	
		Caff+L-thea	-5.39±8.67	28.8±8.20	34.5±6.92	
	Tension	Placebo	-34.0±8.30	9.48±6.41	21.9±9.71	F(3, 66)=2.58 p=0.06
		Caffeine	-27.1±8.70	11.7±8.71	12.8±9.19	
		L-theanine	-15.1±8.69	-12.2±5.54	5.52±7.02	
		Caff+L-thea	-30.7±7.13	14.5±8.58	7.13±6.63	

There were a number of treatment effects on the caffeine research visual analogue scales. Due to a data capture error with 1 dataset this analysis looks at only 23 participants.

3.3.5.3 'Alertness' factor

Subjective ratings of 'alertness' were significantly increased following caffeine at 30 minutes [t(66)=2.21, p=0.03] and at 30 minutes [t(66)=2.99, p=0.004] and 90 minutes [t(66)=2.57, p=0.01] following the combination, see Fig 3.4c.

3.3.5.4 'Tired'

The effect of treatment on 'alertness' was largely due to decreases in 'tiredness'. Caffeine decreased 'tired' ratings at 30 minutes [t(66)=3.33, p=0.001] and at 90 minutes [t(66)=2.56, p=0.01]. The combination also improved ratings at both time points [30 minutes: t(66)=3.52, p=0.0008; 90 minutes: t(66)=3.86, p=0.0003].

3.3.5.5 'Headache'

Caffeine significantly decreased 'headache' ratings at both 30 minutes [t(66)=2.58, p=0.01] and 90 minutes [t(66)=2.81, p=0.006]. The combination also had this effect at 90 minutes [t(66)=3.26, p=0.002]. However, L-theanine increased ratings of 'headache' at 90 minutes post-dose [t(66)=3.58, p=0.0007], see Fig 3.4d.

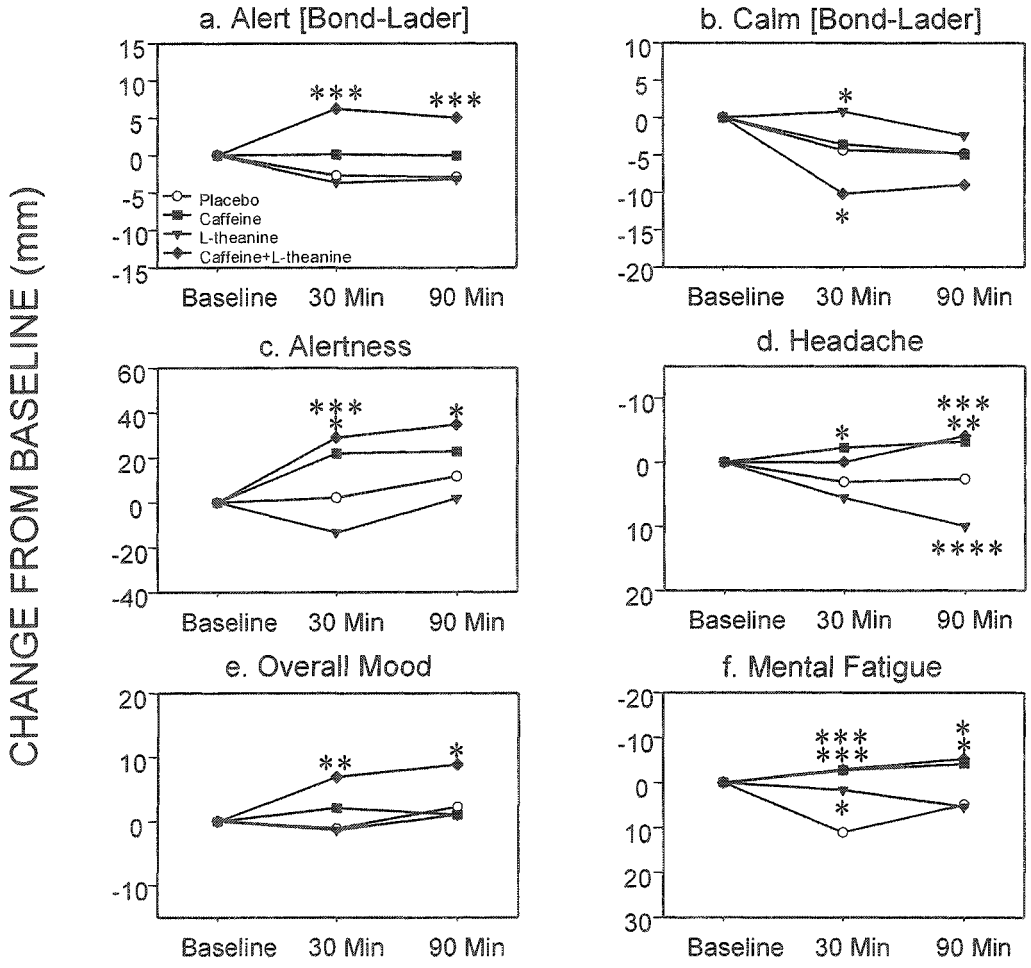

Figure 3.4 Mean change from baseline mood ratings following placebo, 150 mg of caffeine, 250 mg of L-theanine, and 150 mg of caffeine plus 250 mg of L-theanine. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

3.3.5.6 'Overall mood'

'Overall mood' was significantly improved following the combination at 30 minutes [t(66)=2.79, p=0.007] and 90 minutes [t(66)=2.28, p=0.03], see Fig 3.4e.

3.3.5.7 'Mental fatigue'

'Mental fatigue' was significantly decreased following all active treatments. Caffeine decreased ratings at both 30 minutes [t(66)=3.22, p=0.002] and 90 minutes [t(66)=2.12, p=0.04]. The combination also had this effect at 30 minutes [t(66)=3.26, p=0.002] and 90 minutes [t(66)=2.36, p=0.02]. L-theanine also decreased ratings but only at 30 minutes post-dose [t(66)=2.20, p=0.03], see Fig 3.4f.

3.4 Discussion

This study provides support for the findings presented in the previous chapter with regards the effects of 150 mg of caffeine. It also represents the first systematic assessment of the neurocognitive effects of L-theanine, either alone or in combination. Compared with caffeine, L-theanine alone had relatively few effects. However, there is evidence that L-theanine may potentiate the effects of caffeine, albeit selectively.

Caffeine alone produced its characteristic psychoactive effect of enhancing attentional performance while reducing 'fatigue'. Specifically, these data follow a similar profile to that reported in Chapter 2 for both consumers and non-consumers of caffeine using the same battery, in that caffeine improved measures of vigilance and numeric working memory reaction time and 'mental fatigue'. The participants in this study were overnight caffeine deprived (as confirmed by analysis of salivary caffeine levels). However, level of habitual caffeine consumption was not assessed. Future studies in this area would benefit from taking a record of caffeine consumption with specific reference to the source of caffeine as this would allow exploration of any differential effects of caffeine with regard to regular caffeine source. For instance, anecdotal evidence seems to suggest that reasons for drinking tea rather than coffee may extend further than simply lower caffeine levels and taste differences. It would also be interesting in the case of L-theanine to establish whether these effects differ with regard to habitual tea use.

Turning to the effects of the L-theanine-caffeine combination, there were a number of measures that appeared to be differentially sensitive to this treatment. In the case of simple reaction time, accuracy of rapid visual information processing and 'alertness' ratings the positive effects of caffeine alone were numerically greater in the caffeine-L-theanine conditions (see Figs 3.2a, 3.2d and 3.4c). On the other hand the effects on choice reaction time were attenuated by combining L-theanine with caffeine. The addition of caffeine to L-theanine led to a reduction in 'calm' ratings, which is an opposite effect to that seen with L-theanine alone. While the mechanisms underlying such effects are not known they do suggest that there are psychopharmacological interactions between caffeine and L-theanine.

This suggestion is further supported when examining several measures that were significantly affected by the combination treatment but not by the individual components. These included speed of rapid visual information processing, correct serial threes subtractions, 'overall mood', and, most dramatically, word recognition reaction time (see Figs 3.2e, 3.3a, 3.4e, and 3.2g). The latter measure suggests improved efficiency of retrieval of verbal material – an effect that was shown in the previous chapter to be associated with caffeine. Self-rated 'alert' (from the Bond-Lader

scales) was also significantly improved by the caffeine-L-theanine combination but not by caffeine alone (Fig 3.4a). This latter finding may be surprising. However, it appears that, in this case, changes in this dimension were manifested as reduced self-rated 'tiredness'. In addition to these positive effects of the caffeine-L-theanine combination, there was also impairment in this condition. While L-theanine alone improved 'calmness' at 30 min, the caffeine-L-theanine combination reduced 'calmness' at the same time point. These mood effects contradict those of Kakuda (2000) who reported an inhibitory effect of L-theanine on caffeine's stimulatory properties in rats. However, the doses used here are far lower than those employed previously (Kakuda 2000).

There were several effects on cognitive performance and mood associated with L-theanine alone. Compared with placebo, L-theanine was associated with faster choice reaction times at both 30 and 90 min (Fig 3.2c). On the other hand L-theanine slowed numeric working memory at 30 minutes (see Fig 3.2f) and had a detrimental effect on performance of serial sevens at 90 minutes (Fig 3.3b). Compared with placebo, L-theanine alone decreased ratings of 'mental fatigue' at 30 minutes (see Fig 3.4f) and increased ratings of 'calmness' (Fig 3.4b). This finding of increased 'calm' ratings appears to support the findings of Lu et al. (2004) of improvements on the single 'tranquil-troubled' subscale taken from the Bond-Lader visual analogue scales. These data also support those of Kobayashi et al. (1998) who suggest that L-theanine increases 'calmness' without increasing 'drowsiness'. It is widely held that tea drinkers find tea both relaxing and refreshing and taken together these mood effects could be interpreted as being broadly consistent with the purported properties of L-theanine, given that 'calmness' was increased whilst 'mental fatigue' was reduced. However, it is worth noting that L-theanine as typically found in tea is at much lower levels than used here. The mechanisms underlying the effects of L-theanine (both with and without caffeine) are presently unknown. However, L-theanine is known to modulate a number of neurotransmitter systems, including dopaminergic and serotonergic pathways (Yokogoshi et al. 1998; Yokogoshi and Terashima 2000). Clearly, more research is needed in order to establish comprehensive behavioural dose-response relationships for L-theanine, and to establish the nature of any interaction between L-theanine and other components of tea drinks.

The levels of L-theanine and caffeine used here are higher than those found in commercially available tea beverages, which are typically in the region of 40 mg of caffeine and 20 mg of L-theanine. In this initial study it was felt that the use of known psychoactive doses was important in order to examine the effects of combining it with caffeine and to establish a neurocognitive profile for L-theanine. Further studies should be aimed at a full dose-ranging study for L-theanine, particularly as at least one study

has reported mild psychostimulant effects in rats following lower rather than higher doses of L-theanine (Kakuda et al. 2000). Additionally, the next stage of studies into L-theanine-caffeine combinations should include examination of the effects of everyday doses both in isolation and combination, at levels and ratios found in real tea beverages. It is also clear that teas contain a host of potentially psychoactive ingredients which, as well as L-theanine and caffeine, include catechins, tannins, and saponins amongst others. Additionally, green tea has been reported to have anti-cholinesterase properties (Okello et al. 2004), although the components responsible for this property are at present unknown.

Clearly, further work is necessary directed at disentangling the contribution of L-theanine to modulations of behaviour both as an isolated component and in combination with other components of tea.

CHAPTER 4. A DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTI-DOSE EVALUATION OF THE ACUTE BEHAVIOURAL EFFECTS OF GUARANÁ IN HUMANS

4.1 Introduction

The plant species guaraná (*Paullinia cupana*) originates from the central Amazonian Basin, and has a long history of local usage, initially as a stimulant by indigenous people (Henman 1982), and more latterly as a ubiquitous ingredient in Brazilian soft drinks. An extensive range of products that include guaraná seed extracts as ingredients are now commercially available in Western markets. Examples include confections (e.g. chocolate products), fruit juice based drinks, 'energy' drinks, dietary and herbal supplements, and, most controversially, natural weight loss products. The putative stimulant properties are generally taken to reflect the presence of caffeine, which comprises 2.5 – 5 % of the extract's dry weight, although other purine alkaloids (theophylline and theobromine) are present in smaller quantities (Weckerle et al. 2003). Guaraná also has a high content of saponins, tannins (Espinola et al. 1997) and catechins (see Carlson and Thompson 1998), and these may well underlie the demonstrated antioxidant properties of the plant (Mattei et al. 1998).

Whilst guaraná is becoming progressively more common as a purported psychoactive food additive, there is a paucity of evidence as to its specific behavioural effects, and it is assumed that any psychoactive properties are attributable to caffeine. Furthermore, guaraná is often added to beverages as a vehicle for 'caffeine' with no regard for the other components within the extract that could have psychoactive properties, or indeed for potential synergies within components. The findings from the previous chapter demonstrate that the effects of caffeine, when co-administered with another component, can be modulated. However, only a few studies have explored the behavioural effects of guaraná. These are presented below.

Two studies, examining the effects of guaraná in rodents, have included behavioural measures. Espinola et al. (1997) demonstrated that chronic (9 months) administration of a lower dose (0.3 mg/ml) of guaraná improved swimming time in mice, when tested following 100 days and 200 days of treatment. This effect was not observed when tested following 10 and 30 days of treatment. This effect was also not seen with a higher dose (3.0 mg/ml) of guaraná or 0.1 mg/ml caffeine. Similarly, chronic administration of 0.3 mg/ml guaraná was shown to reverse memory deficits in retention on a passive avoidance task in rats treated with 3.0 mg/kg scopolamine. Again, this effect was not demonstrated following 3.0 mg/ml guaraná. The authors state

that these findings of improved physical and mental performance must be due to substances other than caffeine within guaraná. Acute effects of guaraná were also considered in this study. 3 mg/kg and 30 mg/kg of guaraná, as well as 1 mg/kg of caffeine, were shown to reverse memory deficits in retention on a passive avoidance task in mice treated with 2 mg/kg of scopolamine. Mattei et al. (1998) also investigated the effects of both acute and chronic administration of guaraná. They found no toxic effects of guaraná, following either chronic or acute administration. They also failed to find any effects on body weight or on modulation of motor activity or pentobarbital-induced sleep parameters. These latter two measures would be expected to be affected following caffeine and this lack of effects may be indicative of components within guaraná moderating the effects of caffeine. The antioxidant capacity of lyophilized guaraná was also considered at concentrations of 0.8, 1.6, 3.3 and 6.6 µg/ml. Increasing inhibition of lipoperoxidation was observed with increasing dose and it was calculated that 1.2 µg/ml was needed to inhibit 50 % of the process. The authors suggest that this antioxidant activity may be due to the tannin content of guaraná.

Only two randomised, placebo-controlled, double-blind studies to date have examined the cognitive effects of guaraná in humans. Using an independent samples design Galduroz and Carlini (1994) investigated the effects of 1000 mg of guaraná, containing 2.1 % caffeine, in 30 normal young volunteers. They failed to find any effects of guaraná using tests of digit span, free recall, digit symbol substitution, cancellation tests, and the mosaic test. They also evaluated sleep interference and 'anxiety' and again found no effects. The authors present possible explanations for their lack of positive results such as task insensitivity – they also failed to find effects of 25 mg of caffeine in the same study, a dose twice that of the lowest known psychoactive dose (Smit and Rogers 2000). Given the lack of data in this area, it is quite possible that any effects could have been missed simply as a result of inappropriate dose selection or the small sample size utilised. Finally, the time course of testing may not have been sufficient, acute testing only being carried out at 1 hour post-treatment and chronic testing following 3 days of treatment administration. In a follow-up study, the same doses and tasks were used to assess chronic (5 months) effects in 45 elderly participants. They found only one improvement, which was a significant effect of guaraná on mosaic performance at 5 months (Galduroz and Carlini 1996).

Given the increasing use of guaraná and the lack of data regarding its behavioural effects in humans, it is important to assess the plant's effects on mood and cognition. In light of the lack of effects found in the limited number of previous investigations in humans it was deemed necessary to try to overcome some of the

possible methodological flaws. The factor of foremost importance being the determination of the optimum dose of guaraná needed to elicit any such effects. Other important issues include task sensitivity and the time course of any effects. In the current randomised, double-blind, placebo-controlled, counterbalanced study, the cognitive and mood effects of multi-doses of a guaraná extract standardised to 11 to 13 % caffeine content (Pharmaton extract PC-102), were assessed. These doses were 37.5 mg, 75 mg, 150 mg and 300 mg and their effects were assessed in healthy young participants utilising the extensive range of tasks previously used to investigate the effects of caffeine (see Chapters 2 and 3). The tasks selected included attentional tasks as well as specific semantic memory and semantic reasoning tasks, which are known to be sensitive to caffeine (e.g. Warburton 1995; Smith et al., 1994; 1999). Additional secondary memory tasks were also included, which although not typically sensitive to caffeine, were shown to be improved by caffeine when combined in the form with L-theanine in Chapter 3 of this thesis. This selection of tasks also allowed a profile of effects of guaraná to be established. In order to assess potential differential time course effects, testing took place pre-dose and at 1 hour, 3 hours and 6 hours thereafter. To allow a sufficient 'wash out' between treatments, testing was conducted at seven day intervals.

4.2 Materials and Methods

4.2.1 Design

A randomised, placebo-controlled, double-blind, balanced-crossover design was employed.

4.2.2 Initial screening

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix IV).

4.2.3 Participants

Thirty undergraduate volunteers entered the study of which 26 completed all phases of the experiment (18 female and 8 male, mean age 21.4 years, SEM 0.64 range 18 – 31 years). Participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session and throughout the day until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

4.2.4 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence. The saliva samples were immediately frozen at -20 °C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

4.2.5 Assessment

The tasks employed were identical to those described in Chapter 3.

4.2.6 Extracts and treatments

4.2.6.1 Extracts

Guaraná – standardised extract: Extraction from *Paullinia Cupana* H. B. et Kunth seeds was undertaken by exhaustive percolation in a 50 % ethanol, 50 % water solvent at temperatures below 50 °C. Following quantitative analysis of the resultant dry extract, standardisation was performed by the addition of maltodextrine (in the range 10 - 20 %) bringing the concentration of alkaloids (caffeine and theobromine) to 11 – 13 %. This process results in seed: extract ratios of between 3 and 7 parts seed to one part extract depending on the concentration of alkaloids in the root.

4.2.6.2 Treatments

On each study day participants consumed one capsule. The capsules contained either; 37.5 mg of guaraná; 75 mg of guaraná; 150 mg of guaraná; 300 mg of guaraná; or 0 mg of guaraná (placebo). All treatments were identical in appearance and scent.

4.2.7 Procedure

Each participant was required to attend a total of six study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session, on the first day, participants were randomly allocated to a treatment regime using a Latin square design, which counterbalanced the order of treatments across the five active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the five active study days comprised four identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, the second took place 1 hour post-dose, the third at 3 hours post-dose, and the fourth at 6 hours-post-dose.

Each testing session lasted approximately 30 minutes and comprised producing a saliva sample, completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales.

4.2.8 Statistics

Baseline salivary caffeine levels were analysed to assess compliance to overnight caffeine abstinence.

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab.

The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising t tests with MSError from an omnibus ANOVA as an error term (Keppel 1991). At each time point (1, 3, and 6 hours post-treatment) data from the placebo condition was compared to that for each of the four active treatments (37.5, 75, 150, 300 mg of guaraná). Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for treatment, assessment, treatment x assessment and participant (Kirk 1968), was carried out to identify main effects and interaction effects on change from baseline data for each measure. Where baseline differences were observed baseline scores were entered as a covariate in an ANCOVA, with terms fitted to the model for treatment, assessment, treatment x assessment and participant. This analysis was conducted using SAS/STAT. To ensure the overall Type I error protection level only those planned comparisons associated with measures that generated a significant main effect or interaction effect ($p<0.05$) on this initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

4.3 Results

4.3.1 Salivary caffeine levels

Salivary analysis revealed that five participants had not complied with instructions to avoid caffeine-containing products. All data from these participants were excluded from further analyses. Data for the remaining 21 participants confirmed compliance with overnight abstinence, mean baseline values were 0.26 µg/ml (SD = 0.62).

4.3.2 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all five conditions for each outcome were subjected to a one-way, repeated-measures ANOVA. There were significant baseline differences in accuracy of the choice reaction time task [F(4, 80)=2.52, p=0.048] and 'headache' ratings [F(4, 80)=3.02, p=0.02].

4.3.3 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 4.1 along with F values and probabilities for effects of treatment. Significant differences on cognitive tasks are presented in Figure 4.1. Only significant main effects for each outcome measure are reported below.

Table 4.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
			1 hour	3 hours	6 hours	
Immediate word recall accuracy (%)	Placebo	47.8±3.48	-1.59±3.20	-6.51±3.55	-5.56±3.35	F(4, 160)<1
	37.5 mg	49.5±3.33	-6.03±3.87	-3.17±3.45	-1.11±3.92	
	75 mg	49.2±4.21	-0.32±2.84	-4.60±3.76	-2.06±2.74	
	150 mg	45.4±3.52	3.17±2.53	-4.76±3.14	-2.06±2.46	
	300 mg	50.6±3.05	-6.19±2.90	-5.71±3.24	-3.49±2.40	
Simple reaction time (ms)	Placebo	288±7.36	13.5±4.42	14.8±4.74	15.8±5.40	F(4, 160)=1.29 p>0.1
	37.5 mg	293±8.61	6.05±7.45	-0.32±6.47	5.94±7.72	
	75 mg	295±7.94	11.9±5.43	6.42±5.73	8.16±6.51	
	150 mg	286±8.82	7.29±6.29	8.91±5.80	13.1±5.67	
	300 mg	288±9.23	6.99±6.81	5.86±4.94	13.5±6.14	
Digit vigilance accuracy (%)	Placebo	95.8±1.08	-2.86±1.51	-4.23±1.71	-4.23±2.05	F(4, 160)<1
	37.5 mg	96.3±0.80	-4.02±2.04	-3.18±1.82	-3.49±1.39	
	75 mg	95.5±1.09	-2.75±1.57	-2.96±1.76	-3.70±2.16	
	150 mg	94.9±1.57	-1.16±1.58	-2.96±2.24	-3.60±2.22	
	300 mg	92.8±2.17	-1.48±1.59	-1.06±1.11	-2.96±1.56	
Digit vigilance reaction time (ms)	Placebo	441±9.46	13.71±5.58	26.4±7.56	22.8±8.53	F(4, 160)=2.11 p=0.08
	37.5 mg	426±9.75	25.4±7.38	23.3±8.08	18.6±7.49	
	75 mg	434±11.9	10.3±8.50	15.9±8.67	13.1±10.2	
	150 mg	436±9.80	5.87±5.25	12.8±6.31	20.5±8.83	
	300 mg	440±11.3	12.3±5.66	2.86±5.64	10.1±6.79	
Digit vigilance false alarms (number)	Placebo	1.10±0.24	-0.24±0.27	-0.19±0.31	-0.29±0.28	F(4, 160)=1.75 p>0.1
	37.5 mg	0.52±0.15	0.24±0.27	0.10±0.24	0.52±0.35	
	75 mg	0.62±0.17	-0.24±0.23	0.19±0.20	0.19±0.22	
	150 mg	0.76±0.24	0.10±0.34	0.29±0.32	-0.52±0.28	
	300 mg	0.76±0.15	0.24±0.27	-0.24±0.25	0.43±0.30	

Choice reaction time accuracy (%)	Placebo	95.2±0.71	0.38±0.75	0.86±0.56	1.62±0.74	**F(4, 160)=2.72 p=0.03**
	37.5 mg	96.8±0.67	0.29±0.66	-0.76±0.68	-0.76±0.73	
	75 mg	96.8±0.54	0.86±0.49	-0.57±0.59	-1.52±0.70	
	150 mg	95.3±0.78	1.05±0.77	0.00±0.64	0.38±0.77	
	300 mg	96.7±0.52	-0.10±0.87	-0.10±0.62	-1.24±1.01	
Choice reaction time (ms)	Placebo	454±11.2	9.55±7.70	8.81±8.76	-4.02±8.70	F(4, 160)<1
	37.5 mg	443±9.39	-0.72±9.75	-0.89±9.56	9.24±9.00	
	75 mg	443±10.6	6.72±6.61	-3.72±6.39	2.34±8.72	
	150 mg	440±10.7	0.01±9.32	3.06±6.80	5.33±8.88	
	300 mg	448±12.3	5.14±11.8	-19.6±7.15	6.84±13.8	
RVIP accuracy (%)	Placebo	59.1±4.82	-3.13±2.87	1.34±3.00	-1.93±3.03	F(4, 160)=1.41 p>0.1
	37.5 mg	60.4±4.12	0.30±1.76	-0.15±2.63	1.04±2.44	
	75 mg	63.3±4.26	-2.38±2.18	-1.34±2.42	-5.50±3.16	
	150 mg	57.3±4.52	2.08±2.13	2.08±2.82	-1.19±2.32	
	300 mg	59.8±4.61	-0.60±1.99	-3.12±2.48	-1.19±2.38	
RVIP reaction time (ms)	Placebo	509±19.4	-18.2±21.4	-0.18±14.7	-16.0±13.5	F(4, 160)=1.21 p>0.1
	37.5 mg	508±16.9	-2.37±18.7	6.55±16.6	-14.4±13.8	
	75 mg	498±21.6	-8.14±15.5	19.2±18.4	24.4±19.6	
	150 mg	518±20.7	-6.76±16.7	6.13±14.9	-32.8±14.0	
	300 mg	503±16.5	9.05±15.6	-4.86±10.1	6.72±12.4	
RVIP false alarms (number)	Placebo	3.24±1.42	1.43±1.51	-0.43±0.46	-0.29±0.37	**F(4, 160)=3.73 p=0.006**
	37.5 mg	2.10±0.82	0.38±0.40	1.00±0.85	0.90±0.74	
	75 mg	1.33±0.38	1.05±0.74	0.24±0.69	1.71±0.77	
	150 mg	4.95±2.55	-0.19±0.74	-0.81±0.75	-1.62±0.99	
	300 mg	3.05±1.21	-1.43±0.69	-0.52±0.75	-0.81±0.79	
Spatial memory (sensitivity index)	Placebo	0.95±0.01	0.01±0.02	-0.10±0.05	-0.05±0.04	F(4, 160)<1
	37.5 mg	0.92±0.02	-0.06±0.07	0.00±0.02	-0.05±0.03	
	75 mg	0.94±0.01	0.02±0.01	-0.06±0.05	-0.03±0.02	
	150 mg	0.95±0.01	-0.05±0.05	-0.01±0.01	-0.08±0.06	
	300 mg	0.96±0.01	-0.04±0.04	-0.01±0.02	-0.03±0.03	
Spatial memory reaction time (ms)	Placebo	584±21.1	-24.1±10.8	34.6±43.8	-37.4±21.0	F(4, 160)=1.16 p>0.1
	37.5 mg	595±24.0	-40.4±15.2	-25.5±16.1	-20.5±24.8	
	75 mg	587±22.7	-29.0±19.1	-32.1±18.7	-25.5±19.6	
	150 mg	578±20.3	-25.3±15.4	-32.1±15.9	-20.1±11.0	
	300 mg	594±25.0	-51.3±14.9	-50.1±14.7	-28.11±18.7	
Logical reasoning accuracy (%)	Placebo	86.7±2.90	0.60±2.09	0.20±1.20	2.38±2.00	F(4, 160)=2.01 p=0.09
	37.5 mg	90.9±2.08	-2.58±1.05	-1.19±1.41	-2.58±1.39	
	75 mg	91.9±1.91	0.79±1.56	-0.40±1.72	0.00±1.59	
	150 mg	91.5±2.90	-2.38±2.02	-2.98±2.07	-2.38±2.34	
	300 mg	91.3±2.46	-0.20±1.20	-1.79±2.77	-1.59±2.59	
Logical reasoning reaction time (ms)	Placebo	2504±152	-118±95.6	202±134	-189±157	F(4, 160)=1.77 p>0.1
	37.5 mg	2700±156	-65.7±118	113±149	72.9±124	
	75 mg	3056±187	-231±147	-175±173	-363±172	
	150 mg	2840±202	26.7±136	-107±139	-227±99.1	
	300 mg	2865±164	6.52±157	-88.1±200	-211±192	
Numeric working memory (sensitivity index)	Placebo	0.91±0.02	0.00±0.02	-0.03±0.02	-0.01±0.02	**F(4, 160)=3.55 p=0.008**
	37.5 mg	0.91±0.02	0.00±0.02	0.01±0.01	-0.02±0.02	
	75 mg	0.93±0.01	-0.04±0.02	-0.05±0.02	-0.02±0.02	
	150 mg	0.89±0.02	0.00±0.01	0.01±0.01	0.02±0.02	
	300 mg	0.92±0.01	-0.02±0.01	-0.03±0.02	-0.01±0.02	
Numeric working memory reaction time (ms)	Placebo	610±33.7	-16.2±11.4	-23.4±21.5	-9.31±28.6	F(4, 160)=2.17 p=0.07
	37.5 mg	629±30.2	-38.8±11.4	-42.5±13.1	-0.21±18.4	
	75 mg	611±25.7	10.7±19.9	8.77±22.4	1.86±15.4	
	150 mg	603±29.9	4.45±11.3	-9.34±14.0	3.25±17.9	
	300 mg	591±27.5	-1.59±12.2	-18.7±12.0	-15.1±20.6	
Delayed word recall accuracy (%)	Placebo	37.0±3.75	-14.0±3.31	-18.7±4.39	-15.9±2.97	F(4, 160)=1.77 p>0.1
	37.5 mg	35.7±3.53	-14.1±2.99	-11.9±3.77	-10.5±4.03	
	75 mg	34.1±4.03	-6.83±2.80	-13.0±3.26	-12.9±2.21	
	150 mg	34.1±3.94	-6.98±2.30	-13.0±2.85	-12.2±2.67	
	300 mg	36.5±3.86	-11.0±2.84	-14.4±3.09	-12.9±3.05	
Delayed word recognition (sensitivity index)	Placebo	0.70±0.03	-0.07±0.03	-0.05±0.04	-0.12±0.04	F(4, 160)<1
	37.5 mg	0.70±0.03	-0.07±0.03	-0.08±0.04	-0.13±0.05	
	75 mg	0.66±0.04	-0.03±0.04	-0.09±0.03	-0.04±0.05	
	150 mg	0.67±0.04	-0.04±0.04	-0.14±0.05	-0.14±0.05	
	300 mg	0.62±0.04	-0.05±0.04	-0.06±0.03	-0.12±0.05	
Delayed word recognition reaction time (ms)	Placebo	712±36.1	40.6±23.3	59.3±21.7	26.2±27.4	**F(4, 160)=2.93 p=0.02**
	37.5 mg	731±26.9	17.8±22.6	63.6±39.4	60.5±29.0	
	75 mg	802±78.5	-10.8±45.5	-19.2±49.9	-69.4±54.4	
	150 mg	720±30.6	18.1±18.0	28.5±28.2	-13.8±17.9	
	300 mg	731±43.6	19.9±34.5	27.5±21.3	13.5±36.8	
Delayed picture recognition (sensitivity index)	Placebo	0.69±0.05	-0.12±0.05	-0.19±0.04	-0.12±0.03	**F(4, 160)=5.08 p=0.001**
	37.5 mg	0.67±0.05	0.04±0.02	-0.01±0.05	-0.02±0.04	
	75 mg	0.60±0.06	0.07±0.04	-0.04±0.05	-0.06±0.05	
	150 mg	0.65±0.05	-0.03±0.05	-0.06±0.06	-0.14±0.06	
	300 mg	0.64±0.07	-0.03±0.05	-0.02±0.04	-0.03±0.05	
Delayed picture recognition reaction time (ms)	Placebo	833±25.9	21.1±25.8	46.7±45.7	-12.3±28.7	F(4, 160)<1
	37.5 mg	866±50.6	-2.68±34.9	-34.6±29.3	41.2±46.5	
	75 mg	874±44.5	29.6±40.2	-27.1±30.4	-32.8±32.7	
	150 mg	848±38.5	-7.08±27.8	-0.93±34.9	13.0±29.5	
	300 mg	853±45.4	-30.7±24.8	20.5±25.2	-1.79±34.1	

4.3.3.1 Choice reaction accuracy

Significant differences in accuracy of the choice reaction time task were no longer apparent following ANCOVA with baseline scores included as a covariate.

4.3.3.2 Rapid Visual Information Processing (RVIP) false alarms

The number of false alarms generated on the RVIP task was significantly reduced at 1 hr following 150 mg [t(160)=2.01, p=0.046] and 300 mg [t(160)=3.55, p=0.0005]. There was also an impairment on this measure at 6 hrs following 75 mg [t(160)=2.49, p=0.01], see Fig 4.1a.

4.3.3.3 Numeric working memory accuracy

Planned comparisons revealed that the significant main effect of treatment on numeric working memory was not in relation to active treatments versus placebo.

4.3.3.4 Delayed word recognition reaction time

Only the 75 mg dose improved speed of word recognition. These effects were apparent at 3 hrs [t(160)=2.72, p=0.007], and 6 hrs [t(160)=3.31, p=0.001] post-dose, see Fig 4.1b.

4.3.3.5 Delayed picture recognition accuracy

Accuracy of picture recognition was improved by all doses of guaraná. These effects were elicited at all time points by the 37.5 mg dose [1 hr: t(160)=3.42, p=0.0008; 3 hrs: t(160)=3.93, p=0.0001; 6 hrs t(160)=2.12, p=0.04]; at 1 hr [t(160)=3.96, p=0.0001] and 3 hrs [t(160)=3.16, p=0.002] following the 75 mg dose. Effects were apparent at 3 hrs post-dose following the 150 mg dose [t(160)=2.69, p=0.008] and the 300 mg dose [t(160)=3.73, p=0.0003], see Fig 4.1c.

4.3.4 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 4.2 along with F values and probabilities for effects of treatment. Significant differences on cognitive tasks are presented in Figure 4.1. Only significant main effects and/or interactions for each outcome measure are reported below.

Table 4.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
			1 hour	3 hours	6 hours	
Sentence verification accuracy (%)	Placebo	93.7±1.62	-0.95±1.38	-1.90±1.31	-0.32±1.93	F(4, 160)<1
	37.5 mg	93.8±1.58	-0.48±1.42	-1.43±1.53	-1.75±1.25	
	75 mg	94.8±1.29	-0.79±1.05	-2.06±1.29	-0.63±1.20	
	150 mg	94.3±1.58	-0.36±1.40	-1.63±1.17	-2.42±1.40	
	300 mg	94.0±1.46	-1.90±1.16	-1.59±1.55	-0.32±1.25	
Sentence verification reaction time (ms)	Placebo	1422±123	-18.7±50.1	-64.2±50.0	-64.8±61.8	F(4, 160)=1.72 p>0.1
	37.5 mg	1403±107	3.48±46.4	-29.8±44.8	-12.6±46.5	
	75 mg	1477±108	-97.7±52.8	-157±50.5	-119±53.9	
	150 mg	1524±205	-8.07±46.4	-173±128	-64.5±56.7	
	300 mg	1379±81.1	11.1±39.0	-52.9±52.8	-81.7±48.7	
Serial threes subtraction correct (number)	Placebo	40.7±2.90	-1.18±1.35	-0.70±1.33	0.49±1.24	**F(4, 160)=4.56 p=0.001**
	37.5 mg	41.4±2.90	0.29±1.40	-0.14±1.37	-1.29±1.34	
	75 mg	39.0±2.96	1.38±1.63	4.00±1.52	3.71±1.45	
	150 mg	39.5±3.17	-0.38±1.40	1.10±1.38	1.43±1.79	
	300 mg	39.5±3.48	2.76±1.24	2.71±1.00	2.71±1.52	
Serial threes subtraction errors (number)	Placebo	3.36±0.71	0.21±0.67	0.50±0.86	0.12±0.51	F(4, 160)=1.56 p>0.1
	37.5 mg	2.48±0.36	0.95±0.52	1.19±0.72	1.67±0.87	
	75 mg	3.10±0.41	0.43±0.51	0.10±0.59	0.00±0.70	
	150 mg	3.24±0.59	1.19±0.52	0.71±0.49	0.52±0.68	
	300 mg	3.00±0.43	0.43±0.50	0.90±0.57	0.71±0.90	
Serial sevens subtraction correct (number)	Placebo	22.9±2.24	0.90±1.20	0.05±1.29	1.15±1.40	F(4, 152)=2.15 p=0.08
	37.5 mg	24.9±2.61	0.15±1.13	-0.90±1.50	-1.25±1.28	
	75 mg	23.6±2.57	-0.15±1.01	1.65±0.74	1.05±1.08	
	150 mg	23.4±2.18	0.90±0.96	1.70±1.16	1.75±0.99	
	300 mg	22.5±2.44	0.30±0.87	3.25±1.06	2.05±1.43	
Serial sevens subtraction errors (number)	Placebo	3.40±0.58	-0.60±0.61	-0.55±0.51	-0.40±0.44	**F(4, 152)=2.75 p=0.03**
	37.5 mg	2.85±0.42	0.45±0.35	0.30±0.61	0.45±0.57	
	75 mg	3.30±0.46	0.75±0.50	0.00±0.43	0.40±0.43	
	150 mg	3.20±0.48	-0.40±0.47	0.25±0.34	0.10±0.38	
	300 mg	3.20±0.36	1.45±0.72	0.15±0.35	0.45±0.58	

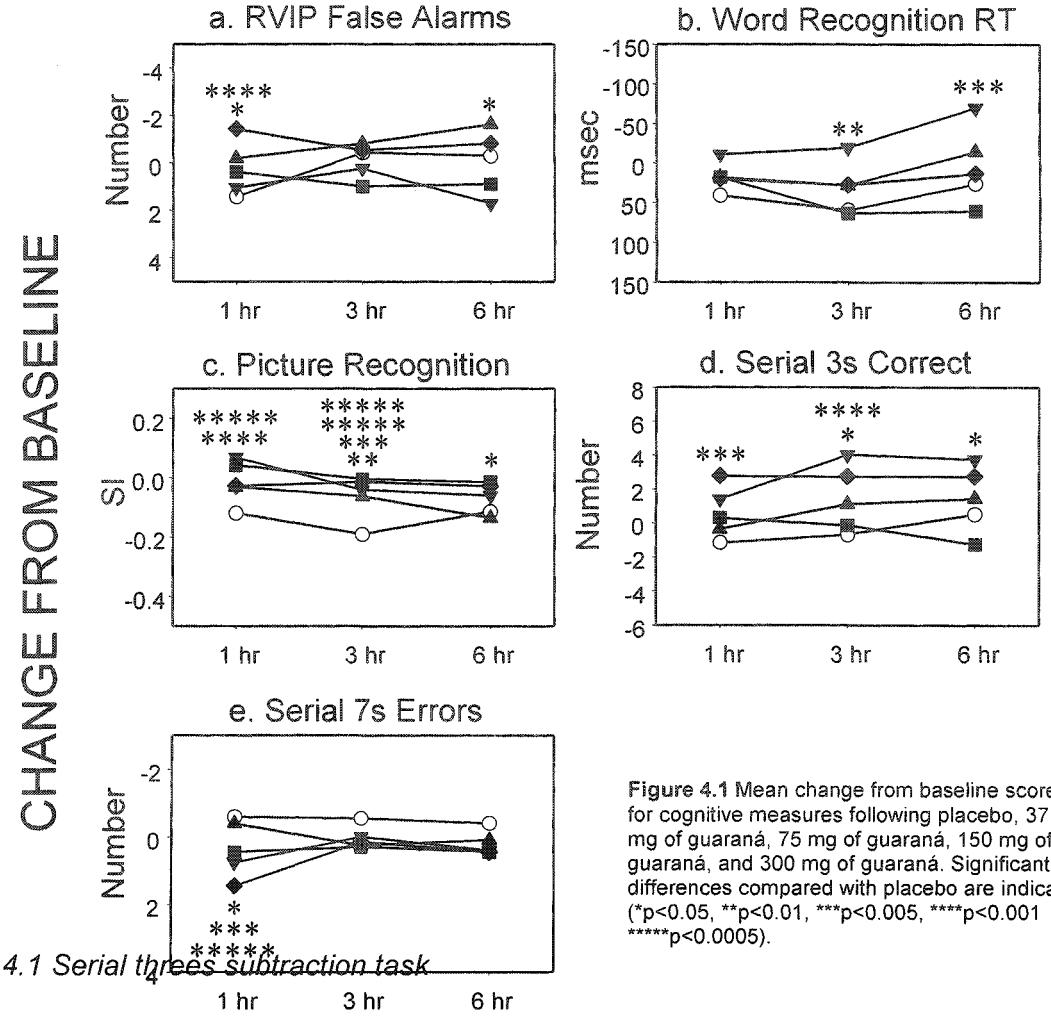

Figure 4.1 Mean change from baseline scores for cognitive measures following placebo, 37.5 mg of guaraná, 75 mg of guaraná, 150 mg of guaraná, and 300 mg of guaraná. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001 *****p<0.0005).

4.3.4.1 Serial threes subtraction task

The number of correct serial threes subtractions was increased following 75 mg of guaraná at 3 hrs [t(160)=3.42, p=0.0008] and 6 hrs [t(160)=2.35, p=0.02] and at 1 hr [t(160)=2.87, p0.0047] and 3 hrs [t(160)=2.49, p=0.01] following 300 mg, see Fig 4.1d.

4.3.4.2 Serial sevens subtraction task

Due to a data capture error one dataset was lost from the analysis of serial sevens.

The number of errors made on the serial sevens task was significantly increased at 1 hr post-treatment following all except the 150 mg dose guaraná [37.5 mg: t(152)=2.27, p=0.02; 75 mg: t(152)=2.91, p=0.004; 300 mg: t(152)=4.43, p=0.00002], see Fig 4.1e.

4.3.5 Subjective mood measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each mood measure are presented in Table 4.3 along with F values and probabilities for effects of treatment. Significant differences on mood measures are presented in Figure 4.2. Only significant main effects and/or interactions for each outcome measure are reported below.

4.3.5.1 Bond-Lader 'Alert' factor

There was a significant increase in Bond-Lader 'alert' ratings following the 300 mg dose at 1 hr [t(160)=2.50, p=0.01] and 3 hrs [t(160)=2.78, p=0.006] post-dose. There were also improvements following 37.5 mg at 3 hrs [t(160)=2.51, p=0.01] and following 150 mg at 1 hr post-dose [t(160)=1.99, p=0.048], see Fig 4.2a.

4.3.5.2 'Bond-Lader 'Content' factor

There were significant improvements in 'content' ratings following 37.5 mg at 1 hr [t(160)=3.2, p=0.002], 3 hrs [t(160)=3.65, p=0.0004], and 6 hrs [t(160)=2.52, p=0.01]; following 75 mg at 1 hr [t(160)=3.19, p=0.002] and 3 hrs [t(160)=2.67, p=0.008]; 150 mg at 1 hr [t(160)=3.26, p=0.001] and 3 hrs [t(160)=2.82, p=0.005]; and following 300 mg at 1 hr [t(160)=2.97, p=0.003] and 3 hrs [t(160)=3.26, p=0.001] post-dose, see Fig 4.2b.

Table 4.3 Baseline and change from baseline mood ratings for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure		Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
				1 hour	3 hours	6 hours	
Bond-Lader factors	**Alert (mm)**	Placebo	56.9±3.73	-4.68±3.02	-11.2±2.84	-8.30±3.92	**F(4, 160)=2.64 p=0.03**
		37.5 mg	60.0±3.79	-3.49±2.78	-3.25±2.89	-6.42±3.65	
		75 mg	59.2±3.60	0.40±2.23	-6.55±2.09	-11.0±3.09	
		150 mg	56.8±4.50	1.61±2.82	-5.85±3.45	-7.10±3.72	
		300 mg	54.0±4.32	3.22±2.73	-2.40±4.03	-2.51±4.01	
	Content (mm)	Placebo	61.7±3.80	-6.25±2.57	-8.19±2.55	-6.45±4.09	**F(4, 160)=4.15 p=0.003**
		37.5 mg	63.7±3.63	0.65±1.53	-0.33±1.90	-1.01±2.70	
		75 mg	64.6±3.43	0.63±1.41	-2.43±1.87	-2.32±2.39	
		150 mg	63.0±3.04	0.78±1.63	-2.10±1.76	-2.65±2.22	
		300 mg	62.9±3.27	0.15±1.77	-1.17±2.64	-2.79±3.35	
	Calm (mm)	Placebo	59.6±3.90	1.69±2.56	-2.57±2.59	3.38±2.34	F(4, 160)=2.14 p=0.08
		37.5 mg	67.2±3.82	-4.17±3.56	-7.86±2.84	-2.02±4.82	
		75 mg	64.9±3.12	-3.45±2.67	-3.45±3.26	-5.21±3.93	
		150 mg	65.4±3.23	-9.05±3.22	-4.64±2.69	-5.69±2.97	
		300 mg	63.2±2.62	-7.31±3.58	-1.81±3.91	-3.26±4.59	
Caffeine research visual analogue scales	Relaxed (mm)	Placebo	55.1±4.70	-3.00±5.83	3.85±3.50	3.10±7.86	F(4, 152)<1
		37.5 mg	58.5±5.32	-2.10±4.87	-7.20±5.91	2.00±7.05	
		75 mg	59.2±5.25	-4.30±5.60	-4.05±3.04	-2.85±4.00	
		150 mg	64.8±3.83	-4.40±4.93	-9.00±5.79	-6.00±4.92	
		300 mg	61.6±5.49	-4.20±5.08	-3.10±4.45	-4.35±6.97	
	Alert (mm)	Placebo	57.9±4.00	-7.10±5.47	-13.1±4.73	-11.7±5.25	F(4, 152)=1.57 p>0.1
		37.5 mg	54.5±5.36	-4.65±5.70	-4.55±7.24	-12.2±6.76	
		75 mg	53.8±5.67	1.95±5.32	-5.45±3.48	-5.55±4.58	
		150 mg	49.9±6.03	4.25±4.68	-3.20±4.97	-5.90±5.71	
		300 mg	56.6±5.65	-3.25±4.64	-5.95±6.28	-7.50±6.40	
	Jittery (mm)	Placebo	26.4±5.08	7.20±3.89	11.4±3.21	-0.50±3.93	**F(4, 152)=4.89 p=0.001**
		37.5 mg	29.0±4.98	0.85±3.72	1.40±3.59	-7.50±5.22	
		75 mg	21.8±4.50	10.2±4.03	13.3±3.90	11.8±3.21	
		150 mg	26.5±4.71	5.30±4.82	5.50±4.66	1.65±3.53	
		300 mg	27.4±4.92	10.6±6.35	12.1±4.27	6.15±4.97	
	Tired (mm)	Placebo	42.6±6.44	5.75±3.79	12.1±6.11	17.3±7.51	**F(4, 152)=2.89 p=0.02**
		37.5 mg	41.7±6.78	7.55±5.20	13.2±6.85	10.9±8.63	
		75 mg	48.9±6.19	-11.0±3.69	-0.75±6.46	5.65±6.17	
		150 mg	43.7±7.17	2.25±3.99	5.90±5.84	8.35±6.24	
		300 mg	44.9±6.68	-2.60±4.64	9.05±7.50	4.50±7.45	
	Tense (mm)	Placebo	35.0±5.42	-1.90±2.90	1.80±5.71	-1.80±5.30	F(4, 152)=1.38 p>0.1
		37.5 mg	32.7±5.40	4.30±5.93	6.75±4.05	-2.95±4.35	
		75 mg	33.0±5.44	4.65±2.67	4.55±3.36	4.20±3.13	
		150 mg	29.0±4.80	7.25±5.27	8.70±4.94	5.40±4.51	
		300 mg	28.9±4.97	7.70±4.02	1.70±4.08	9.55±6.41	
	Headache (mm)	Placebo	15.1±3.56	7.45±4.26	11.4±5.87	9.75±5.51	**F(4, 152)=3.89 p=0.004**
		37.5 mg	15.0±4.01	6.45±3.43	2.65±4.23	1.85±3.98	
		75 mg	19.5±4.55	-1.30±2.96	2.80±3.54	2.15±4.34	
		150 mg	26.1±5.91	-0.15±2.27	-3.80±4.48	-3.15±4.01	
		300 mg	25.5±5.49	-2.50±4.61	2.90±5.58	-0.15±5.65	
	Overall (mm)	Placebo	59.8±5.23	-3.25±4.39	-4.80±3.72	-2.65±4.84	F(4, 152)=1.42 p>0.1
		37.5 mg	64.0±3.85	-4.10±2.33	-2.55±5.05	4.05±3.34	
		75 mg	62.9±3.26	3.50±3.00	1.05±3.47	-2.80±3.67	
		150 mg	64.4±4.55	-0.85±3.12	-2.30±3.67	-5.95±3.59	
		300 mg	67.0±3.68	-2.95±1.97	-6.25±3.90	-6.75±3.86	
	Mental fatigue (mm)	Placebo	25.8±5.18	5.55±3.67	18.0±4.04	20.2±7.00	F(4, 152)=1.08 p>0.1
		37.5 mg	27.2±5.27	6.30±3.76	10.5±7.71	15.5±6.83	
		75 mg	33.7±6.02	-3.05±4.16	12.9±5.38	11.6±6.34	
		150 mg	34.5±6.19	2.40±3.91	9.30±5.44	11.4±4.85	
		300 mg	31.7±5.43	5.00±3.53	8.70±4.65	14.0±5.34	
Caffeine research visual analogue scales factors	**Alertness (mm)**	Placebo	57.7±4.33	-6.43±3.58	-12.6±4.58	-14.5±5.72	**F(4, 152)=2.74 p=0.03**
		37.5 mg	56.4±5.25	-6.10±5.09	-8.85±6.54	-11.5±6.89	
		75 mg	52.4±5.00	6.45±3.96	-2.35±3.80	-5.60±4.03	
		150 mg	53.1±5.95	1.00±2.90	-4.55±4.65	-7.13±4.98	
		300 mg	55.9±5.18	-0.33±3.97	-7.50±6.27	-6.00±6.23	
	Tension (mm)	Placebo	40.0±4.52	0.55±3.57	-1.03±3.55	-2.45±5.81	F(4, 152)=1.54 p>0.1
		37.5 mg	37.1±4.88	3.20±4.90	6.98±4.20	-2.48±4.94	
		75 mg	36.9±4.81	4.48±3.42	4.30±2.42	3.53±2.87	
		150 mg	32.1±3.57	5.83±4.12	8.85±3.81	5.70±3.63	
		300 mg	33.7±4.60	5.95±4.15	2.40±3.25	6.95±5.84	

There were a number of treatment effects on the caffeine research visual analogue scales. Due to a data capture error with one dataset the following analyses look at only 20 participants.

4.3.5.3 'Alertness' factor

'Alertness' was significantly increased following 75 mg at 1 hr [t(152)=2.65, p=0.009] and 3 hrs [t(152)=2.11, p=0.04] post-dose, see Fig 4.2c.

4.3.5.4 'Tired'

The effects on 'alertness' were largely due to decreases in 'tired' ratings following 75 mg at 1 hr [t(152)=2.86, p=0.00049]; 3 hrs [t(152)=2.19, p=0.03] and 6 hrs [t(152)=1.99, p=0.048] post-dose. The 300 mg dose also led to improvements at 6 hrs post dose [t(152)=2.18, p=0.03].

4.3.5.5 'Jittery'

There was a significant decrease in 'jittery' ratings following 37.5 mg at 3 hrs post-dose [t(152)=2.35, p=0.02]. However, 75 mg significantly increased 'jittery' ratings at 6 hrs post-treatment [t(152)=2.89, p=0.004], see Fig 4.2d.

4.3.5.6 'Headache'

Significant differences in 'headache' ratings were no longer apparent following ANCOVA with baseline scores included as a covariate.

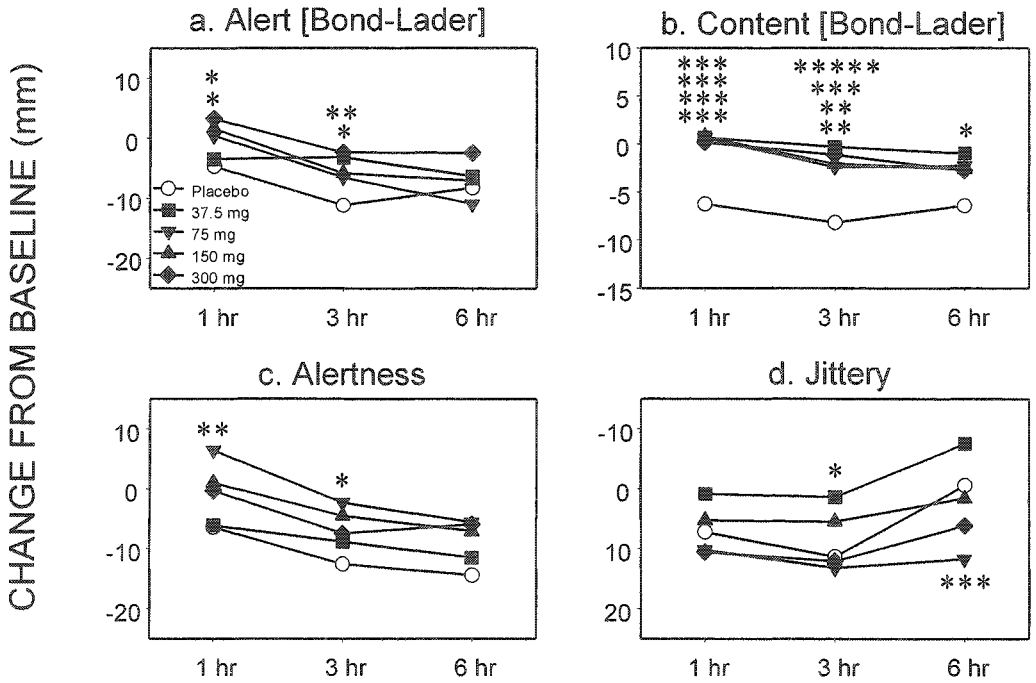

Figure 4.2 Mean change from baseline mood ratings following placebo, 37.5 mg of guaraná, 75 mg of guaraná, 150 of mg of guaraná, 300 mg of guaraná. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

4.4 Discussion

The results of the current study show that guaraná can improve cognitive performance and mood in comparison to placebo in healthy young participants. The study provides the first demonstration of acute cognitive and mood effects of guaraná. Guaraná increased speed of word recognition, improved accuracy of picture recognition, and increased the number of correct serial threes subtractions. There was also an impairment observed, which took the form of an increase in the number of errors on the serial sevens subtraction task. In terms of mood, increases in 'alertness' (Bond-Lader and caffeine research visual analogue scales) and improved 'content' ratings were observed. 'Jittery' ratings and number of false alarms on an RVIP task were differentially affected in relation to dose.

The effects of guaraná were most notable following administration of the 75 mg dose. Following this treatment, improvements were seen to speed of word recognition, accuracy of picture recognition, correct serial threes subtractions, and self-ratings of 'alertness' (caffeine research visual analogue scales) and 'content' (Bond-Lader). There were also some detrimental effects. The number of errors in the serial sevens subtraction task increased (however, no effect was observed on the number of correct responses), false alarms on RVIP were increased (again with no effects on the number of correct responses, or reaction time) and 'jittery' ratings were also increased. However, the impairments seen were only at a single time point, whereas improvements were always at two time points or more. Greater improvements were observed following the lowest dose on picture recognition and 'content' ratings' as well as single time point improvements to 'alert' (Bond-Lader) and 'jittery' ratings. However, no improvements to word recognition, serial threes, or 'alertness' (caffeine research visual analogue scales) were observed with this dose and the impairment to serial sevens was again observed. The highest dose also produced a number of effects but these were mainly single time point effects, with the exception of serial threes subtractions, 'alert' ratings (Bond-Lader) and 'content' ratings (which were improved by all doses at more than one time point). The 150 mg dose was the only treatment not to impair serial sevens performance but it also produced the fewest effects in general.

These findings suggest that the 75 mg treatment in the current study was the most effective. Given that the guaraná extract contained only 11 to 12 % caffeine, it seems unlikely that even the effects of the highest dose, containing around 36 mg of caffeine, could be solely attributed to the effects of caffeine. The fact that the 75 mg dose (containing only 9 mg of caffeine), is generally more beneficial lends further support to this posit. Espinola et al.'s (1997) observations in rodents that doses of guaraná with minimal total caffeine content were more beneficial than tenfold doses of

guaraná also provide support for the suggestion that guaraná's caffeine content alone does not account for all of its behavioural effects. Obviously, the lower doses of guaraná utilised in this study also contain lower doses of any other potential active ingredients, but since so little is known about the other constituents of guaraná – it's effects so often being attributed to caffeine – it is difficult to assess what level of these constituents is the optimum, or indeed what level is present. It is also possible that at the higher doses, the effects of the higher levels of caffeine are in some way masking the effects of the other active ingredients. As in the previous chapter, this highlights the complexity of studying naturally concomitant psychoactives. Given that it is known that saponins and tannins have effects that may, directly or indirectly, affect behaviour, it is extremely likely that any effects of caffeine within guaraná are concomitant with effects of other components.

In the current study the tasks that were improved by guaraná are secondary memory tasks and serial threes. Reference to the previous chapters and the literature with regards caffeine demonstrates that secondary memory tasks are not generally accepted as being sensitive to the effects of caffeine. Whilst improvements to secondary memory reaction time and serial threes were evinced in Chapter 3, this was only observed when caffeine was combined with L-theanine. As well as the measures affected not being those typically susceptible to caffeine, it is also surprising that none of the well-established effects of caffeine were observed here, such as improved simple reaction time or vigilance performance. It should be pointed out that very little is known about the effects of very low doses of caffeine (equivalent to those employed here) and further exploration of these effects is warranted. The increases in ratings of 'alert' and 'content' are broadly in keeping with the literature pertaining to caffeine and the effects relating to 'alert' are supportive of the findings from the previous two chapters.

In relation to the time course of the findings, the greatest number of effects was observed at 1 and 3 hours post-treatment. However, there were a number of effects apparent at 6 hours post-treatment, which poses somewhat of a challenge to the supposition that guaraná's effects are merely the effects of caffeine. The effects of caffeine are typically tested between 15 and 45 minutes post-treatment and, very rarely more than 2 hours post-dose. With a half-life of around 6 hours it is theoretically possible that caffeine could still exert effects at 6 hours. However, this possibility has not yet been explored. Some effects here were also not apparent until 3 or 6 hours post-dose. Again, this does not fit with the generally accepted time course of the effects of caffeine. This may, to some extent, explain the lack of positive findings of Galduroz and Carlini (1994) given that they only tested acute effects at 1 hour post-dose.

The findings from the current study suggest that, within the range of doses employed, lower doses are more psychoactive than higher ones. This implies a role for components within guaraná other than caffeine and raises questions of the advisability of standardising guaraná extracts to caffeine content (as in the extract used here). However, further exploration of this extract, including investigating the other properties of the seed, is needed in order to fully understand its action.

CHAPTER 5. IMPROVED COGNITIVE PERFORMANCE FOLLOWING ADMINISTRATION OF GUARANÁ EXTRACT: COMPARISON AND INTERACTION WITH PANAX GINSENG

5.1 Introduction

The research described in the current chapter was carried out in collaboration with Pharmaton SA. As detailed in the previous chapter, guaraná is becoming increasingly common as a food additive in Western markets despite a lack of research into its specific behavioural effects. Initially, in some cases, it was added to foods as a substitute for caffeine, with products containing guaraná often being marketed as caffeine-free. This is despite the fact that guaraná naturally contains around 4 % guaranine, which is chemically identical to caffeine. Guaraná's purported stimulant effects are usually assigned to this guaranine content and it is often claimed to be released more slowly and to have a more subtle and long-lasting effect than caffeine. This slower absorption is variously attributed to the fat content of guaraná making it insoluble in water, the presence of tannins, which caffeine binds to, and saponins. However, Bempong and Houghton (1992) studied the absorption rate of caffeine from guaraná and found no significant difference in absorption between pure caffeine and caffeine from guaraná, in a number of comparisons using different pH levels either in capsule form or in solution.

These findings suggest that, as with mateine from yerba mate, and theine from tea, guaranine is merely caffeine under another name. However, the results from Chapter 4 illustrate that the effects of guaraná are not indicative of a caffeine effect. Guaraná with a caffeine content ranging from 4.5 to 36 mg of caffeine did not evince the well accepted effects of caffeine on vigilance performance and simple reaction time, or the expected dose-response. In addition, secondary memory and serial threes subtraction performance was improved despite a lack of effects seen with caffeine on these measures previously. It is plausible, however, that effects may manifest in different ways dependent upon the other constituents of these plants and, as highlighted in Chapter 3, the effects of caffeine-containing products should not be attributed to caffeine without regard for possible effects of other concomitant compounds, or at least some interaction between caffeine and these other constituents. In the case of guaraná other possible active components include tannins, saponins (Espinola et al. 1997) and catechins (see Carlson and Thompson 1998).

Henman (1982) states that guaraná is used in cases of physical and mental stress. This suggests a possible resistogen or adaptogen property (i.e. offering

protection against the physiological effects of physical or psychological stressors) of the plant, which is similar to that of ginseng (Mattei et al. 1998). Espinola et al. (1997) employed a forced swimming exercise, which has previously been indicated to be a measure of adaptogenic properties (Brekhman 1980), and found improvements on this measure following guaraná. The authors suggest that these adaptogenic effects of guaraná are attributable to a non-specific action of saponins (high-molecular weight glycosides combining a sugar element and a steroid aglycone or triterpene molecule), of which ginseng also contains a high content. Further support for a similar action of guaraná and ginseng comes from the common practice of combining the two.

Panax ginseng is a member of the plant genus *Panax* (Araliaceae family). It is indigenous to the Far East (most notably China and Korea), was first cultivated around 11 BC, and has a medical history (originally as a wild herb) stretching back more than 5000 years (Yun 2001). It is currently consumed worldwide for its putative beneficial properties, which include positive effects on physical parameters, cognitive performance, and well-being. The major active constituents of the *Panax* genus are thought to be saponins, in this case species unique triterpenoid glycosides known as ginsenosides, of which over 30 individual examples, many of which exist only in minute amounts, have been identified (Tachikawa et al. 1999). The individual and combined ginsenosides have been shown to exhibit both a plethora of physiological effects in vitro and to modulate physical and mnemonic performance in animals (for review see: Kennedy and Scholey 2003).

A number of recent double-blind, placebo-controlled, crossover studies have also examined the behavioural effects of acute administration of a standardised ginseng extract (G115) to humans. In the first of these experiments (Kennedy et al. 2001), the cognitive and mood effects of three separate single doses (comparing 200, 400 and 600 mg) of ginseng were assessed in healthy young participants. The results showed benefits in memory performance following all three doses of ginseng, with this effect most apparent following the middle dose. The two less mnemonically beneficial doses were, however, associated with slower performance on attention tasks. This finding of longer response latency on attention tasks was in contrast to the findings of a subsequent electroencephalography (EEG) experiment in which a 200 mg dose of ginseng was shown to significantly shorten evoked P300 response latency, and provoke a stronger pattern of beneficial topographic EEG effects than *Ginkgo biloba* (Kennedy et al. 2003b). Similarly, a recent study (Reay et al. 2005) reported faster performance on a mental arithmetic task during an extended period of cognitive demand, with a concomitant reduction in blood glucose levels, following this dose.

Given the potential for saponins to play a part in any effects of guaraná it was felt that a comparison of the behavioural effects of guaraná with those of *Panax ginseng* (whose major active constituents are thought to be saponins) would help to elucidate any contributing factor that they present. The aim of this study was therefore, to examine the behavioural effects of ginseng and guaraná in order to compare the profile of tasks affected by the two treatments in an attempt to delineate the underlying mechanisms of action. The findings from the previous chapter led to the conclusion that 75 mg of guaraná was the most effective dose of those tested. It seemed appropriate to further investigate potential nootropic properties in this dose of guaraná allowing a partial replication of findings. The effects of this dose were compared to 200 mg of *Panax ginseng* (G115) which, as outlined above, has previously been shown to be a psychoactive dose of this extract. Further to this, a product combining the two was also tested, which allowed assessment of the common, commercially available, combination of guaraná with *Panax ginseng*. In order to assess potential differential time course effects, testing took place pre-dose, and at 1 hour, 2.5 hours, 4 hours and 6 hours thereafter. This schedule of testing is slightly different to that employed in Chapter 4 but it is in line with a wealth of previous research carried out using this battery examining the effects of *Panax ginseng*.

5.2 Materials and Methods

5.2.1 Design

A randomised, placebo-controlled, double-blind, balanced-crossover design was employed.

5.2.2 Initial screening

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix IV).

5.2.3 Participants

Twenty-eight undergraduate volunteers took part in the study (19 female and 9 male, mean age 21.4 years, SEM 0.77, range 18 - 34). Participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session and throughout the day until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

5.2.4 Assessment

The tasks employed were identical to those described in Chapter 3.

5.2.5 Extracts and treatments

5.2.5.1 Extracts

Panax ginseng – standardised extract G115: Extraction from selected roots of *Panax ginseng* C. A. Meyer was undertaken by exhaustive percolation in a 40 % ethanol, 60 % water solvent at temperatures below 40 °C. Following quantitative analysis of the resultant dry extract, standardisation was performed by the addition of excipients (lactose at a range of 43 – 68 %, and 2 % silicon dioxide) bringing the

concentration of ginsenosides to 4 %. This process results in root: extract ratios of between 3 and 7 parts root to one part extract depending on the concentration of ginsenosides in the root.

Guaraná – see Chapter 4.

5.2.5.2 Treatments

On each study day participants received two capsules. The individual capsules contained a total of either; 75 mg of guaraná extract; 200 of mg *Panax ginseng* G115; a combination of 75 mg of guaraná and 200 mg of ginseng; or 0 mg of guaraná and 0 mg of ginseng (placebo). All treatments were identical in appearance and scent.

5.2.6 Procedure

Each participant was required to attend a total of five study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the four active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the four active study days comprised five identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, the second took place 1 hour post-dose, the third at 2.5 hours post- dose, the fourth at 4 hours-post-dose and the final session took place at 6 hours post-dose.

Each testing session lasted approximately 30 minutes and comprised completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales.

5.2.7 Statistics

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab.

The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising t tests with MSError from an omnibus ANOVA as an error term (Keppel 1991). At each time point (1, 2.5, 4, and 6 hours post-treatment) data from the placebo condition was compared to that for each of the three active treatments (guaraná, ginseng, and guaraná/ginseng). This allowed comparison of the profile for each active treatment. Although this did not allow analyses of the differences between these active treatments, this is reflective of the main focus of study, which is, the effects of the single doses. Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for treatment, assessment, treatment x assessment and participant (Kirk 1968), was carried out to identify main effects and interaction effects on change from baseline data for each measure. Where baseline differences were observed baseline scores were entered as a covariate in an ANCOVA, with terms fitted to the model for treatment, assessment, treatment x assessment and participant and this was analysed using SAS/STAT. To ensure the overall Type I error protection level only those planned comparisons associated with measures that generated a significant main effect or interaction effect ($p<0.05$) on this initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated. Due to the number of comparisons, effects on measures that generated only a single significant result were interpreted with caution.

5.3 Results

5.3.1 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, guaraná, ginseng, and guaraná/ginseng) for each outcome were subjected to a one-way, repeated-measures ANOVA. There was a significant baseline difference in RVIP accuracy, with significantly poorer performance in the ginseng condition [$F(3, 81)=3.14$, $p=0.03$].

5.3.2 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 5.1 along with F values and probabilities for effects of treatment. Significant differences on cognitive outcomes from the CDR battery are presented in Figure 5.1. Only planned comparisons for those measures that showed a significant main effect are reported below.

5.3.2.1 Digit vigilance reaction time

Guaraná significantly improved digit vigilance reaction time at 1 hr [$t(243)=2.56$, $p=0.01$], 4 hrs [$t(243)=2.39$, $p=0.02$], and 6 hrs [$t(243)=4.24$, $p<0.0001$]. This effect was apparent at 6 hrs following ginseng [$t(243)=2.84$, $p=0.0049$] and at 4 hrs [$t(243)=2.26$, $p=0.03$], and 6 hrs [$t(243)=3.26$, $p=0.001$] following the combination, see Fig 5.1a.

5.3.2.2 Choice reaction time accuracy

Accuracy of choice reaction was significantly impaired following guaraná at 1 hr [$t(243)=3.05$, $p=0.003$], and 4 hrs post-dose [$t(243)=2.63$, $p=0.009$], see Fig 5.1b.

Table 5.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
			1 hour	2.5 hours	4 hours	6 hours	
Immediate word recall accuracy (%)	Placebo	50.4±2.63	-5.71±2.29	-6.79±2.37	-5.71±2.62	-6.79±2.11	F(3, 243) =1.25 p>0.1
	Guaraná	49.9±2.82	-4.52±3.18	-4.05±2.48	-7.26±1.93	-7.26±2.46	
	Ginseng	45.2±3.15	-6.55±1.90	-2.86±2.49	-3.93±3.10	-0.12±3.44	
	Guar+Gins	49.8±2.67	-3.81±2.40	-6.67±2.79	-5.12±2.58	-9.17±2.95	
Simple reaction time (ms)	Placebo	286±10.3	10.3±7.08	8.63±9.09	21.3±8.70	11.5±8.60	F(3, 243) =1.91 p>0.1
	Guaraná	280±5.95	10.1±4.82	12.2±6.17	18.0±5.59	18.4±5.82	
	Ginseng	280±6.75	8.14±4.28	12.1±6.54	23.4±7.25	18.3±6.23	
	Guar+Gins	286±7.28	5.58±6.63	2.35±7.03	9.39±7.63	7.28±5.81	
Digit vigilance accuracy (%)	Placebo	94.5±1.37	0.56±0.78	0.08±0.83	-4.21±1.62	-3.49±1.90	F(3, 243) =1.05 p>0.1
	Guaraná	94.6±1.39	-0.71±1.09	-1.75±1.40	-0.71±0.98	-0.63±1.00	
	Ginseng	95.0±1.29	-1.27±1.21	-1.82±1.11	-2.94±1.89	-3.81±1.11	
	Guar+Gins	94.2±1.44	-0.56±1.65	-1.35±1.73	-2.78±1.47	-0.63±1.44	

Digit vigilance reaction time (ms)	Placebo	426±8.23	21.5±4.63	13.8±5.89	24.8±6.08	36.8±6.39	**F(3, 243) =5.41 p=0.001**
	Guaraná	432±7.03	5.42±5.48	14.4±6.55	9.86±5.93	10.2±6.13	
	Ginseng	436±10.3	18.4±5.73	19.9±6.35	15.3±7.60	19.0±6.83	
	Guar+Gins	433±9.10	11.0±6.62	3.01±6.70	10.6±7.48	16.3±7.71	
Digit vigilance false alarms (number)	Placebo	1.29±0.26	-0.04±0.29	0.21±0.33	-0.21±0.20	0.04±0.34	F(3, 243)<1
	Guaraná	0.96±0.25	-0.04±0.27	0.61±0.33	0.04±0.26	0.21±0.27	
	Ginseng	1.21±0.24	-0.29±0.22	-0.07±0.32	-0.04±0.28	0.21±0.37	
	Guar+Gins	1.14±0.28	-0.04±0.25	-0.04±0.31	-0.18±0.30	-0.14±0.25	
Choice reaction time accuracy (%)	Placebo	95.3±0.69	0.71±0.84	0.21±0.48	0.00±0.74	0.00±0.63	**F(3, 243) =5.56 p=0.001**
	Guaraná	96.5±0.67	-1.36±0.58	-0.93±0.71	-1.79±0.84	-0.64±0.65	
	Ginseng	94.7±0.90	2.00±0.74	0.64±0.65	0.07±0.59	0.21±0.79	
	Guar+Gins	95.6±0.63	0.36±0.71	-0.50±0.63	0.00±0.76	-0.14±0.82	
Choice reaction time (ms)	Placebo	422 ±11.7	12.6±9.17	15.5±7.19	24.7±10.9	17.8±11.7	**F(3, 243) =3.48 p=0.02**
	Guaraná	426±13.2	-5.60±6.92	0.95±9.23	6.73±10.9	7.32±10.9	
	Ginseng	434±11.6	4.17±6.03	6.71±9.52	-8.16±8.95	1.23±8.51	
	Guar+Gins	425±9.59	13.0±9.82	-0.02±6.80	11.7±7.09	14.8±6.59	
RVIP accuracy (%)	Placebo	63.8±3.73	-4.02±1.99	-4.02±2.23	-0.22±1.95	-7.14±2.28	F(3, 243) =2.24 p=0.08
	Guaraná	64.4±4.35	-2.23±2.70	-1.68±2.24	-5.47±1.98	-5.25±2.25	
	Ginseng	57.8±3.82	-0.45±2.60	-1.56±2.29	-1.56±2.15	0.00±2.70	
	Guar+Gins	60.1±4.26	2.12±1.87	0.00±2.17	-1.79±2.55	-4.46±3.00	
RVIP reaction time (ms)	Placebo	497±13.2	14.5±12.1	-0.55±18.1	16.2±13.0	-9.26± 10.5	F(3, 243) =1.90 p>0.1
	Guaraná	519±20.3	-31.4±9.15	-13.6±8.63	-19.1±9.91	-4.80±9.24	
	Ginseng	524±18.8	-14.6±18.2	-12.7±14.0	-14.8±13.4	-21.1±13.0	
	Guar+Gins	496±17.9	2.82± 11.3	-1.80±11.2	-13.5±22.4	-31.8±34.1	
RVIP false alarms (number)	Placebo	1.89±0.65	0.50±0.28	0.29±0.41	-0.36±0.47	0.21±0.34	F(3, 243)<1
	Guaraná	1.43±0.33	-0.04±0.33	-0.36±0.30	0.50±0.39	0.21±0.38	
	Ginseng	1.54±0.38	0.18±0.32	0.64±0.33	0.64±0.38	0.32±0.31	
	Guar+Gins	1.54±0.35	0.00±0.31	0.26±0.48	0.25±0.23	0.57±0.40	
Spatial memory (sensitivity index)	Placebo	0.96±0.01	-0.01±0.02	-0.04±0.05	-0.02±0.02	-0.04±0.02	F(3, 243) =2.11 p=0.1
	Guaraná	0.95±0.02	-0.05±0.03	-0.05±0.02	-0.05±0.03	-0.04±0.03	
	Ginseng	0.95±0.01	-0.06±0.03	-0.03±0.02	-0.01±0.01	-0.10±0.04	
	Guar+Gins	0.92±0.02	0.00±0.02	-0.01±0.02	-0.02±0.04	-0.01±0.03	
Spatial memory reaction time (ms)	Placebo	611±21.8	-7.79±27.3	-14.9±15.3	-7.74±32.6	19.1±31.9	F(3, 243)<1
	Guaraná	621±29.6	16.5±36.8	-14.6±14.9	-32.8±15.7	7.63±36.8	
	Ginseng	615±36.2	-34.8±33.0	-29.5±33.5	-59.6±34.6	13.1±53.2	
	Guar+Gins	622±30.6	40.5±31.8	-26.8±22.2	-55.2±28.2	-2.81±31.3	
Logical reasoning (%)	Placebo	76.0±4.34	-1.34±1.66	0.60±2.26	-1.64±1.80	1.49±1.44	F(3, 234)<1
	Guaraná	76.6±3.86	-0.30±1.74	0.46±1.42	-0.59±1.83	1.64±2.15	
	Ginseng	75.3±4.34	3.13±1.51	2.53±1.90	0.89±1.80	0.75±2.14	
	Guar+Gins	77.5±4.13	0.89±1.24	2.23±1.61	1.49±1.74	0.30±1.65	
Logical reasoning reaction time (ms)	Placebo	2856±312	-139±183	-67.8±179	-124±130	-339±255	F(3, 234) =1.18 p>0.1
	Guaraná	2803±260	-200±111	-432±186	-261±139	-267±210	
	Ginseng	2858±283	-26.8±222	-167±209	-495±192	-242±221	
	Guar+Gins	3106±339	-279±287	-462±238	-414±222	-496±182	
Numeric working memory (sensitivity index)	Placebo	0.90±0.02	-0.02±0.02	-0.02±0.02	-0.02±0.02	-0.01±0.02	F(3, 243) =2.52 p=0.06
	Guaraná	0.89±0.02	0.01±0.02	0.01±0.02	-0.01±0.02	-0.01±0.02	
	Ginseng	0.92±0.02	-0.02±0.01	-0.01±0.01	-0.04±0.01	-0.01±0.01	
	Guar+Gins	0.92±0.01	-0.04±0.02	-0.04±0.01	-0.01±0.01	-0.04±0.01	
Numeric working memory reaction time (ms)	Placebo	601±23.9	5.99±13.1	-2.17±19.9	5.60±21.5	-14.8±17.8	**F(3, 243 =4.03 p=0.008**
	Guaraná	608±26.6	3.39±12.7	-13.6±10.1	-4.49±22.6	-27.3±17.5	
	Ginseng	636±30.6	-20.3±24.1	-47.5±25.6	-43.9±26.3	-60.0±27.7	
	Guar+Gins	612±21.3	-3.10±21.0	-2.41±13.2	-11.3±12.6	-42.3±14.8	
Delayed word recall accuracy (%)	Placebo	36.0±2.49	-10.5±2.63	-14.6±3.20	-15.0±2.58	-14.5±2.53	F(3, 243) =1.71 p>0.1
	Guaraná	35.0±2.74	-7.62±2.60	-10.7±2.88	-13.0±3.11	-14.8±3.07	
	Ginseng	32.3±3.15	-11.1±2.76	-9.29±2.85	-12.7±2.93	-8.33±3.74	
	Guar+Gins	36.0±3.16	-12.3±2.93	-14.6±3.04	-11.9±2.78	-15.8±2.89	
Delayed word recognition (sensitivity index)	Placebo	0.67±0.04	-0.06± 0.05	-0.11±0.04	-0.11±0.04	-0.07±0.03	F(3, 243) =1.13 p>0.1
	Guaraná	0.62±0.04	-0.03±0.03	-0.02±0.04	-0.07±0.05	-0.06±0.04	
	Ginseng	0.64±0.04	-0.08±0.05	-0.08±0.05	-0.11±0.06	-0.10±0.04	
	Guar+Gins	0.67±0.04	-0.07±0.04	-0.10±0.03	-0.09±0.04	-0.11±0.04	
Delayed word recognition reaction time (ms)	Placebo	691±24.1	114±42.0	10.9±17.5	76.0±42.0	53.0±27.7	**F(3, 243) =4.74 p=0.003**
	Guaraná	718±27.4	48.8±30.9	42.8±30.2	63.6±39.0	-7.09±28.9	
	Ginseng	761±30.8	-0.94±33.6	-47.7±37.3	-18.3±34.5	-13.0±29.0	
	Guar+Gins	749±37.9	67.0±64.1	-21.2±36.9	-27.8±32.0	-27.7±24.7	
Delayed picture recognition (sensitivity index)	Placebo	0.65±0.04	-0.12±0.05	-0.11±0.04	-0.10±0.04	-0.11±0.05	**F(3, 243) =3.98 p=0.008**
	Guaraná	0.60±0.05	-0.01±0.03	0.02±0.05	-0.04±0.03	-0.02±0.04	
	Ginseng	0.62±0.04	-0.05±0.03	-0.03±0.04	-0.0±0.03	-0.02±0.04	
	Guar+Gins	0.62±0.05	-0.05±0.04	-0.08±0.05	-0.06±0.05	-0.05±0.04	
Delayed picture recognition reaction time (ms)	Placebo	789±27.4	47.6±23.6	19.7±23.2	51.8±18.1	21.8±29.6	**F(3, 243) =4.91 p=0.002**
	Guaraná	827±29.1	-6.28±33.2	-45.5±21.5	-34.0±20.8	-28.2±26.1	
	Ginseng	805±26.1	50.3±34.0	14.9±22.4	-2.43±23.9	-7.15±21.7	
	Guar+Gins	827±28.0	8.97±35.1	9.16±31.4	-6.16±27.5	-14.3±26.1	

5.3.2.3 Choice reaction time

Speed of choice reaction was, however, improved at 1 hr [t(243)=2.19, p=0.03], and 4 hrs [t(243)=3.97, p=0.03] following guaraná. Ginseng also speeded choice reaction time at 4 hrs [t(243)=2.00, p=0.0001], and 6 hrs [t(243)=3.05, p=0.047], see Fig 5.1c.

5.3.2.4 Rapid Visual Information Processing (RVIP) Accuracy

ANCOVA with baseline scores included as a covariate revealed there were no significant differences on this measure.

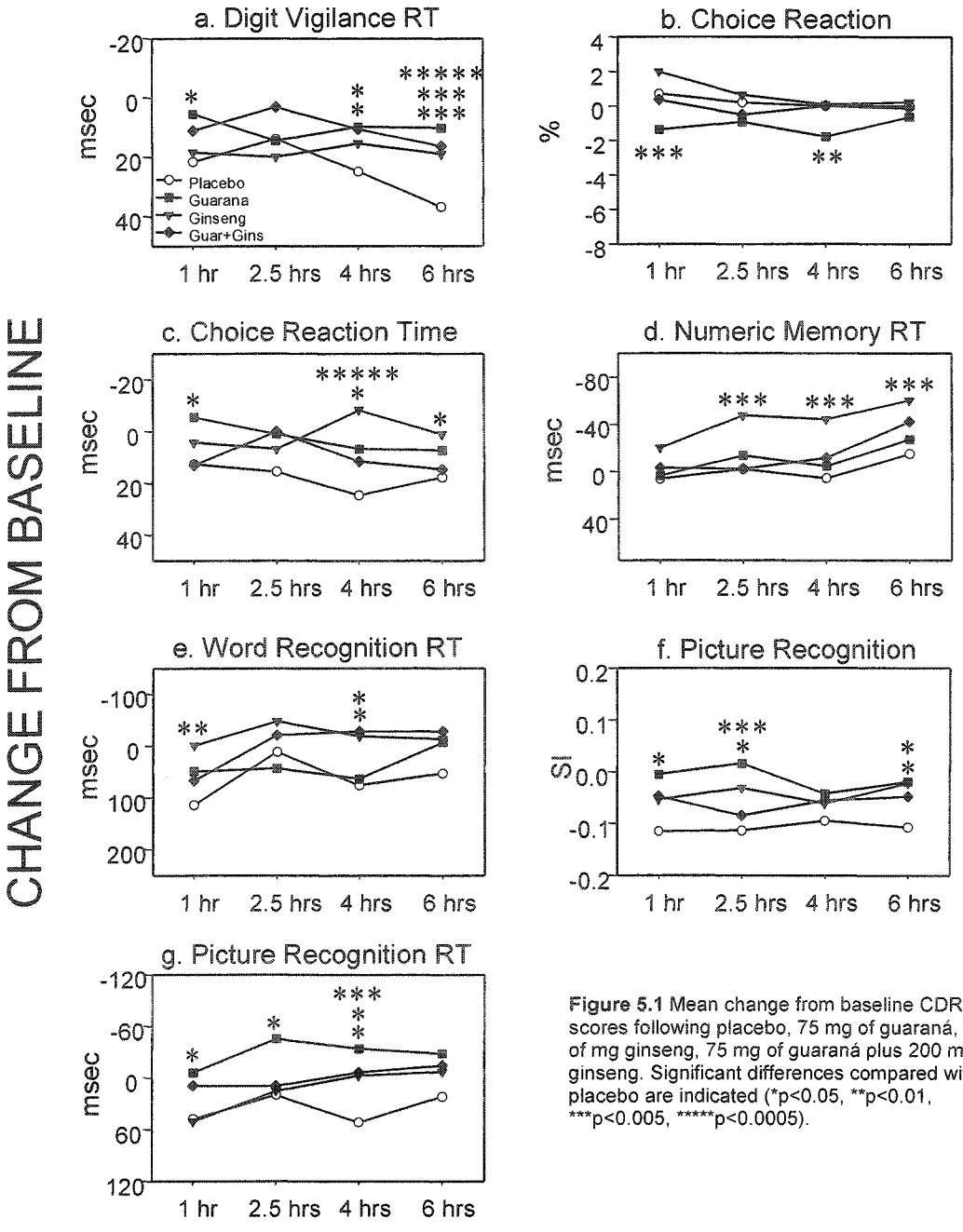

Figure 5.1 Mean change from baseline CDR scores following placebo, 75 mg of guaraná, 200 of mg ginseng, 75 mg of guaraná plus 200 mg of ginseng. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

5.3.2.5 Numeric working memory reaction time

There was a significant improvement in numeric working memory reaction time following ginseng at 2.5 hrs [t(243)=2.99, p=0.003], 4 hrs [t(243)= 3.26, p=0.004], and 6 hrs [t(243)=2.98, p=0.003] post-dose, see Fig 5.1d.

5.3.2.6 Delayed word recognition reaction time

Ginseng improved word recognition reaction time at 1 hr [t(243)=2.69, p=0.008], and 4 hrs [t(243)=2.21, p=0.03]. Following the combination speed was increased at 4 hrs post-dose [t(243)=2.44, p=0.02], see Fig 5.1e.

5.3.2.7 Delayed picture recognition accuracy

Accuracy of picture recognition was improved at 1 [t(243)=2.73, p=0.007], 2.5 [t(243)=3.22, p=0.001] and 6 hrs [t(243)=2.17, p=0.03] post-dose following guaraná. Ginseng also led to improvements to this measure at 2.5 [t(243)=2.05, p=0.04] and 6 hrs [t(243)=2.10, p=0.04] see Fig 5.1f.

5.3.2.8 Delayed picture recognition reaction time

Speed of picture recognition was improved following guaraná at 1 hr [t(243)=2.04, p=0.04], 2.5 hrs [t(243)=2.46, p=0.01], and 4 hrs [t(243)=3.24, p=0.001]. Speed was also increased at 4 hrs post-dose following ginseng [t (243)=2.05, p=0.04] and the combination speed [t (243)=2.19, p=0.03], see Fig 5.1g.

5.3.3 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on sentence verification and serial subtractions are presented in Table 5.2 along with F values and probabilities for effects of treatment. Significant differences on cognitive tasks are presented in Figure 5.2. Only planned comparisons for those measures that showed a significant main effect are reported below.

5.3.3.1 Sentence verification reaction time

The speed of performing the sentence verification task was significantly improved for all three conditions. This effect was evident at 2.5 hrs [t(243)=3.05, p=0.003], 4 hrs [t(243)=2.20, p=0.03], and 6 hrs [t(243)=2.09, p=0.04] following guaraná. Ginseng led to increased speed at all time points (1 hr [t(243)=2.73, p=0.007], 2.5 hrs [t(243)=3.24, p=0.001], 4 hrs [t(243)=3.06, p=0.002], 6 hrs [t(243)=2.84, p=0.0049]). The same effect for the combination was restricted to the 2.5 hr time point [t(243)=2.81, p=0.005], see Fig 5.2e.

Table 5.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
			1 hour	2.5 hours	4 hours	6 hours	
Sentence verification accuracy (%)	Placebo	96.2±0.68	-0.71±0.74	-0.83±0.87	-1.43±0.87	-0.71±0.79	F(3, 243)<1
	Guaraná	96.0±0.96	-1.79±1.39	0.36±0.73	-1.55±1.09	-0.24±0.80	
	Ginseng	95.0±1.14	0.12±1.17	0.24±1.06	0.83±0.99	-1.07±0.92	
	Guar+Gins	95.7±0.91	-1.19±1.23	0.83±0.78	-1.43±0.99	0.00±1.18	
Sentence verification reaction time (ms)	Placebo	1273±58.9	-26.4±21.6	22.4±39.9	-3.64±41.4	-33.8 ±40.6	**F(3, 243) =5.35 p=0.001**
	Guaraná	1352±69.8	-35.3±44.8	-92.9±32.6	-86.7±45.1	-113±43.3	
	Ginseng	1360±90.1	-130±57.8	-100±72.0	-120±62.1	-141±47.7	
	Guar+Gins	1303±50.3	-11.1±51.7	-83.8±31.6	-46.7±30.4	-15.3±27.1	
Serial threes subtraction correct (number)	Placebo	39.9±2.50	1.04±1.02	1.54±1.55	-0.61±1.51	2.29±1.15	**F(3, 243) =2.81 p=0.04**
	Guaraná	37.0±2.82	2.25±1.61	5.00±1.08	3.75±1.38	3.75±1.80	
	Ginseng	38.9±2.45	0.75±1.10	2.93±1.44	1.96±1.47	3.54±1.62	
	Guar+Gins	38.0±2.50	1.79±1.10	2.68±1.60	1.29±1.68	4.57±1.03	
Serial threes subtraction errors (number)	Placebo	2.57± 0.36	1.14±0.36	1.04±0.45	1.36±0.62	0.64±0.44	**F(3, 243) =3.76 p=0.01**
	Guaraná	2.93±0.37	0.25±0.54	-0.14±0.36	0.29±0.47	0.36±0.51	
	Ginseng	2.96±0.31	0.82±0.41	0.36±0.41	0.50±0.47	0.46±0.52	
	Guar+Gins	3.36±0.39	0.11±0.49	0.64±0.51	-0.04±0.56	-0.61±0.42	
Serial sevens subtraction correct (number)	Placebo	24.2±2.42	-2.32±0.78	0.11±1.03	1.54±0.69	0.64±0.93	**F(3, 243) =5.92 p=0.001**
	Guaraná	21.9±2.64	1.21±0.91	3.21±0.80	2.32±1.06	2.86±0.96	
	Ginseng	22.8±2.27	1.61±1.23	1.79±1.27	1.07±1.04	2.43±0.98	
	Guar+Gins	21.1±2.37	2.57±1.22	2.79±1.24	2.00±1.19	3.61±1.47	
Serial sevens subtraction errors (number)	Placebo	3.04±0.31	0.86±0.35	0.96±0.50	-0.04±0.42	0.29±0.44	**F(3, 243) =4.95 p=0.002**
	Guaraná	2.79±0.31	1.39±0.47	0.32±0.30	1.07±0.56	0.50±0.44	
	Ginseng	2.82±0.42	0.18±0.56	0.43±0.48	0.89±0.41	0.68±0.45	
	Guar+Gins	3.82±0.68	-0.57±0.74	-0.75±0.73	-0.36±0.62	-0.36±0.84	

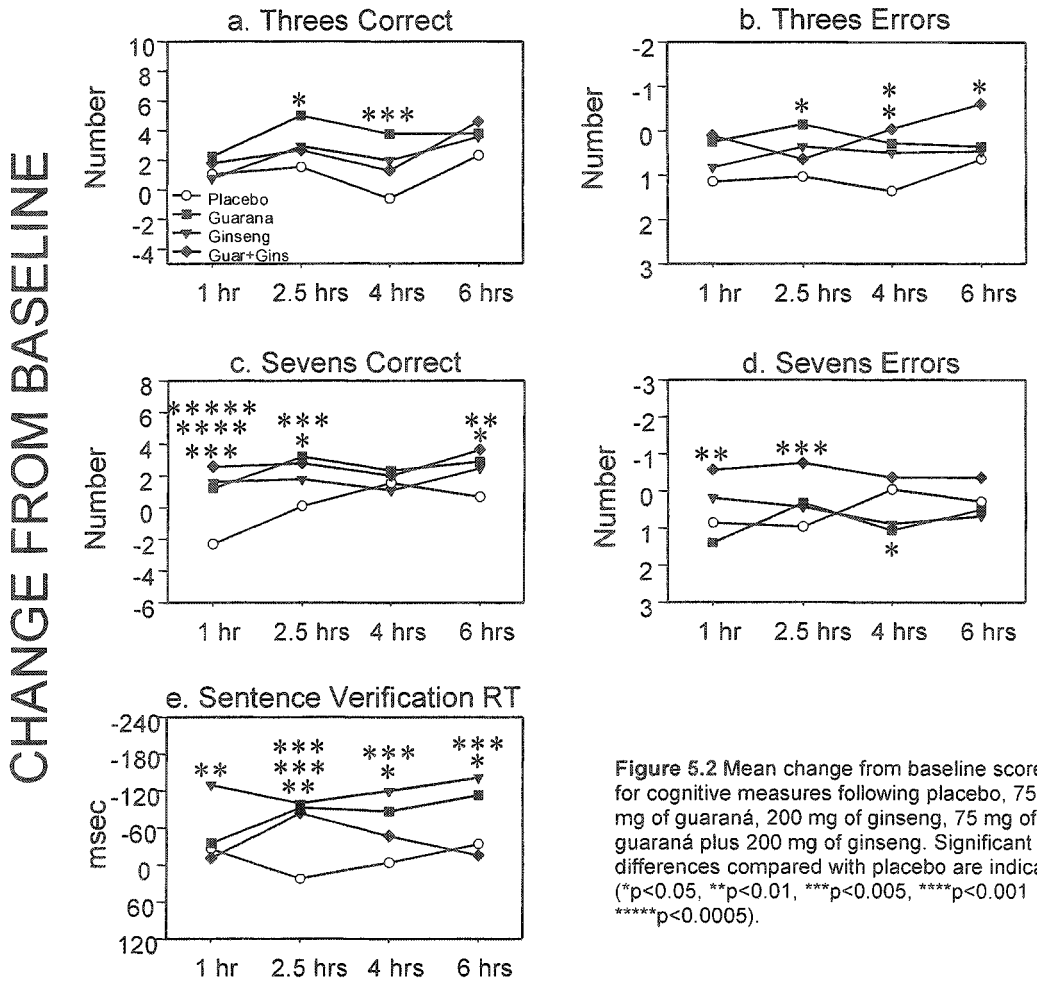

Figure 5.2 Mean change from baseline scores for cognitive measures following placebo, 75 of mg of guaraná, 200 mg of ginseng, 75 mg of guaraná plus 200 mg of ginseng. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001 *****p<0.0005).

5.3.3.2 Serial threes subtraction task

There was a significant increase in the number of correct serial threes subtractions following guaraná at 2.5 hrs [t(243)=2.54, p=0.01], and 4 hrs [t(243)=3.14, p=0.002], see Fig 5.2a. Errors were also decreased following guaraná at 2.5 hrs [t(243)=2.17, p=0.03], and 4 hrs [t(243)=1.97, p=0.049] and following the combination at 4 hrs [t(243)=2.57, p=0.01], and 6 hrs [t(243)=2.30, p=0.02], see Fig 5.2b.

5.3.3.3 Serial sevens subtraction task

The number of correct responses on the serial sevens task was increased following guaraná at 1 hr [t(243)=3.34, p=0.001], 2.5 hrs [t(243)=2.94, p=0.004], and 6 hrs [t (243)=2.09, p=0.04]. Ginseng only produced this effect at 1 hr [t(243)=3.72, p=0.00096] whereas the guaraná/ginseng combination also led to improvements at the 1 hr [t(243)=4.63, p<0.0001], 2.5 hr [t(243)=2.53, p=0.01], and 6 hr [t (243)=2.80, p=0.005] testing sessions, see Fig 5.2c. The combination also led to decreases in errors at 1 hr [t(243)=2.78, p=0.006], and 2.5 hrs [t(243)=3.33, p=0.001] errors were, however, increased following guaraná at 4 hrs [t(243)=2.15, p=0.03], see Fig 5.2d.

5.3.4 Mood assessment

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 5.3 along with F values and probabilities for effects of treatment. Only significant main effects for each outcome measure are reported below. Significant differences in mood are presented in Figure 5.3.

Due to data capture errors, which led to the loss of 4 datasets, analyses of Bond-Lader mood ratings incorporated the data from 24 participants. Similarly, the caffeine research visual analogue scales analysis was based on 27 participants.

5.3.4.1 *Bond-Lader 'Calm' factor*

'Calm' ratings were significantly decreased following ginseng at 1 hr [t(207)=3.70 p=0.0002], and 6 hrs post-dose [t(207)=2.07, p=0.04], see Fig 5.3a.

5.3.4.2 'Relaxed'

'Relaxed' ratings were significantly decreased following guaraná at 1 hr [t(234)=2.90 p=0.004] and following the combination at 4 hrs [t(234)=3.37 p=0.001], and 6 hrs post-dose [t(234)=2.69, p=0.008], see Fig 5.3b.

5.3.4.3 'Tired'

'Tired' ratings were significantly decreased at 2.5 hrs following guaraná [t(234)=3.50 p=0.001] and following the combination [t(234)=3.00 p=0.003], see Fig 5.3c.

Table 5.3 Baseline and change from baseline mood ratings for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure		Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
				1 hour	2.5 hours	4 hours	6 hours	
Bond-Lader factors	Alert	Placebo	54.7±4.22	-5.35±3.59	-7.08±-1.44	-11.5±-2.34	-15.0±-3.07	F(3, 207) =2.06 p>0.1
		Guaraná	55.9±4.07	-3.61±1.56	-2.58±-0.53	-5.23±-1.07	-8.14±-1.66	
		Ginseng	55.3±3.86	-5.75±2.40	-7.18±-1.47	-12.9±-2.63	-7.29±-1.49	
		Guar+Gins	58.0±3.61	-4.02±2.09	-5.86±-1.20	-8.86±-1.81	-10.9±-2.23	
	Content	Placebo	62.9±4.11	0.33±2.29	-0.76±-0.15	-4.12±-0.84	-3.88±-0.79	F(3, 207) =1.04 p>0.1
		Guaraná	65.8±3.05	-3.42±1.38	-2.03±-0.41	-1.89±-0.39	-4.87±-0.99	
		Ginseng	62.3±3.19	-3.78±1.61	-1.43±-0.29	-1.32±-0.27	-4.52±-0.92	
		Guar+Gins	63.4±3.29	-0.50±1.63	-0.42±-0.09	-1.63±-0.33	-0.51±-0.10	
	Calm	Placebo	62.9±3.21	3.52±2.07	-0.71±-0.14	-2.75±-0.56	-0.23±-0.05	**F(3, 207) =4.60 p=0.004**
		Guaraná	66.1±3.46	-0.25±2.65	-4.56±-0.93	-6.35±-1.30	-5.94±-1.21	
		Ginseng	65.1±3.00	-7.75±3.18	-5.23±-1.07	-4.75±-0.97	-6.46±-1.32	
		Guar+Gins	59.6±2.92	2.75±3.32	2.58±0.53	-0.81±-0.17	-4.08±-0.83	
Caffeine research visual analogue scales	**Relaxed**	Placebo	59.6±4.86	1.81±5.03	-2.30±-0.44	5.78±1.11	1.26±0.24	**F(3, 234) =4.49 p=0.004**
		Guaraná	69.6±3.00	-5.30±3.01	-8.15±-1.57	-6.70±-1.29	-4.33±-0.83	
		Ginseng	62.0±3.95	-5.07±4.16	0.93±0.18	1.00±0.19	0.04±0.01	
		Guar+Gins	67.0±3.35	-2.59±2.69	-4.44±-0.85	-8.74±-1.68	-10.3±-1.99	
	Alert	Placebo	53.7±4.41	-3.04±5.12	-7.52±-1.45	-7.67±-1.47	-9.63±-1.85	F(3, 234) =1.68 p>0.1
		Guaraná	57.8±3.93	1.59±4.26	3.15±0.61	-2.41±-0.46	-8.89±-1.71	
		Ginseng	52.7±4.03	-1.67±4.27	-1.70±-0.33	-10.7±-2.05	-5.74±-1.10	
		Guar+Gins	58.1± 3.74	-3.19±4.01	-6.37±-1.23	-10.1±-1.94	-10.7±-2.06	
	Jittery	Placebo	23.7±4.26	-3.37±3.84	-1.26±-0.24	-2.44±-0.47	-2.67±-0.51	F(3, 234) =1.19 p>0.1
		Guaraná	22.7±4.43	2.15±3.74	1.74±0.33	2.56±0.49	1.04±0.20	
		Ginseng	22.1± 4.29	6.41±3.43	2.78±0.53	-1.85±-0.36	-2.70±-0.52	
		Guar+Gins	26.2± 3.96	-2.78±2.72	-2.37±-0.46	2.74±0.53	1.81±0.35	
	Tired	Placebo	51.7±5.71	1.81±5.33	8.07±1.55	12.9±2.48	14.3±2.76	**F(3, 234) =3.60 p=0.01**
		Guaraná	51.9±5.48	-5.85±4.13	-7.78±-1.50	4.11±0.79	11.8±2.27	
		Ginseng	50.5±5.35	1.07±3.93	7.00±1.35	15.3±2.93	10.9±2.09	
		Guar+Gins	50.4± 4.76	-2.30±4.19	-5.52±-1.06	4.63±0.89	11.3±2.17	
	Tense	Placebo	26.6±4.13	-1.44±2.28	3.41±0.66	4.59±0.88	4.93±0.95	F(3, 234)<1
		Guaraná	26.6± 4.25	1.41±4.38	6.78±1.30	2.00±0.38	3.70±0.71	
		Ginseng	29.7±4.81	2.78±4.27	2.33±0.45	-4.74±-0.91	1.89±0.36	
		Guar+Gins	32.4± 4.50	-0.78±3.78	1.56±0.30	1.00±0.19	-1.37±-0.26	
	Headache	Placebo	16.0± 4.00	1.30±2.71	6.89±1.32	8.93±1.72	8.37±1.61	F(3, 234) =2.52 p=0.06
		Guaraná	12.9± 3.64	0.48±1.80	0.96±0.19	3.96±0.76	9.04±1.74	
		Ginseng	13.7±4.10	4.56±1.79	5.81±1.12	10.2±1.95	11.2±2.16	
		Guar+Gins	15.8± 4.27	1.11±1.66	-0.04±-0.01	3.15±0.61	8.52±1.64	
	Overall mood	Placebo	65.9±3.69	-0.67±3.04	-1.26±-0.24	-4.41±-0.85	-2.19±-0.42	F(3, 234) =1.61 p>0.1
		Guaraná	69.9±3.37	-4.78±2.70	-2.19±-0.42	-7.70±-1.48	-6.22±-1.20	
		Ginseng	65.0±3.60	-0.44±2.56	-0.89±-0.17	-4.81±-0.93	-3.78±-0.73	
		Guar+Gins	68.6±3.39	-0.19±2.61	0.07±0.01	-2.56±-0.49	-2.00±-0.38	
	Mental fatigue	Placebo	34.5±5.33	9.33±4.87	17.3±3.33	22.4±4.32	22.8±4.39	F(3, 234) =2.31 p=0.08
		Guaraná	35.7±4.73	6.11±2.24	6.22±1.20	14.7±2.83	21.6±4.15	
		Ginseng	36.3±4.59	8.52±3.62	13.3±2.56	19.4±3.73	19.0±3.65	
		Guar+Gins	37.5±4.59	6.15±4.52	9.41±1.81	12.7±2.44	16.2±3.12	
Caffeine research visual analogue scales factors	Alertness	Placebo	51.0±4.18	-2.43±4.67	-7.80±-1.50	-10.3±-1.98	-12.0±-2.30	F(3, 234) =2.49 p=0.06
		Guaraná	52.9±4.36	3.72±3.63	5.46±1.05	-3.26±-0.63	-10.4±-1.99	
		Ginseng	51.1±4.31	-1.37±3.81	-4.35±-0.84	-13.0±-2.49	-8.31±-1.60	
		Guar+Gins	53.8±3.63	-0.44±3.42	-0.43±-0.08	-7.37±-1.42	-11.0±-2.12	
	Tension	Placebo	33.5±3.85	-1.63±2.99	2.85±0.55	-0.59±-0.11	1.83±0.35	F(3, 234) =1.53 p>0.1
		Guaraná	28.5±3.06	3.35±3.01	7.46±1.44	4.35±0.84	4.02±0.77	
		Ginseng	33.9±4.05	3.93±3.29	0.70±0.14	-2.87±-0.55	0.93±0.18	
		Guar+Gins	32.7±3.64	0.91±2.74	3.00±0.58	4.87±0.94	4.48±0.86	

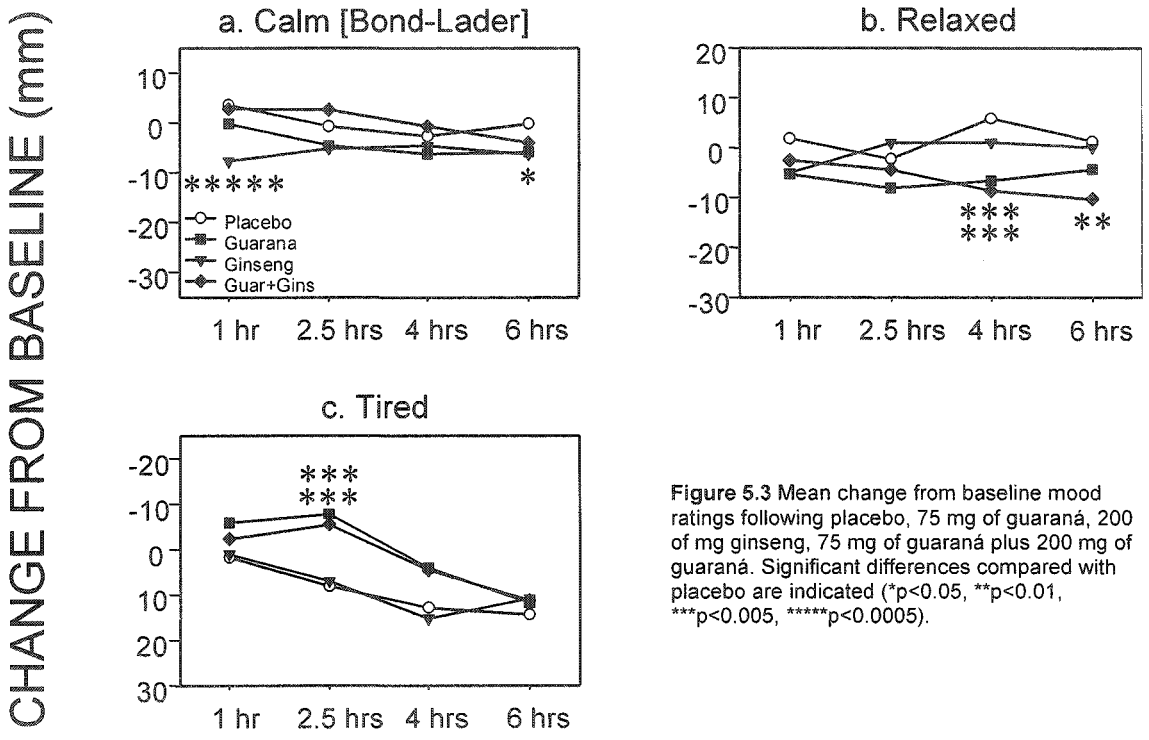

Figure 5.3 Mean change from baseline mood ratings following placebo, 75 mg of guaraná, 200 of mg ginseng, 75 mg of guaraná plus 200 mg of guaraná. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

5.4 Discussion

The results of the current study show that single doses of both guaraná and ginseng, and a combination of the two, can improve cognitive performance and mood in comparison to placebo in healthy young participants. These improvements took the form both of gross improvement of performance on several tasks, and an attenuation of the decline in performance invariably seen in placebo conditions during multiple completions of elements of the CDR test battery.

With regards guaraná, a number of improvements were found in terms of mood and cognition. Improvements to accuracy of picture recognition were observed and reaction times for digit vigilance, picture recognition and sentence verification were faster when compared to placebo. The number of correct serial threes subtractions was increased and the number of errors decreased. An improvement to choice reaction time coupled with impairment to accuracy on the same measure at the same time points following guaraná is highly indicative of a speed-accuracy trade-off. Similarly, an increase in serial sevens errors was observed at 4 hours, with increases in correct responses at 1 hour and 2.5 hours, suggests that there may also have been a speed-accuracy trade-off at the 4 hour time point on this task.

The improvements to secondary memory and serial threes subtractions evinced here replicate those results presented in Chapter 4, as does the impairment to serial sevens. The only additional effect seen here was an improvement to digit vigilance reaction time and sentence verification reaction time, and the only cognitive effect not seen here, which was demonstrated in the previous chapter, is increased RVIP false alarms, which was previously only observed at a single time point. This study, therefore, provides support for the findings presented in Chapter 4.

Turning to mood measures, reductions in 'tired' ratings were seen here, as previously observed, and a reduction in 'relaxed' ratings here and increased 'jittery ratings' seen in the previous chapter could be a manifestation of the same shift in mood. However, these mood effects were only observed at one time point. Improvements to 'content' ratings were not replicated. One possible explanation for differences between the effects seen here and those observed with the same dose in Chapter 4 is the addition of an extra testing session in the current chapter with testing at 2.5 hours and 4 hours rather than at 3 hours.

As mentioned in Chapter 4 the guaraná extract employed contained only 11 to 12 % caffeine so it seems unlikely that the potential maximum dose of less than 10 mg of caffeine could itself account for the performance effects seen here. The tasks affected are, again, mainly secondary memory tasks and serial subtractions, which are generally not perceived as sensitive to the effects of caffeine. A number of the effects

of guaraná were again still apparent at 6 hours, such as improvements to speed of digit vigilance and sentence verification and accuracy of picture recognition. Given that caffeine, including when derived from guaraná, has a half-life of approximately 6 hours in non-smoking humans (Haller et al. 2002), it would presumably have decayed to sub-active levels by this time point. However, in this study there were also some improvements to tasks that would be expected as a result of caffeine in isolation. Digit vigilance reaction time was faster as was reaction time on the sentence verification task, which is a measure of semantic memory. In addition, with the exception of the effects seen on the speed of sentence verification, the effects of guaraná are largely dissimilar to those of ginseng. The most striking effects of ginseng here are seen in terms of faster numeric working memory response, a measure that was unaffected by guaraná. These findings indicate that, although caffeine cannot be solely responsible for the effects seen with guaraná on cognition, it is also unlikely that these effects could be attributed to common saponin properties. Some of the cognitive and mood effects of guaraná in the current study are broadly in keeping with the effects of caffeine. In the case of mood, an example is decreases in 'tired' ratings coupled with decreased 'relaxed' ratings. However, the effects on 'relaxed' ratings would not be expected at such a low dose. These findings suggest that caffeine may be involved in some of the effects seen with guaraná but these effects are likely to be modulated by other components within the extract.

In the case of ginseng the results again replicate a direct mnemonic effect for this dose (Kennedy et al. 2001). In terms of accuracy, improvement was restricted to picture recognition and a single time point for serial sevens. However, the effect of this dose on the speed of task performance was somewhat more marked and extended across the CDR battery, with faster performance of picture and word recognition as well as numeric working memory. Reaction times were also improved at all time points on the sentence verification task, thus extending the range of memory tasks shown to be sensitive to ginseng. In terms of attention, improvements were seen in speed of digit vigilance and choice reaction time performance but the effect on digit vigilance was only seen at a single time point. 'Calm' ratings were also reduced at 1 hour and 6 hours post-dose. These results are consistent with the demonstration of significantly reduced P300 latency (Kennedy et al. 2003b).

The ginseng/guaraná combination was associated with faster digit vigilance task performance at 4 and 6 hours and faster word recognition, picture recognition and sentence verification task performance at one time point only. Reductions in errors were also seen on serial threes and sevens as was an increase in correct serial sevens responses. As these tasks (in particular serial sevens), have previously been rated as

the most subjectively demanding tasks within the entire battery utilised here, this does potentially signal a utility for the combination in situations of intense cognitive demand. Indeed, given that guaraná is often consumed for its stimulant properties at times of increased mental demand, this possibility should be further explored. Beyond this, whilst the general pattern of effects following the combination could be described as reflecting elements of the effects from the single ingredients, it could not be described, on the basis of the current data, as offering clear evidence for a synergistic relationship between the two. However, this does not preclude the possibility of synergistic effects at different doses. The current study employed a typical dose of guaraná (75 mg), which was substantially lower than that of ginseng (200 mg) – although this is not an untypical dose. It is possible that changing the ratios of the two might potentiate any behavioural effects.

It should also be noted that the cognitive and mood effects of chronic administration of ginseng have not been adequately addressed as yet, and that this is one of the first investigations of the cognitive effects of guaraná in healthy young humans. It is entirely feasible that the effects of extracts of either or both might increase with chronic dosage. Mattei et al. (1998) note that, on the basis of their saponin contents and observations from animal studies, guaraná, and ginseng might both be classed as 'resistogens' or 'adaptogens' (i.e. offering protection against the physiological effects of physical or psychological stressors). If this is the case then the effects of both may increase with chronic dosage, and the possibility exists for a synergistic relationship between their components, which would confer an added advantage over time, potentially both in terms of general health and cognitive performance.

CHAPTER 6. A COMPARISON OF BEHAVIOURAL EFFECTS OF GUARANÁ WITH THOSE OF A MATCHED DOSE OF CAFFEINE

6.1 Introduction

A number of products on the market, such as energy drinks and confections, as well as food supplements, contain guaraná extract. Guaraná contains only around 4 % caffeine. Despite this, and despite the presence of other components within guaraná, which possess possible psychoactive properties, these products usually describe this extract simply as caffeine and any reference to guaraná is only included as 'from guaraná extract' in parentheses. This assumes caffeine to be the active ingredient in guaraná. A similar practice has also been employed in two recent placebo-controlled trials of the effects of energy drinks on behaviour. Scholey and Kennedy (2004) examined the effects of an energy drink containing 37.5 mg glucose, 75 mg of caffeine, and flavouring levels of herbs. The effects of this complete drink and the effects of its constituent parts were compared to placebo. They failed to find any effects of caffeine on DSST, speed of attention (including simple reaction time and digit vigilance), working memory, serial subtractions, or mood. 75 mg of caffeine did increase speed of word recognition and produced a trend towards improved accuracy of choice reaction. A proportion of the caffeine within this study came from guaraná extract and it is interesting to note that the tasks affected appear to be more in line with those affected by guaraná than those affected by caffeine. However, although the authors refer to the possibility that caffeine from guaraná may engender a different profile of effects to pure caffeine, the results are referred to throughout as representing the effects of caffeine.

Smit et al. (2004) also considered the effects of energy drinks containing caffeine on performance of a 20-minute simple reaction time task, immediate word recall, a 10-minute RVIP task, delayed word recall, and visual analogue mood scales. Treatments took the form of two energy drinks (150 ml and 250 ml), 150 ml water, 250 ml water, and a no drink control. The energy drinks were iso-caloric and both contained 75 mg of caffeine but in the case of the 250 ml drink, part of this was in the form of guaraná extract. The findings showed that although both energy drinks led to an improvement on the mildly fatiguing simple reaction time task, the effects of the drink containing guaraná extract were apparent within 5 minutes of consumption. This effect was not observed with the energy drink containing pure caffeine. In addition, the drink containing pure caffeine led to improved RVIP performance when compared to the no drink control. This effect was not observed with the drink containing guaraná extract. Both energy drinks increased 'energetic arousal' and decreased 'bored' ratings but only the drink containing guaraná led to reductions in 'tense' ratings and increases in

'overall mood' ratings. The authors suggest that given the profile of effects it is most likely that the effects observed are the result of caffeine. However, they also state that the effects of the drink containing pure caffeine appear stronger than those of the drink containing guaraná and the reason for this difference between the energy drinks is unclear. The two drinks obviously differed in volume, which may have been a factor. They also differed in taste and carbonation, and contained slightly different levels of vitamins. The effects of these factors are considered by the authors and it seems unlikely that they are responsible for the differences. However, the impact that the guaraná extract may have had on the findings is not considered.

The findings from Scholey and Kennedy (2004) do not show the improvements to simple reaction time or digit vigilance reaction time following 75 mg of caffeine that would be expected from the caffeine literature and from the findings of Chapter 2 of this thesis utilising the same dose and tasks. They also found evidence of faster word recognition, which was also observed following guaraná in Chapter 4 of this thesis, and which fits with the findings from both Chapters 4 and 5 in terms of representing an improvement to secondary memory. The results from Smit et al. (2004), showing differences between energy drinks containing equal amounts of caffeine from different sources, suggest that, although guaraná has some similar effects to caffeine, these effects are in some way modulated by other components within guaraná. This is emphasised by the finding of stronger effects following pure caffeine than following caffeine with guaraná, although it is possible that other factors are responsible for these differences. Further support for the assertion that caffeine cannot be solely responsible for the action of guaraná comes from Campos et al. (2005) who found that, although caffeine (10 and 20 mg/kg) and guaraná (25 and 59 mg/kg) were able to decrease immobility time in rodents during a forced swimming test, only caffeine could block the effects of cyclopentyl adenosine (CPA), an adenosine agonist. This suggests that at least some of the effects of guaraná must be explained through a different mechanism to that of caffeine, as guaraná does not show the same antagonism of adenosine as caffeine.

In Chapter 4 of this thesis, the lowest dose of guaraná shown to be psychoactive contained ~ 4.5 mg of caffeine. There is no evidence of behavioural effects of doses of caffeine as low as this. In fact the average cup of decaffeinated coffee contains between 3 – 5 mg of caffeine, and this is generally believed to be below the psychoactive threshold. The findings in Chapter 4 suggest that not only does 37.5 mg of guaraná, containing only 4.5 mg of caffeine, possess psychoactive properties but 75 mg of guaraná, containing 9 mg of caffeine, appears to be more beneficial than guaraná doses containing 18 mg and 36 mg of caffeine. Although 12.5 mg of caffeine

has been shown to improve RVIP performance in certain populations and to speed simple reaction time (Smit and Rogers 2000), there is no evidence of doses lower than this having any effects, and no reason to believe that lower doses would have greater benefits than the amount found in an average cup of tea (36 mg). Additionally, a number of the effects of guaraná demonstrated in Chapters 4 and 5 were still apparent at six hours post-treatment. This is not typically what would be expected given that the half-life of caffeine is around 6 hours. Finally, the cognitive tasks that have been shown to be sensitive to guaraná are not those typically susceptible to the effects of caffeine. As shown in Chapter 2, caffeine affects measures relating to attention, vigilance and working memory with the effects on attention and vigilance being well accepted and working memory effects being suggested but not so well established (see Smith 2002 for review). However, guaraná has been shown to affect secondary memory measures and performance of serial subtractions, both of which have previously been shown to be insensitive to caffeine (see Chapter 2). There is, however, some overlap in the effects of caffeine and guaraná with the results from Chapter 2 showing improvements to accuracy of a sentence verification task following caffeine and the results of Chapter 5 showing improvements to this measure following guaraná, albeit in terms of speed rather than accuracy. Also, the findings from Chapter 4 showed some effects of guaraná on RVIP, which is a task that shows fairly robust effects of caffeine (e.g. Yeomans et al. 2002). The previous two chapters also showed that guaraná is similar to caffeine in its ability to increase subjective 'arousal' ratings manifested either in terms of increased 'alertness', reduced 'tiredness', or an amelioration of 'mental fatigue' ratings.

As seen in Chapter 3 it is possible that concomitant components within a substance can modulate the effects of one another. In the case of caffeine it was seen that by combining it with L-theanine a different profile of effects was produced on a number of measures. In the case of guaraná there are a number of other components that could be modulating any of the effects of caffeine, such as flavonoids, tannins, or saponins (Weckerle et al. 2003). It is also possible that the effects seen with guaraná are actually not related to caffeine and that one or more of its other components are responsible for the observed effects.

In order to attempt to clarify the role that caffeine plays in the effects of guaraná a randomised, placebo-controlled, double-blind, balanced-crossover study of the cognitive and mood effects of a single dose of guaraná (75 mg), a matched caffeine dose (9 mg) and placebo was carried out. In line with Chapter 4, testing took place pre-dose and at 1 hour, 3 hours and 6 hours thereafter. This design allowed us to find out if there were any differences between the profile of effects observed following guaraná

and a matched caffeine dose. In addition, it allowed us to establish if 9 mg of caffeine is capable of producing any psychoactive effects, and if so what the time course of these effects is, specifically to ascertain whether any effects are still apparent 6 hours after administration.

6.2 Materials and Methods

6.2.1 Design

A randomised, placebo-controlled, double-blind, balanced-crossover design was employed.

6.2.2 Initial screening

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix IV).

6.2.3 Participants

Twenty-six undergraduate volunteers completed all phases of the experiment (14 male and 12 female, mean age 20.9 years, SEM 0.42, range 18 - 29). Assessment of habitual caffeine consumption (using the caffeine consumption questionnaire employed in Chapter 2, see Appendix I) showed that 14 of these were habitual caffeine consumers (> 50 mg per day). Participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session and throughout the day until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

6.2.4 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence and immediately prior to all post-treatment assessments to confirm effective caffeine absorption. The saliva samples were immediately frozen at -20 °C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure

caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

6.2.5 Assessment

The tasks employed were identical to those described in Chapter 3.

6.2.6 Extracts and treatments

6.2.6.1 Extracts

Guaraná – see Chapter 4.

6.2.6.2 Treatments

On each study day participants received one capsule. The individual capsules contained a total of either; 75 mg of guaraná extract; 9 mg of caffeine hydrochloride BP (Merck, Darmstadt, Germany); or 0 mg of guaraná and 0 mg of caffeine (placebo). All treatments were identical in appearance and scent.

6.2.7 Procedure

Each participant was required to attend a total of four study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the three active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the three active study days comprised four identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day. The second took place 1 hour post-dose, the third at 3 hours post-dose, and the fourth at 6 hours-post-dose.

Each testing session lasted approximately 30 minutes and comprised producing a saliva sample, completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales.

6.2.8 Statistics

Salivary caffeine levels were analysed to assess compliance to caffeine abstinence and effective caffeine absorption.

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab.

The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising t tests with MSError from an omnibus ANOVA as an error term (Keppel 1991). At each time point (1, 3, and 6 hours post-treatment) data from the placebo condition was compared to that for each of the two active treatments (caffeine, guaraná). Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for treatment, assessment, treatment x assessment and participant (Kirk 1968), was carried out to identify main effects and interaction effects on change from baseline data for each measure. To ensure the overall Type I error protection level only those planned comparisons associated with measures that generated a significant main effect or interaction effect ($p<0.05$) on this initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

6.3 Results

6.3.1 Salivary caffeine levels

Salivary analysis revealed that all participants had complied with instructions to avoid caffeine-containing products. Mean baseline values were 0.57 μg/ml (SD = 0.88). Five datasets were unusable and were excluded from any salivary analysis. Analysis of post-treatment salivary caffeine levels revealed significantly higher levels at 1 hr [$t(80)=4.41$, $p<0.0005$], 3 hrs [$t(80)=3.67$, $p<0.0005$], and 6 hrs [$t(80)=4.41$, $p<0.0005$] following administration of caffeine, see Fig 6.1.

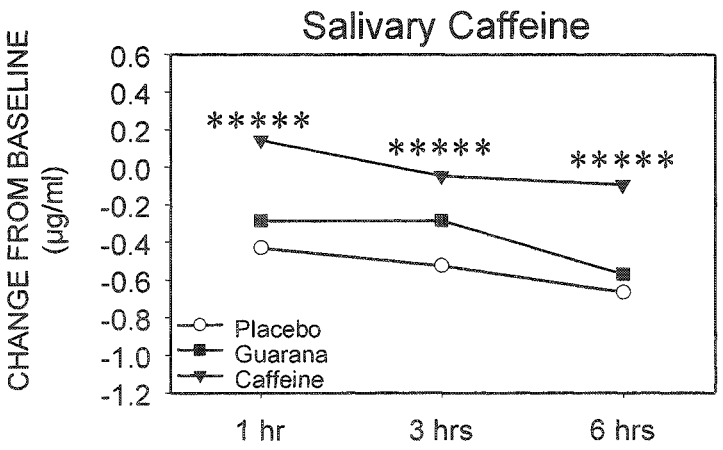

Figure 6.1 Mean change from baseline salivary caffeine levels following placebo, 9 mg of caffeine, and 75 mg of guaraná. Significant treatment effects compared with placebo are indicated (*****p<0.0005).

6.3.2 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose baseline scores for all three treatments (placebo, 9 mg of caffeine, 75 mg of guaraná) for each outcome (individual CDR task scores, sentence verification scores, serial subtraction scores and mood scale scores) were subjected to a one-way, repeated-measures, ANOVA. There were no significant differences at baseline.

6.3.3 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 6.1 along with F values and probabilities for effects of treatment. Only significant main effects for each outcome measure are reported below. Significant differences on cognitive tasks from the CDR battery are presented in Figure 6.2.

Table 6.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
			1 hour	3 hours	6 hours	
Immediate word recall accuracy (%)	Placebo	40.8±2.92	1.54±3.81	0.38±3.30	-1.92±3.07	F(2, 100)<1
	75 mg guaraná	43.1±2.37	-0.38±2.35	-4.49±2.64	-3.46±2.54	
	9 mg caffeine	41.7±2.30	-2.82±3.36	-1.79±2.71	-0.90±3.04	
Simple reaction time (ms)	Placebo	273±6.02	-0.19±5.12	10.9±4.00	11.9±4.47	**F(2, 100) =5.50 p=0.005**
	75 mg guaraná	273±4.59	12.9±4.44	5.37±4.72	15.4±6.08	
	9 mg caffeine	283±6.13	-5.37±3.72	-1.96±5.30	5.17±6.23	
Digit vigilance accuracy (%)	Placebo	94.1±1.29	-0.77±0.93	1.54±1.26	-0.08±1.12	**F(2, 100) =5.85 p=0.003**
	75 mg guaraná	96.2±0.90	-0.26±0.86	-2.74±1.54	-4.27±1.33	
	9 mg caffeine	95.6±0.95	-1.97±1.00	-2.74±1.37	-1.80±1.01	
Digit vigilance reaction time (ms)	Placebo	437±8.95	-2.18±5.68	2.10±6.73	13.1±6.75	**F(2, 100) =3.36 p=0.04**
	75 mg guaraná	425±6.36	9.59±6.06	13.2±5.71	25.4±6.71	
	9 mg caffeine	428±7.20	16.4±6.05	12.0±8.37	17.1±5.01	
Digit vigilance false alarms (number)	Placebo	0.96±0.19	-0.04±0.21	0.15±0.33	-0.23±0.19	**F(2, 100) =9.39 p<0.001**
	75 mg guaraná	0.62±0.15	0.54±0.30	0.58±0.30	1.08±0.37	
	9 mg caffeine	0.96±0.23	-0.27±0.23	-0.12±0.21	0.19±0.25	
Choice reaction time accuracy (%)	Placebo	95.3±0.63	0.46±0.70	0.62±0.72	-0.69±0.73	F(2, 100)<1
	75 mg guaraná	94.9±0.82	1.23±0.74	-0.46±0.82	-1.08±0.78	
	9 mg caffeine	95.8±0.68	0.77±0.53	-0.54±0.68	0.23±0.63	
Choice reaction time (ms)	Placebo	430±8.19	-13.8±7.52	-4.86±7.57	6.21±9.66	F(2, 100)<1
	75 mg guaraná	437±9.22	7.23±6.89	3.29±6.81	-0.43±7.86	
	9 mg caffeine	434±10.7	4.09±7.55	-5.28±7.57	1.71±7.51	
RVIP accuracy (%)	Placebo	73.7±3.33	-3.73±3.05	-3.73±3.25	-7.45±3.54	F(2, 100) =1.69 p>0.1
	75 mg guaraná	68.4±4.16	-3.24±2.44	-1.80±2.50	-0.24±2.87	
	9 mg caffeine	70.9±3.56	-3.00±2.06	-1.08±2.19	-2.29±2.42	
RVIP reaction time (ms)	Placebo	512±20.1	-13.9±11.1	-33.5±15.0	-13.4±12.9	F(2, 100) =2.15 p>0.1
	75 mg guaraná	501±17.4	-2.13±13.0	-7.73±16.6	6.89±14.7	
	9 mg caffeine	505±18.4	-12.2±13.1	-4.09±11.7	-17.2±12.9	
RVIP false alarms (number)	Placebo	3.69±2.10	0.35±0.48	-0.08±0.40	-0.19±0.69	F(2, 100) =2.88 p=0.06
	75 mg guaraná	2.81±1.19	-0.04±0.55	0.73±0.51	0.35±0.54	
	9 mg caffeine	3.23±1.52	-0.12±0.42	-0.50±0.39	-0.42±0.40	
Spatial memory (sensitivity index)	Placebo	0.94±0.01	-0.02±0.02	-0.01±0.02	-0.03±0.02	F(2, 100)<1
	75 mg guaraná	0.93±0.01	0.01±0.02	0.01±0.01	-0.04±0.03	
	9 mg caffeine	0.93±0.02	0.02±0.02	-0.03±0.03	-0.02±0.02	
Spatial memory reaction time (ms)	Placebo	533±22.3	-30.3±14.5	-15.3±17.9	-6.20±24.1	F(2, 100)<1
	75 mg guaraná	529±14.1	-33.7±10.3	-21.7±10.7	-7.99±17.6	
	9 mg caffeine	519±18.0	-33.3±8.60	-8.02±10.8	8.54±15.8	
Logical reasoning accuracy (%)	Placebo	89.3±3.14	0.16±1.67	-1.60±2.18	-0.48±2.02	F(2,100)<1
	75 mg guaraná	89.4±2.29	1.28±1.63	-1.76±1.57	-2.56±1.79	
	9 mg caffeine	89.9±1.84	-1.28±1.69	-0.96±1.66	-2.40±1.79	
Logical reasoning reaction time (ms)	Placebo	2768±233	-180±115	-108±106	-206±138	F(2, 100)<1
	75 mg guaraná	2750±407	-112±79.7	-315±160	-170±122	
	9 mg caffeine	2744±319	-87.3±102	-184±111	-189±146	
Numeric working memory (sensitivity index)	Placebo	0.91±0.01	-0.01±0.01	-0.01±0.01	-0.06±0.02	F(2, 100) =2.20 p>0.1
	75 mg guaraná	0.91±0.01	-0.01±0.01	-0.01±0.01	-0.01±0.02	
	9 mg caffeine	0.90±0.02	0.03±0.02	0.00±0.02	-0.02±0.03	
Numeric working memory reaction time (ms)	Placebo	581±19.8	10.2±12.2	23.5±18.2	46.6±38.4	**F(2, 100) =7.78 p=0.001**
	75mg guaraná	579±21.4	-8.95±7.51	6.41±9.28	5.62±16.5	
	9 mg caffeine	600±20.8	-26.9±11.4	-30.6±14.3	-19.0±15.2	
Delayed word recall accuracy (%)	Placebo	27.9±2.56	-6.28±3.25	-10.0±3.74	-11.3±3.03	F(2, 100)<1
	75 mg guaraná	26.7±2.39	-10.3±3.23	-9.10±2.76	-10.5±3.17	
	9 mg caffeine	28.6±2.10	-13.6±2.68	-12.7±2.37	-7.05±2.92	
Delayed word recognition (sensitivity index)	Placebo	0.57±-0.03	0.05±0.05	-0.05±0.04	-0.12±0.04	F(2, 100)<1
	75 mg guaraná	0.58±-0.08	0.05±0.05	-0.09±0.04	-0.14±0.04	
	9 mg caffeine	0.55±-0.04	0.04±0.04	-0.03±0.04	-0.12±0.04	
Delayed word recognition reaction time (ms)	Placebo	731±26.9	-7.80±20.1	54.7±17.8	12.6±38.8	F(2, 100)=1.11 p>0.1
	75 mg guaraná	708±30.5	30.1±22.9	11.7±17.1	17.3±22.3	
	9 mg caffeine	702±23.8	43.8±35.7	73.1±48.6	29.7±25.0	
Delayed picture recognition (sensitivity index)	Placebo	0.64±0.04	-0.04±0.04	-0.06±0.03	-0.05±0.03	F(2,100)<1
	75 mg guaraná	0.63±0.04	0.00±0.04	-0.05±0.05	-0.06±0.04	
	9 mg caffeine	0.64±0.04	-0.05±0.03	-0.04±0.04	-0.06±0.04	
Delayed picture recognition reaction time (ms)	Placebo	842±26.7	-25.5±23.3	28.6±27.6	15.1±34.9	F(2, 100) =1.34 p>0.1
	75 mg guaraná	824±29.8	-12.3±21.0	2.22±29.1	-14.1±24.9	
	9 mg caffeine	813±25.8	12.5±20.8	13.1±23.5	47.8±33.4	

6.3.3.1 Simple reaction time

Performance of the simple reaction time task was significantly impaired following administration of guaraná [t(100)=2.43, p=0.02] at 1 hr post-dose, and significantly improved following caffeine [t(100)=2.38, p=0.02] at 3 hrs, see Fig 6.2a.

6.3.3.2 Digit vigilance

Accuracy of digit vigilance was significantly impaired following caffeine at 3 hrs [t(100)=3.98, p=0.0001], and at 3 hrs [t(100)=3.98, p=0.0001] and 6 hrs following guaraná [t(100)=3.91, p=0.0002], see Fig 6.2b. Speed of digit vigilance was also impaired following caffeine at 1 hr [t(100)=2.98, p=0.004], see Fig 6.2c. Guaraná significantly increased the number of false alarms on the digit vigilance task at 6 hrs [t(100)=4.48, p<0.0001], see Fig 6.2d.

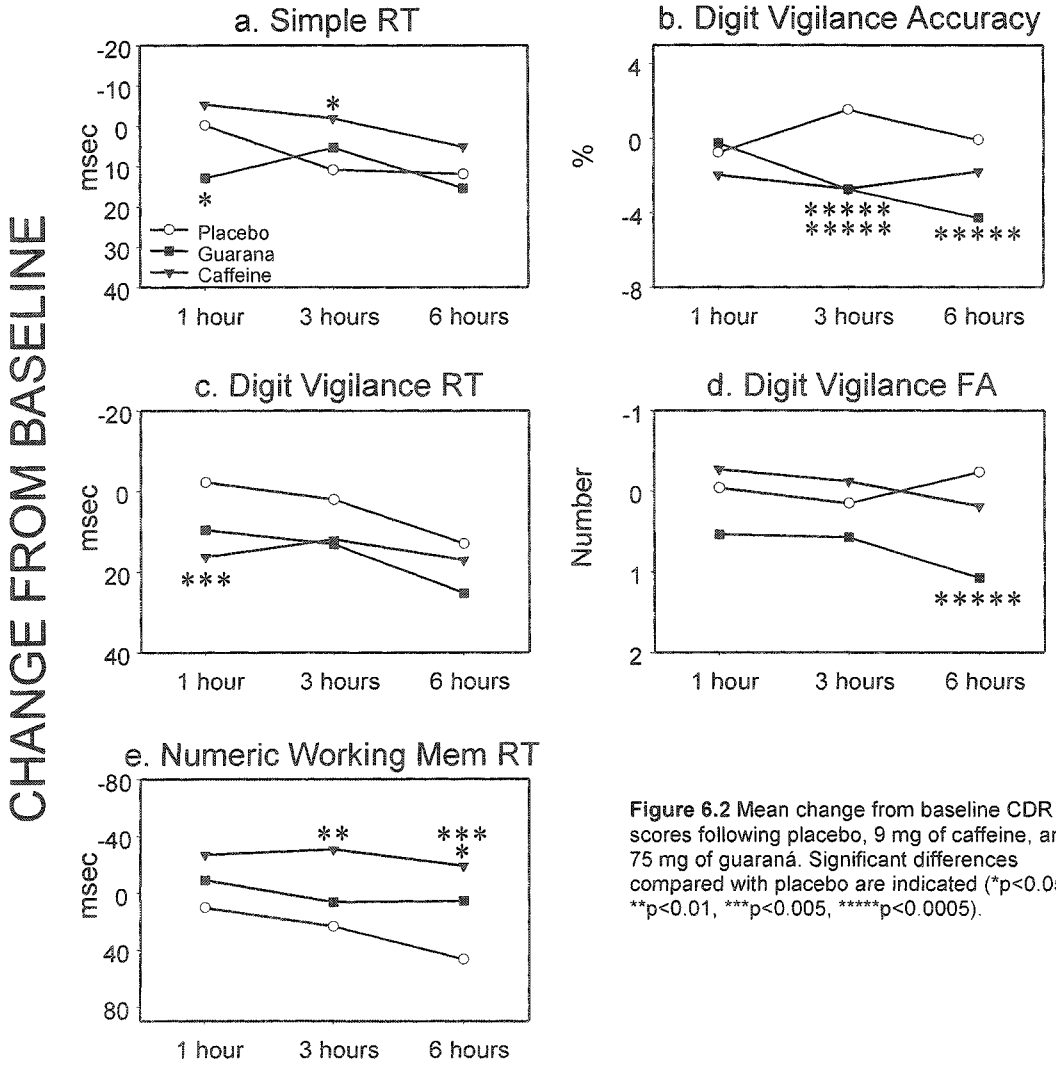

Figure 6.2 Mean change from baseline CDR scores following placebo, 9 mg of caffeine, and 75 mg of guaraná. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

6.3.3.3 Numeric working memory reaction time

Speed of numeric working memory was significantly improved following caffeine at 3 hrs [t(100)=2.77, p=0.007] and 6 hrs [t(100)=3.36, p=0.001], and at 6 hrs following guaraná [t(100)=2.10, p=0.04], see Fig 6.2e.

6.3.4 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 6.2 along with F values and probabilities for effects of treatment. Significant differences are presented in Figure 6.3. Only significant main effects and/or interactions for each outcome measure are reported below.

Table 6.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
			1 hour	3 hours	6 hours	
Sentence verification accuracy (%)	Placebo	94.7±1.66	0.00±1.18	-0.13±1.65	-1.92±1.06	F(2,100)<1
	75 mg guaraná	95.3±0.93	-1.67±0.98	-0.64±1.20	-1.92±0.91	
	9 mg caffeine	95.1±1.19	-0.38±0.85	-0.13±0.95	-0.77±1.00	
Sentence verification reaction time (ms)	Placebo	1431±95.2	-65.6±28.0	-87.7±31.3	-93.4±48.3	**F(2, 100) =4.63 p=0.01**
	75 mg guaraná	1435±156	-141±64.6	-92.4±56.4	-59.6±48.8	
	9 mg caffeine	1535±155	-180±59.2	-186±72.5	-187±58.4	
Serial threes subtraction correct (number)	Placebo	40.4±3.02	4.00±1.17	3.62±1.34	1.12±1.64	F(2, 100) =1.29 p>0.1
	75 mg guaraná	41.6±2.61	1.54±1.31	1.85±1.50	1.54±1.62	
	9 mg caffeine	41.2±2.94	1.96±1.32	2.12±0.92	0.19±1.25	
Serial threes subtraction errors (number)	Placebo	3.04±0.43	0.27±0.53	0.46±0.60	0.65±0.55	**F(2, 100) =3.45 p=0.03**
	75 mg guaraná	3.65±0.43	0.58±0.62	-0.69±0.48	-0.62±0.55	
	9 mg caffeine	2.54±0.39	0.92±0.51	0.88±0.57	0.85±0.50	
Serial sevens subtraction correct (number)	Placebo	26.5±2.66	-0.08±0.86	0.65±1.00	-0.15±1.00	F(2, 100) =2.82 p=0.06
	75 mg guaraná	26.2±2.59	0.81±0.93	1.96±0.95	0.73±1.17	
	9 mg caffeine	24.7±2.42	2.81±0.91	1.12±0.93	1.58±1.05	
Serial sevens subtraction errors (number)	Placebo	2.88±0.36	0.19±0.54	0.15±0.39	0.15±0.39	F(2, 100) =2.84 p=0.06
	75 mg guaraná	2.85±0.40	-0.08±0.30	0.58±0.44	0.65±0.45	
	9 mg caffeine	3.50±0.36	-0.50±0.33	-0.54±0.42	-0.19±0.58	

6.3.4.1 Sentence verification reaction time

Speed of sentence verification was significantly improved following guaraná at 1 hr [t(100)=2.00, p=0.048], and at 1 hr [t(100)=3.05, p=0.003], 3 hrs [t(100)=2.62, p=0.01] and 6 hrs [t(100)=2.50, p=0.01] following caffeine, see Fig 6.3a.

6.3.4.2 Serial threes subtractions errors

The number of errors on the serial threes subtraction task was significantly reduced following guaraná at 3 hrs [t(100)=2.22, p=0.03] and 6 hrs [t(100)=2.44, p=0.02], see Fig 6.3b.

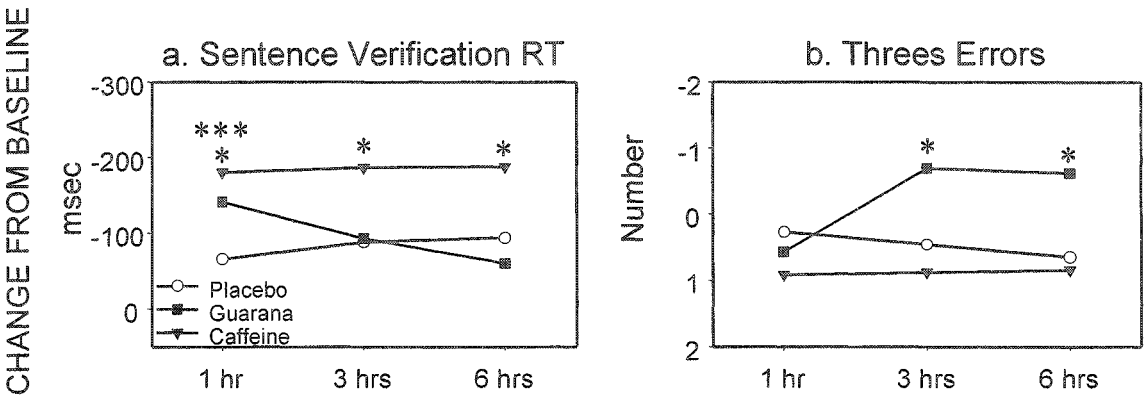

Figure 6.3 Mean change from baseline scores on cognitive measures following placebo, 9 mg of caffeine, and 75 mg of guaraná. Significant differences compared with placebo are indicated (*p<0.05, ***p<0.005).

6.3.5 Subjective mood measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 6.3 along with F values and probabilities for effects of treatment. Significant differences are presented in Figure 6.4. Only significant main effects and/or interactions for each outcome measure are reported below.

6.3.5.1 'Mental fatigue'

'Mental fatigue' was significantly reduced following both guaraná [t(100)=3.39, p=0.001] and caffeine [t(100)=2.68, p=0.009] at 6 hrs post-dose, see Fig 6.4.

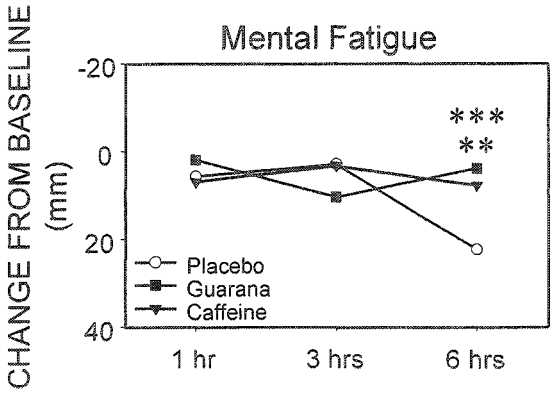

Figure 6.4 mean change from baseline mental fatigue ratings following placebo, 9 mg of caffeine, and 75 mg of guaraná. Significant differences compared with placebo are indicated (**p<0.01, ***p<0.005).

Table 6.3 Baseline and change from baseline scores for mood for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text).[x] denotes treatment x time interaction. Significant measures are shown in bold.

Measure		Treatment	Pre-dose baseline score	Post-dose change from baseline score			Treatment effect
				1 hour	3 hours	6 hours	
Bond-Lader factors	Alert (mm)	Placebo	57.9±3.68	-7.20±2.76	-5.60±3.36	-13.6±2.76	F(2, 100)
		75 mg guaraná	57.5±4.24	-4.07±3.07	-8.88±4.08	-7.03±4.70	=1.44
		9 mg caffeine	57.8±3.72	-4.19±3.36	-3.35±3.29	-6.37±4.15	p>0.1
	Content (mm)	Placebo	64.4±3.16	-3.81±1.60	-3.46±2.32	-10.1±2.56	F(2, 100)
		75 mg guaraná	66.5±3.05	-3.39±1.50	-5.76±2.92	-5.91±2.71	=0.13
		9 mg caffeine	66.2±3.28	-5.63±2.02	-4.55±2.43	-6.96±2.91	p>0.1
	Calm (mm)	Placebo	67.2±3.14	-4.65±2.36	-5.19±3.00	-1.31±2.72	F(2, 100)
		75 mg guaraná	67.2±3.10	-2.29±3.82	-3.63±2.62	-7.00±3.86	=1.52
		9 mg caffeine	64.7±3.59	-0.21±3.53	-1.06±3.03	0.12±2.93	p>0.1
Caffeine research visual analogue scales	Relaxed (mm)	Placebo	66.3±3.86	-1.00±3.70	-4.23±3.35	-3.77±4.06	
		75 mg guaraná	67.3±4.48	-7.04±3.93	-4.38±5.45	-7.42±5.43	F(2, 100)<1
		9 mg caffeine	69.5±4.04	-8.54±4.61	-3.85±3.39	-8.38±3.89	
	Alert (mm)	Placebo	59.2±4.50	-10.8±5.57	-5.96±5.49	-18.7±5.52	F(2, 100)
		75 mg guaraná	57.7±5.26	-3.54±5.51	-9.62±5.73	-2.96±6.48	=2.71
		9 mg caffeine	55.7±5.05	2.92±4.19	-3.12±5.41	-7.88±5.83	p=0.07
	Jittery (mm)	Placebo	22.1±4.25	0.27±2.80	-0.65±4.35	3.77±3.46	
		75 mg guaraná	17.2±3.83	2.31±3.23	3.73±3.30	3.54±3.60	F(2, 100)<1
		9 mg caffeine	21.2±4.26	4.85±6.19	-0.58±4.86	-0.38±3.77	
	Tired (mm)	Placebo	38.8±5.89	-2.77±3.84	4.92±5.39	19.9±7.08	F(2, 100)
		75 mg guaraná	36.7±5.78	7.15±5.74	14.6±6.94	15.9±7.25	=1.05
		9 mg caffeine	38.4±5.73	3.08±6.06	0.54±6.20	15.3±7.81	p>0.1
	Tense (mm)	Placebo	29.6±5.03	-4.23±4.58	-6.73±5.77	-1.69±5.03	F(2, 100)
		75 mg guaraná	21.4±4.31	5.27±5.09	-0.12±4.24	5.58±3.97	=2.88
		9 mg caffeine	22.7±4.59	2.88±4.60	2.23±4.00	0.85±4.07	p=0.06
	Headache (mm)	Placebo	11.6±3.33	-0.54±3.15	-0.42±3.54	11.0±5.85	
		75 mg guaraná	13.2±4.68	4.42±2.96	4.42±4.01	4.12±4.92	F(2, 100)<1
		9 mg caffeine	16.4±4.74	-2.85±4.15	1.42±5.02	5.69±5.94	
	Overall (mm)	Placebo	65.5±4.23	-0.19±2.44	2.42±3.62	-7.35±3.43	F(2, 100)
		75 mg guaraná	72.3±3.62	-5.23±2.90	-7.92±3.59	-6.12±3.16	=3.04
		9 mg caffeine	63.9±4.45	-3.77±3.51	2.77±4.53	0.35±4.77	p=0.05
	Mental Fatigue (mm)[x]	Placebo	28.7±4.75	5.62±3.78	2.92±5.07	22.4±5.39	F(2, 100)
		75 mg guaraná	33.7±4.84	1.88±4.07	10.4±4.92	3.92±6.58	=1.21
		9 mg caffeine	31.7±4.81	6.85±4.56	3.23±3.11	7.81±4.48	p>0.1
Caffeine research visual analogue scales factors	Alertness (mm)	Placebo	60.2±4.56	-4.02±3.92	-5.44±4.70	-19.3±5.17	F(2, 100)
		75 mg guaraná	60.5±5.17	-5.35±5.17	-12.1±5.68	-9.44±6.40	=1.06
		9 mg caffeine	58.6±4.98	-0.08±4.53	-1.83±5.03	-11.6±6.28	p>0.1
	Tension (mm)	Placebo	31.7±3.71	-1.62±3.01	-1.25±3.92	1.04±3.80	F(2, 100)
		75 mg guaraná	27.1±4.11	6.15±3.87	2.13±4.47	6.50±4.00	=2.44
		9 mg caffeine	26.6±3.98	5.71±4.04	3.04±3.23	4.62±2.96	p=0.09

6.4 Discussion

The results of the current study showed that 75 mg of guaraná and a matched caffeine dose are both capable of improving cognitive performance in comparison to placebo in healthy young participants, although some impairment was also noted with each active treatment.

The effects of 75 mg of guaraná partly replicated the findings from previous chapters. Performance of serial threes subtractions was improved, here in terms of a reduction in errors. Previously observed improvement has been seen on this measure in terms of increased correct responses (Chapter 4 and Chapter 5), and a reduction in errors (Chapter 5). Previously observed speed of sentence verification task performance (Chapter 5) was also demonstrated here, albeit at a single time point. Increases in subjective ratings relating to 'arousal' were demonstrated in earlier chapters in terms of increased 'alert' ratings (Chapter 4) and decreased 'tired' ratings (Chapter 5). In the current study these effects were seen in the shape of an amelioration of 'mental fatigue' but this effect was only apparent at 6 hours post-treatment.

Impairment to simple reaction time and accuracy of digit vigilance (percent accuracy and false alarms) evinced here following guaraná does not support the findings from previous chapters (in fact an improvement to speed of digit vigilance was reported in Chapter 5). Additionally, improvement relating to tasks measuring secondary memory, which showed a robust effect in Chapters 4 and 5, is not replicated here. Inconsistencies in the effects on simple reaction time, reaction time of secondary memory tasks and accuracy of digit vigilance (%) may be explained by spurious placebo effects. Comparing mean data on these measures, as shown in Tables 4.1 (pages 113 - 114), 5.1 (pages 129 - 130), and 6.1 (page 148), reveals that the effects of 75 mg of guaraná are remarkably similar across studies. However, in the case of the current study, there are quite marked and unexpected improvements following placebo, as compared to placebo data in Chapters 4 and 5.

The effects of caffeine at this dose have not previously been explored and, as such, this represents the first demonstration of effects of such a low dose. However, the effects presented are very similar to those demonstrated in Chapters 2 and 3. Improvements to simple reaction time evinced following 9 mg were also produced in Chapter 2 following 75 mg of caffeine, and in Chapter 3 following 150 mg. These findings also replicate Smit and Rogers (2000) who found improvements to simple reaction time following 12.5 mg of caffeine that were comparable to those of 50 mg and 100 mg. Improvements to speed of numeric working memory seen here also replicate those found following 150 mg in Chapters 2 and 3. In terms of the effects on sentence

verification, improvements to speed were demonstrated in the current study, whereas in Chapter 2 improvements to accuracy on this task were shown following both 75 mg and 150 mg of caffeine. However, improvements to speed of digit vigilance found in Chapters 2 and 3 were not replicated here. In fact, impairment was seen on this measure at 1 hour post-treatment and accuracy of this task was also impaired at 3 hours post-treatment. This again can be explained by the lack of decline in placebo performance ordinarily seen during multiple completions of elements of the CDR test battery. This effect also has some bearing on the finding of improved simple reaction time at only a single time point following caffeine and on the lack of alerting mood effects following caffeine. As with guaraná, the alerting effects previously seen with caffeine (increased 'alertness' and decreased 'mental fatigue' - Chapters 2 and 3) are not apparent here with the exception of an amelioration of 'mental fatigue' at 6 hours.

In terms of the time course of the behavioural effects of caffeine, these have not previously been explored beyond 3 hours after treatment. A number of effects were apparent at 6 hours in the current study. In the case of cognitive improvements to speed of numeric working memory and speed of sentence verification, these effects were evident earlier in the day and remained until the final testing session. However, in the case of amelioration of 'mental fatigue', this effect only became apparent at 6 hours suggesting that the effects of caffeine apparent at this time are the result of an offsetting of decline. In fact, looking at figures 6.2 – 6.4 (pages 149 and 151), it can be seen that the effects of caffeine remain fairly constant throughout the day, as shown by the relative flatness of the caffeine curve on each measure.

The results covered so far suggest that, contrary to the conclusions of previous chapters, caffeine is indeed capable of producing cognitive and mood benefits at the dose of 9 mg, and that some of these effects are still apparent at 6 hours after administration. Turning to the tasks affected by the two treatments, aside from digit vigilance performance, which may be influenced by anomalous placebo data, there were some similarities in the tasks affected. Both treatments improved speed of numeric working memory and speed of sentence verification. However, in the case of guaraná, these effects were only apparent at one time point, whereas the effects were more robust following caffeine. Additionally, both treatments attenuated decrements in 'mental fatigue' seen at 6 hours. Some differences also exist in the tasks affected, with differential effects in favour of caffeine on speed of simple reaction and a significant improvement to serial threes following guaraná, which was not evident following caffeine.

Taken together these results suggest that, although caffeine is capable of producing effects at the level present in 75 mg of guaraná (9 mg), and despite some of

these effects being observed at 6 hours post-treatment, differences in the tasks affected or in the manner of the effects suggest that the effects of guaraná should not solely be attributed to caffeine. In terms of the measures affected by both treatments, the size of effects favours caffeine and simple reaction time is improved by caffeine but not guaraná, but the opposite is true for serial threes subtractions. Overall the results suggest that, rather than any synergy between caffeine and other components within guaraná, these effects may reflect an attenuation of the effects of caffeine by other components. The salivary caffeine data shown in Fig 6.1 support this contention, given that 9 mg of caffeine significantly increased salivary caffeine levels, whereas guaraná containing the same amount of caffeine did not. It is also possible that the effects of guaraná are related to a separate underlying mechanism independent of caffeine. The finding of improvements to serial threes, which is not evident with caffeine, supports this notion. However, it is unlikely that what has now been shown to be a psychoactive level of caffeine is not having any effects, and it is more likely that caffeine and at least one other component within guaraná are exerting effects, which lead to an attenuation of the effects of caffeine on a number of measures but also has the potential for potentiation on other tasks.

CHAPTER 7. A LOW-DOSE-RANGING STUDY OF THE BEHAVIOURAL EFFECTS OF CAFFEINE

7.1 Introduction

The behavioural effects of caffeine have been investigated for more than a century. Recent research in this area has examined the effects of caffeine loads of 250 – 300 mg, with some as high as 500 mg (e.g. Kaplan et al. 1997). Although of interest, these studies are not reflective of normal acute consumption levels of caffeine and, in line with this, the majority of studies now evaluate the effects of lower doses, typically in the range of 75 – 150 mg or 1 – 2 mg/kg (equivalent to one to two teaspoons of instant coffee respectively). Such doses produce fairly well characterised effects, including increased 'alertness' (Quinlan et al. 2000; Rogers et al. 2003) and improvements to measures of reaction time and sustained attention (Richardson et al. 1995; Yeomans et al. 2002). These same effects were also demonstrated in response to a 75 and 150 mg of caffeine challenge in non-consumers in Chapter 2 and in Chapter 3 in response to 150 mg. However, these levels are at the upper end of typical caffeine doses found in a single serving of dietary caffeine, and only a few studies have considered the effects of acute doses lower than 75 mg, these are reviewed briefly below.

In the first of two experiments Quinlan et al. (2000) compared the effects of caffeinated beverages presented as one or two cups of tea (containing 37.5 or 75 mg of caffeine respectively); or one or two cup of coffee (75 and 150 mg) with hot water and a no drink control. Significantly decreased heart rate and increased systolic and diastolic blood pressure were observed in response to caffeine. Caffeine also significantly increased ratings of 'energetic arousal' and 'hedonic tone', whilst decreasing 'sedation'. There were no differential dose effects on any measure. In a second experiment 25, 50, 100, and 200 mg of caffeine were found to significantly decrease heart rate in an inverse dose-dependent fashion. Systolic and diastolic blood pressure was again increased but in this case ≥100 mg was required to increase systolic pressure. Ratings of 'energetic arousal' were also increased with a U-shaped dose-response curve and 'sedation' ratings were decreased by all except 50 mg of caffeine. These findings suggest that, at least for some measures, doses as low as 25 mg of caffeine are as effective as higher doses with intermediate doses having no effect.

The effects of lower doses of caffeine have also been demonstrated in non-habitual or low caffeine consumers. Smith et al. (1999b) examined the effects of 40 mg

of caffeine in a variety of beverages (tea; coffee; cola; diet cola; water; sparkling water) compared with decaffeinated equivalents. Caffeine improved attentional and mnemonic function and increased 'alertness' and 'anxiety' in low and high habitual caffeine consumers alike. Lieberman et al. (1987) also reported comparable effects on a visual reaction time task following 32, 128, and 256 mg of caffeine with lesser effects following 64 mg. All doses also improved speed of four-choice auditory reaction time, this time with comparable effects following 32 and 128 mg and greater effects following 64 and 256 mg. All of these effects were independent of consumer status.

The lowest reported psychoactive dose of caffeine in humans is 12.5 mg (Smit and Rogers 2000), which, as measured by speeded simple reaction time, was equipotent to doses of 50 and 100 mg of caffeine in both high (>200 mg/day) and low (<100 mg/day) regular caffeine consumers. Interestingly, this effect was not apparent following 25 mg of caffeine. In the same study improvements were seen on a vigilance working memory task following 12.5, 25, 50, and 100 mg of caffeine but only in regular high caffeine consumers. Additionally, an increase in 'energetic arousal' was reported, for 100 mg only. Thus the lowest and highest doses from Lieberman et al. (1987) and Smit and Rogers (2000) showed comparable effects on two measures, whereas a reduced or lack of effect was seen with an intermediate dose. The study presented in Chapter 6 demonstrated improvements to performance following 9 mg caffeine, which represents the lowest known psychoactive dose of caffeine. However, despite extensive research in this area, the lower threshold for psychoactive effects has not been established.

As well as limited evidence with regards the lowest dose of caffeine capable of engendering psychoactive effects, very little is known about the time course for any effects of caffeine. Peak salivary levels of caffeine are reached around 40 minutes post-ingestion (Liguori et al. 1997) and, as such, studies of acute caffeine ingestion have focused on the effects 30 to 120 minutes after administration. However, given that the half-life of caffeine is around 5 hours (Blanchard and Sawers 1983), it is clear that the possibility exists of psychoactive effects beyond 2 hours post-ingestion. This is borne out by the results from the previous chapter, which showed reliable effects in terms of improved attentional processing, reduced 'mental fatigue', and elevated salivary caffeine levels following 9 mg of caffeine, that were still apparent at 6 hours post-consumption.

The current study, therefore, examined the behavioural effects of low doses of caffeine (2.5, 5, and 10 mg). Despite a lack of any empirical evidence, doses such as these have been assumed to be below the level required for psychoactive effects. In order to further explore the pharmacokinetics of such doses, the current study also

explored the time-dependent effects of caffeine pre-dose and at 1 hour, 3 hours, 6 hours, and 9 hours thereafter.

7.2 Materials and Methods

7.2.1 Design

A randomised, placebo-controlled, double-blind, balanced-crossover design was employed.

7.2.2 Initial screening

Prior to participation in the study, volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session of the morning and throughout the testing session. In order to aid compliance to caffeine abstinence instructions, participants were provided with a list of common caffeine-containing products (see Appendix IV).

7.2.3 Participants

Twenty undergraduate volunteers (7 male and 13 female, mean age 20.7 years, SEM 0.53, range 18 - 26) took part in the study. Assessment of habitual caffeine consumption (using the caffeine consumption questionnaire employed in Chapter 2, see Appendix I) showed that 10 of these were habitual caffeine consumers (> 50 mg per day). Participants abstained from caffeine and alcohol for a minimum of 12 hours prior to the first testing session and throughout the day until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

7.2.4 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence and immediately prior to all post-treatment assessments to confirm effective caffeine absorption. The saliva samples were immediately frozen at -20 °C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure

caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

7.2.5 Assessment

The tasks employed were identical to those described in Chapter 3.

7.2.6 Autonomic measures

Heart rate and blood pressure were monitored using the Boso medicus prestige (Bosch and Sohn, Jungingen, Germany). This is a fully automatic upper arm monitor that measures heart rate (bpm) and systolic and diastolic blood pressure (mmHg).

7.2.7 Treatments

Participants received four drinks containing 0 mg (placebo), 2.5 mg, 5 mg, and 10 mg of caffeine hydrochloride BP (Merck, Darmstadt, Germany) on separate occasions. In each case the caffeine was presented in a 150 ml drink containing 30 ml of Robinsons Special R Apple and Blackcurrant Juice Drink with no added sugar (Robinsons Soft Drinks Ltd, Chelmsford, UK). Five minutes was allowed for drink consumption. All treatments were identical in appearance and scent.

7.2.8 Procedure

Each participant was required to attend a total of five study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the four active study days comprised five identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, and was immediately followed by the day's treatment. Further testing sessions began at 1 hour, 3 hours, 6 hours, and 9 hours following consumption of the day's treatment. Each testing session lasted approximately 35 minutes and comprised

producing a saliva sample, completion of the CDR test battery, Bond-Lader mood scales, a sentence verification task, serial subtractions (threes and sevens) and caffeine research visual analogue mood scales, followed by heart rate and blood pressure monitoring.

7.2.9 Statistics

Salivary caffeine levels were analysed to assess compliance to caffeine abstinence and effective caffeine absorption.

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab.

The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising t tests with MSError from an omnibus ANOVA as an error term (Keppel 1991). At each time point (1, 3, 6, and 9 hours post-treatment) data from the placebo condition was compared to that for each of the three active treatments (2.5, 5, 10 mg of caffeine). Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for treatment, assessment, treatment x assessment and participant (Kirk 1968), was carried out to identify main effects and interaction effects on change from baseline data for each measure. Where baseline differences were observed baseline scores were entered as a covariate in an ANCOVA, with terms fitted to the model for treatment, assessment, treatment x assessment and participant and this was analysed using SAS/STAT. To ensure the overall Type I error protection level only those planned comparisons associated with measures that generated a significant main effect or interaction effect ($p<0.05$) on this initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated. Due to the number of comparisons, effects on measures that generated only a single significant result were interpreted with caution.

7.3 Results

7.3.1 Salivary caffeine levels

Salivary analysis revealed that all participants had complied with instructions to avoid caffeine overnight. Mean baseline values were 0.02 μg/ml (SD = 0.13). Data from 6 time points were unusable and were excluded from the analyses. Analysis of post-treatment salivary caffeine levels revealed significantly higher salivary caffeine levels at 1 hr post-treatment following 10 mg of caffeine [t(117)=3.68, p=0.0004] see Fig 7.1.

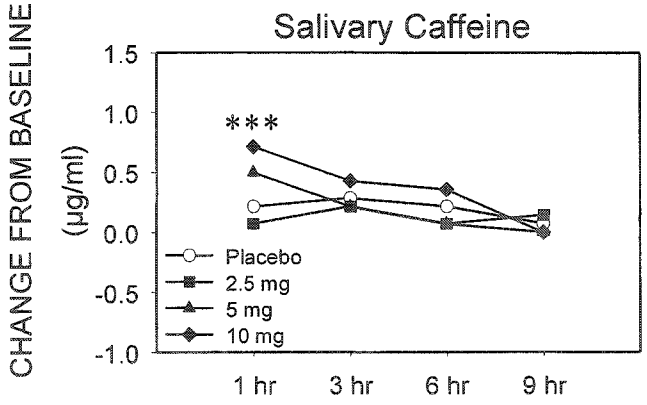

Figure 7.1 Mean change from baseline salivary caffeine levels following placebo, 2.5 mg of caffeine, 5 mg of caffeine, and 10 mg of caffeine. Significant treatment effects compared with placebo are indicated (***p<0.005).

7.3.2 Baseline scores

Prior to analysis of change from baseline data, mean pre-dose baseline scores for all four treatments (placebo, 2.5 mg of caffeine, 5 mg of caffeine, 10 mg of caffeine) for each outcome (individual CDR task scores, sentence verification scores, serial subtraction scores and mood scale scores) were subjected to a one-way, repeated-measures ANOVA that revealed significant differences in speed of numeric working memory [t(57)=5.49, p=0.002] and serial threes errors [t(57)=4.42, p=0.007].

7.3.3 CDR assessment battery

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 7.1 along with F values and probabilities for effects of treatment. Only significant main effects for each outcome measure are reported below. Significant differences on cognitive measures are presented in Figure 7.2.

Table 7.1 Baseline and change from baseline scores for each measure from the CDR battery for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
			1 hour	3 hours	6 hours	9 hours	
Immediate word recall accuracy (%)	Placebo	52.7±3.92	-10.8±3.58	-5.83±3.73	-6.83±3.18	-11.5±2.61	F(3, 171) =2.30 p=0.08
	2.5 mg	51.0±4.42	-1.67±3.16	-1.83±4.09	-6.33±4.06	-7.50±4.26	
	5 mg	54.8±3.70	-8.17±3.24	-9.83±3.92	-9.50±3.54	-11.3±4.17	
	10 mg	52.0±3.32	-5.17±3.05	-8.00±2.65	-3.17±2.86	-9.50±3.76	
Simple reaction time (ms)	Placebo	287±7.12	1.41±6.29	2.46±5.00	7.37±6.51	9.32±5.83	F(3, 171) =1.87 p>0.1
	2.5 mg	278±8.15	13.4±5.43	14.7±3.70	17.4±6.11	18.2±5.52	
	5 mg	280±7.51	-0.47±8.22	0.97±5.54	7.46±13.4	0.57±7.87	
	10 mg	284±6.77	-1.61±6.66	3.99±7.21	-1.91±5.36	46.9±29.2	
Digit vigilance accuracy (%)	Placebo	94.5±1.16	-0.55±1.58	-3.22±1.70	-3.11±1.61	-0.78±1.57	F(3, 171) =1.50 p>0.1
	2.5 mg	94.8±0.89	-2.22±1.19	-2.00±1.18	-4.33±1.62	-4.00±1.94	
	5 mg	95.3±0.84	-1.44±1.23	-2.00±1.45	-1.89±1.56	-0.78±1.35	
	10 mg	96.0±1.36	-2.89±1.44	-2.33±1.84	-2.78±1.32	-4.78±1.84	
Digit vigilance reaction time (ms)	Placebo	431±11.9	8.48±5.13	19.2±8.36	18.5±7.52	28.5±10.3	F(3, 171) =1.45 p>0.1
	2.5 mg	436±10.6	9.31±5.88	6.00±7.00	13.4±8.46	16.3±7.47	
	5 mg	421±9.15	10.9±5.17	8.80±6.50	17.9±7.92	21.4±7.34	
	10 mg	437±11.6	1.57±9.08	13.4±8.62	11.4±7.37	8.84±8.27	
Digit vigilance false alarms (number)	Placebo	1.00±0.21	0.25±0.40	0.15±0.37	0.05±0.33	0.15±0.26	**F(3, 171) =4.86 p=0.003**
	2.5 mg	1.30±0.29	-0.35±0.29	0.40±0.36	0.55±0.29	0.50±0.34	
	5 mg	1.20±0.30	-0.35±0.35	-0.10±0.28	-0.45±0.32	0.15±0.38	
	10 mg	0.85±0.23	0.60±0.28	0.70±0.44	0.00±0.27	0.40±0.29	
Choice reaction time accuracy (%)	Placebo	94.4±0.95	-0.20±0.63	0.10±0.67	0.20±0.71	0.70±1.02	F(3, 171) =1.04 p>0.1
	2.5 mg	94.6±0.84	-0.10±1.12	0.40±0.89	-0.10±0.75	0.70±0.80	
	5 mg	95.3±0.82	0.40±0.82	-0.60±0.70	-1.10±0.88	-0.20±0.86	
	10 mg	95.5±0.65	-0.20±0.72	0.00±0.81	-0.60±0.99	-1.70±0.72	
Choice reaction time (ms)	Placebo	422±10.8	13.0±6.82	18.8±10.7	10.1±5.29	31.2±10.8	F(3, 171) =1.80 p>0.1
	2.5 mg	417±9.18	9.66±8.29	8.75±7.09	30.8±13.0	10.3±6.86	
	5 mg	425±15.0	-2.22±6.35	14.9±9.22	8.01±9.12	-3.73±8.72	
	10 mg	426±9.88	7.95±7.78	18.8±16.1	10.0±10.5	11.8±8.16	
RVIP accuracy (%)	Placebo	60.2±4.84	-2.19±2.13	-1.72±2.82	-4.69±2.54	-3.44±2.60	F(3, 171) =2.25 p=0.08
	2.5 mg	60.2±4.54	3.28±2.07	-0.78±2.24	-4.38±2.83	-4.37±2.37	
	5 mg	61.6±3.87	1.09±2.35	-1.10±2.28	-5.78±2.81	0.63±3.01	
	10 mg	55.8±4.98	4.69±2.77	3.44±3.01	-1.56±2.98	-1.41±3.02	
RVIP reaction time (ms)	Placebo	521±24.8	-18.1±12.6	4.81±15.3	13.5±17.6	-10.1±19.8	F(3, 171)<1
	2.5 mg	520±22.8	-19.6±18.0	-11.1±18.1	-24.5±18.8	-4.15±17.7	
	5 mg	518±18.1	-4.97±15.4	9.59±16.4	-2.71±17.9	-10.2±15.7	
	10 mg	527±21.9	-21.3±24.9	-7.53±21.7	12.4±14.6	-11.2±22.6	
RVIP false alarms (number)	Placebo	2.10±0.56	-0.10±0.41	-0.50±0.42	-0.20±0.55	-0.30±0.36	**F(3, 171) =4.58 p=0.004**
	2.5 mg	1.40±0.56	0.70±0.33	0.85±0.45	0.45±0.28	0.65±0.65	
	5 mg	2.30±0.66	0.00±0.44	0.10±0.50	-0.65±0.51	-0.85±0.65	
	10 mg	2.05±0.84	-0.45±0.49	-0.50±0.29	0.30±0.32	0.80±0.45	
Spatial memory (sensitivity index)	Placebo	0.92±0.02	-0.03±0.02	-0.10±0.04	-0.03±0.02	-0.03±0.02	F(3, 171)<1
	2.5 mg	0.91±0.02	-0.05±0.04	-0.05±0.02	-0.01±0.02	-0.03±0.03	
	5 mg	0.93±0.01	-0.03±0.03	-0.07±0.02	-0.04±0.03	-0.02±0.02	
	10 mg	0.89±0.02	-0.08±0.05	-0.02±0.03	-0.10±0.06	-0.01±0.03	
Spatial memory reaction time (ms)	Placebo	534±17.0	-18.6±11.1	-12.4±16.3	3.07±22.6	-44.9±13.4	F(3, 171) =2.04 p>0.1
	2.5 mg	533±19.0	-8.02±11.4	2.28±19.6	6.50±18.5	-43.2±12.6	
	5 mg	554±22.1	-27.9±14.8	-20.0±16.9	-26.2±23.9	-63.8±16.0	
	10 mg	552±22.7	-11.6±18.8	13.1±20.0	39.9±42.4	-53.2±22.0	
Logical reasoning (%)	Placebo	86.7±3.11	-1.25±1.69	-1.25±2.03	-0.21±1.99	-0.83±1.93	F(3, 171) =1.78 p>0.1
	2.5 mg	89.0±2.90	-2.08±2.02	-2.29±2.70	-1.46±1.92	-5.63±2.76	
	5 mg	88.5±2.55	-2.08±2.02	-1.67±2.06	-0.21±2.14	-0.62±1.58	
	10 mg	87.5±2.65	-0.42±1.35	1.25±1.42	0.00±2.05	-0.42±2.72	
Logical reasoning reaction time (ms)	Placebo	2499±168	-191±90.0	50.2±133	-97.1±140	-347±108	**F(3, 171) =6.21 p<0.001**
	2.5 mg	2255±161	-6.47±83.0	7.32±101	91.1±100	-21.3±134	
	5 mg	2520±212	-101±78.8	-70.9±67.0	-6.83±138	-222±115	
	10 mg	2756±176	-163±135	-355±136	-198±172	-474±146	
Numeric working memory (sensitivity index)	Placebo	0.90±0.02	-0.04±0.01	-0.04±0.02	-0.03±0.02	-0.06±0.03	**F(3, 171) =3.71 p=0.01**
	2.5 mg	0.89±0.02	-0.07±0.03	-0.04±0.02	-0.08±0.03	-0.03±0.02	
	5 mg	0.90±0.01	-0.03±0.01	-0.04±0.02	-0.05±0.02	-0.03±0.02	
	10 mg	0.88±0.03	0.01±0.03	-0.04±0.03	0.01±0.02	-0.01±0.03	
Numeric working memory reaction time (ms)	Placebo	570±21.2	-10.3±13.4	-0.03±13.6	-0.57±15.7	-7.80±16.8	**F(3, 171) =9.47 p<0.001**
	2.5 mg	550±17.1	5.62±11.8	5.17±11.4	26.3±18.9	1.19±9.98	
	5 mg	548±20.4	18.8±11.6	28.9±17.0	24.9±19.8	4.35±14.9	
	10 mg	620±30.7	-42.9±24.2	-17.1±16.1	-28.5±26.2	-59.0±24.5	
Delayed word recall accuracy (%)	Placebo	37.3±4.32	-10.2±3.77	-14.2±4.21	-16.5±3.45	-23.2±4.34	**F(3, 171) =6.33 p<0.001**
	2.5 mg	32.7±3.65	-8.50±3.22	-10.3±3.04	-14.2±2.80	-13.3±3.51	
	5 mg	40.2±4.03	-18.2±3.75	-22.5±4.46	-23.0±3.89	-23.0±4.83	
	10 mg	37.5±2.94	-16.0±3.11	-18.8±2.76	-19.8±3.37	-15.8±3.53	

Delayed word recognition (sensitivity index)	Placebo	0.57±0.06	-0.06±0.05	-0.09±0.05	-0.12±0.04	-0.12±0.05	F(3, 171)<1
	2.5 mg	0.63±0.04	-0.10±0.05	-0.08±0.05	-0.13±0.04	-0.16±0.04	
	5 mg	0.62±0.04	-0.10±0.04	-0.13±0.04	-0.14±0.05	-0.19±0.06	
	10 mg	0.63±0.05	-0.07±0.05	-0.13±0.05	-0.15±0.04	-0.16±0.04	
Delayed word recognition reaction time (ms)	Placebo	688±35.2	8.41±24.0	13.4±40.0	-11.8±32.5	17.8±42.7	**F(3, 171) =6.73 p<0.001**
	2.5 mg	675±24.2	51.3±21.3	46.8±26.3	22.8±33.2	8.74±20.9	
	5 mg	657±29.5	103±29.9	71.6±35.8	64.4±31.4	11.6±21.1	
	10 mg	726±30.1	-37.0±23.2	-14.4±27.3	-16.7±28.1	-11.3±37.6	
Delayed picture recognition (sensitivity index)	Placebo	0.67±0.06	-0.07±0.04	-0.08±0.06	-0.08±0.06	-0.08±0.06	F(3, 171) =1.57 p>0.1
	2.5 mg	0.66±0.05	-0.06±0.05	-0.07±0.05	-0.08±0.05	-0.09±0.05	
	5 mg	0.62±0.05	0.00±0.05	-0.03±0.04	-0.03±0.05	-0.03±0.05	
	10 mg	0.69±0.04	-0.12±0.04	-0.13±0.04	-0.05±0.05	-0.07±0.04	
Delayed picture recognition reaction time (ms)	Placebo	831±46.9	-39.9±45.9	-59.3±46.5	-52.9±41.8	-80.9±52.3	**F(3, 171) =5.59 p=0.001**
	2.5 mg	778±29.3	16.6±33.7	10.3±22.6	-9.77±27.6	-4.16±36.7	
	5 mg	756±27.9	34.0±24.7	36.6±23.3	47.0±37.2	-14.2±25.4	
	10 mg	799±31.4	-13.01±30.5	-18.3±39.1	18.3±35.9	-44.0±35.7	

7.3.3.1 Digit vigilance false alarms

Planned comparisons revealed that the significant main effect of treatment on digit vigilance false alarms was not in relation to active treatments versus placebo.

7.3.3.1.2 Rapid Visual Information Processing (RVIP) false alarms

The number of false alarms on the RVIP task were significantly increased at 3 hrs [t(171)=3.09, p=0.002] and 9 hrs [t(171)=2.17, p=0.03] following 2.5 mg and at 9 hrs following 10 mg [t(171)=2.52, p=0.01], see Fig 7.2a.

7.3.3.3 Logical reasoning reaction time

Speed of logical reasoning was significantly improved following 10 mg at 3 hrs [t(171)=3.70, p=0.0003] but significantly impaired following 2.5 mg at 9 hrs [t(171)=2.98, p=0.002], see Fig 7.2b.

7.3.3.4 Numeric working memory accuracy

10 mg of caffeine significantly improved accuracy of numeric working memory at 1 hr [t(171)=319, p=0.03] and 9 hrs [t(171)=2.27, p=0.02] post-treatment but 2.5 mg significantly impaired it at 6 hrs [t(171)=2.10, p=0.04], see Fig 7.2c.

7.3.3.5 Numeric working memory reaction time

The effect on numeric working memory reaction time was no longer apparent when baseline scores were entered as a covariate.

7.3.3.6 Delayed word recall

Accuracy of delayed word recall was significantly impaired following 5 mg of caffeine at 1 hr [t(171)=2.03, p=0.04] and 3 hrs [t(171)=2.12, p=0.04] but significantly improved following 2.5 mg at 9 hrs [t(171)=2.50, p=0.01], see Fig 7.2d.

7.3.3.7 Delayed word recognition reaction time

Speed of word recognition was significantly impaired following 5 mg of caffeine at 1 hr [t(171)=3.13, p=0.002] and 6 hrs [t(171)=2.51, p=0.01], see Fig 7.2e.

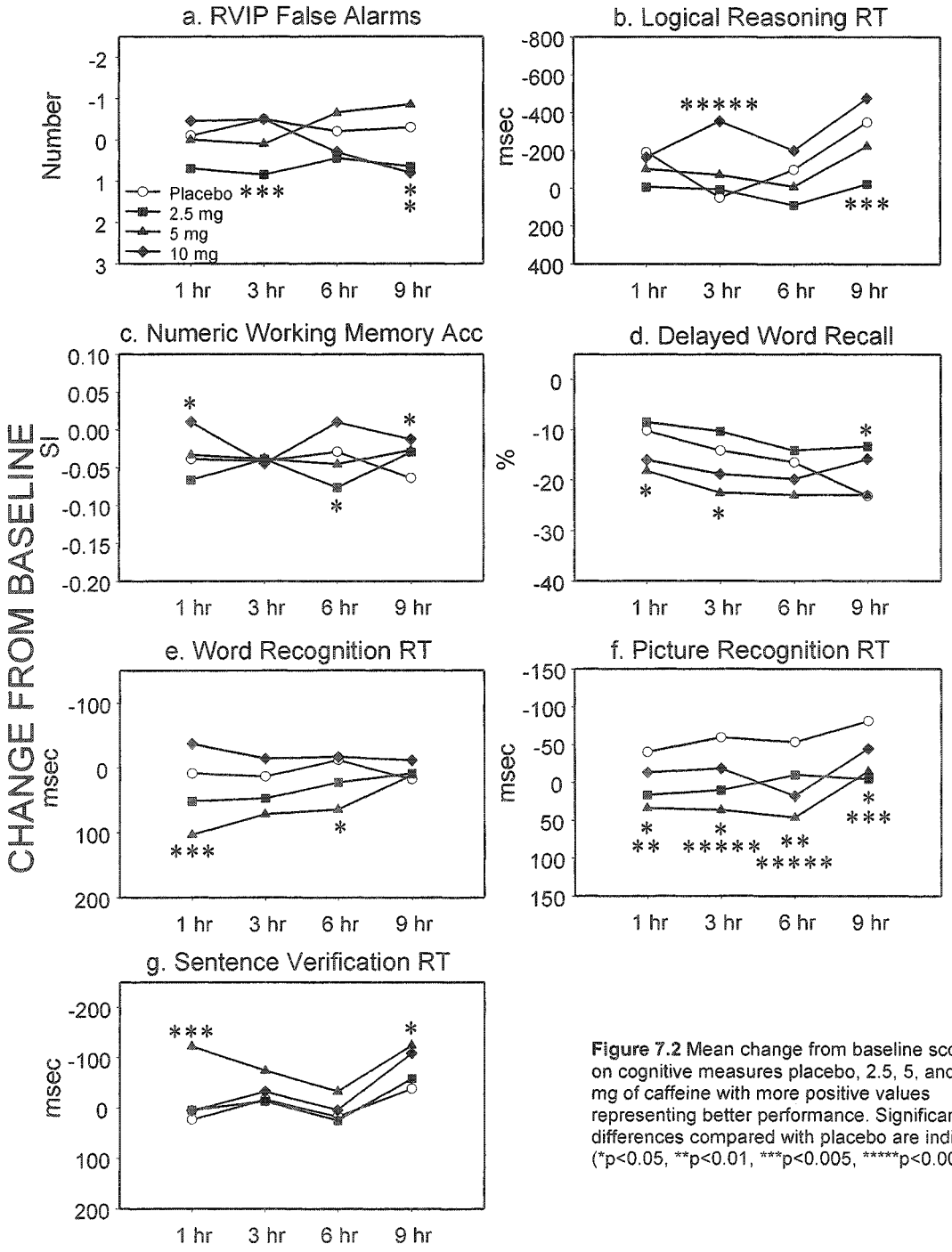

Figure 7.2 Mean change from baseline scores on cognitive measures placebo, 2.5, 5, and 10 mg of caffeine with more positive values representing better performance. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

7.3.3.8 Delayed picture recognition reaction time

Speed of picture recognition was significantly impaired following all doses of caffeine: at 1 hr [t(171)=2.10, p=0.04], 3 hrs [t(171)=2.59, p=0.01], and 9 hrs [t(171)=2.85, p=0.0048] following 2.5 mg. At 1 hr [t(171)=2.75, p=0.007], 3 hrs [t(171)=3.57, p=0.00047], 6 hrs [t(171)=3.72, p=0.0003], and 9 hrs [t(171)=2.48, p=0.01] following 5 mg, and at 6 hrs following 10 mg [t(171)=2.65, p=0.009], see Fig 7.2f.

7.3.4 Other cognitive measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 7.2 along with F values and probabilities for effects of treatment. Significant differences are presented in Figure 7.2. Only significant main effects and/or interactions for each outcome measure are reported below.

7.3.4.1 Sentence verification reaction time

Sentence verification reaction time was significantly improved 1 hr [t(171)=3.36, p=0.001], and 9 hrs [t(171)=1.99, p=0.048] following 5 mg, see Fig 7.2g.

7.3.4.2 Serial threes subtraction task

The effect on number of errors on the serial threes subtraction task was no longer apparent when baseline scores were entered as a covariate.

Table 7.2 Baseline and change from baseline scores for sentence verification and serial subtractions for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
			1 hour	3 hours	6 hours	9 hours	
Sentence verification accuracy (%)	Placebo	94.8±1.46	-2.17±1.44	-1.00±1.63	-2.00±1.36	-1.67±1.20	F(3, 171) =2.55 p=0.06
	2.5 mg	96.5±1.07	-2.67±0.96	-3.33±1.19	-3.17±1.22	-1.67±1.15	
	5 mg	93.5±1.70	-1.00±1.66	-0.67±1.04	-1.00±1.28	0.67±1.36	
	10 mg	95.4±0.80	-0.28±1.51	-1.61±1.13	0.22±1.08	-1.11±1.42	
Sentence verification reaction time (ms)	Placebo	1154±70.1	22.7±35.7	-17.3±35.2	17.1±31.5	-38.3±37.2	**F(3, 171) =2.67 p=0.048**
	2.5 mg	1077±55.8	3.95±24.7	-13.4±23.3	25.0±40.9	-58.1±27.0	
	5 mg	1265±140	-122±52.3	-74.3±58.5	-32.5±58.6	-124±80.1	
	10 mg	1207±115	4.92±70.3	-32.5±70.2	4.12±68.9	-108±64.9	
Serial threes subtraction correct (number)	Placebo	40.7±2.39	0.25±1.69	2.00±1.26	1.85±1.58	1.70±2.21	F(3, 171) =2.41 p=0.07
	2.5 mg	42.0±2.57	-0.60±1.70	1.75±1.74	-0.75±1.87	2.00±1.33	
	5 mg	38.9±2.90	2.65±1.56	1.35±1.46	0.00±1.86	4.15±1.99	
	10 mg	37.8±2.82	1.60±1.78	4.05±1.98	4.20±1.55	4.80±1.74	
Serial threes subtraction errors (number)	Placebo	3.20±0.48	0.35±0.52	0.25±0.49	0.20±0.57	0.40±0.68	**F(3, 171) =12.06 p<0.001**
	2.5 mg	2.60±0.53	1.50±0.46	1.00±0.47	0.90±0.56	0.65±0.58	
	5 mg	4.70±0.61	-1.10±0.64	-0.70±0.56	-1.10±0.60	-1.65±0.66	
	10 mg	3.80±0.47	0.05±0.47	-0.75±0.61	-0.35±0.44	-0.75±0.61	
Serial sevens subtraction correct (number)	Placebo	24.4±2.11	-0.75±1.17	0.35±0.83	0.10±0.99	-0.45±1.47	F(3,171) =2.40 p=0.07
	2.5 mg	25.0±2.04	1.40±0.97	1.05±1.03	-2.10±1.35	1.50±1.04	
	5 mg	24.4±2.24	-0.80±1.19	0.05±1.11	-2.10±1.27	-2.30±1.20	
	10 mg	23.9±2.55	-0.80±1.24	1.15±1.08	1.00±1.45	1.15±1.10	
Serial sevens subtraction errors (number)	Placebo	3.10±0.48	0.50±0.69	0.80±0.63	0.45±0.48	0.75±0.57	F(3, 171)<1
	2.5 mg	3.35±0.40	0.55±0.47	0.05±0.41	1.25±0.63	0.15±0.65	
	5 mg	2.70±0.24	0.95±0.60	0.25±0.48	1.20±0.55	1.65±0.52	
	10 mg	3.20±0.36	0.85±0.45	0.85±0.42	-0.10±0.46	1.05±0.51	

7.3.5 Subjective mood measures

Mean pre-dose baseline scores, and change from baseline scores for each condition on each outcome measure are presented in Table 7.3 along with F values and probabilities for effects of treatment. Only significant main effects for each outcome measure are reported below. Significant differences in mood are presented in Figure 7.3.

Table 7.3 Baseline and change from baseline scores for mood for each treatment condition. Means ±SEM are presented with F and p values from the primary ANOVA of treatment effects (see text). Significant measures are shown in bold.

Measure		Treatment	Pre-dose baseline score	Post-dose change from baseline score				Treatment effect
				1 hour	3 hours	6 hours	9 hours	
Bond-Lader factors	**Alert**	Placebo	54.3±3.61	-5.20±2.00	-1.23±3.51	-3.03±4.04	-3.48±3.77	**F(3, 171) =2.71 p=0.046**
		2.5 mg	55.1±3.14	-3.48±3.38	-1.87±2.60	-9.71±3.39	-7.53±2.25	
		5 mg	57.6±3.21	-6.78±3.46	-8.74±3.15	-14.9±3.68	-8.13±4.54	
		10 mg	55.9±3.83	-4.54±2.27	-6.06±2.12	-9.84±2.81	-7.78±4.07	
	Content	Placebo	62.2±3.59	1.00±1.32	1.71±2.56	1.20±2.24	2.63±3.95	**F(3, 171) =4.59 p=0.004**
		2.5 mg	66.5±2.99	-1.59±2.15	-1.58±2.58	-1.90±2.47	-2.28±2.68	
		5 mg	66.8±3.26	-3.29±2.78	-2.23±2.76	-6.88±2.87	-2.43±2.89	
		10 mg	61.8±3.83	0.70±1.29	-0.88±1.18	-1.27±1.29	2.53±2.04	
	Calm	Placebo	60.3±3.44	2.05±1.97	-0.98±2.68	-3.30±3.51	-0.05±3.27	F(3, 171)<1
		2.5 mg	64.9±3.64	0.43±2.29	-2.45±2.33	-2.88±2.61	3.28±4.04	
		5 mg	65.0±3.83	-0.58±4.64	-4.58±2.82	-0.33±3.83	-1.15±3.56	
		10 mg	62.8±3.48	1.48±2.47	-1.45±2.37	-0.95±2.60	-4.53±4.85	
Caffeine research visual analogue scales	Relaxed	Placebo	62.6±5.86	-0.25±4.58	-4.85±4.53	-6.40±5.00	-3.15±4.68	F(3, 171) =1.09 p>0.1
		2.5 mg	65.7±4.60	-8.50±3.66	-6.65±6.44	-4.40±6.25	-2.10±6.44	
		5 mg	68.6±3.67	-10.1±5.08	-11.8±5.28	-13.2±5.79	-4.90±4.33	
		10 mg	63.8±5.06	-3.20±2.88	-9.85±5.93	-2.65±5.21	-12.9±7.51	
	Alert	Placebo	53.1±5.55	-4.50±3.27	-3.45±4.10	-0.70±5.52	-6.40±4.17	F(3, 171)<1
		2.5 mg	53.1±4.41	0.05±4.55	-0.50±3.63	-15.0±4.83	-5.85±3.40	
		5 mg	48.6±4.83	4.20±3.95	-1.60±3.80	-9.65±4.70	-0.80±5.36	
		10 mg	50.8±5.83	-5.00±5.02	-0.50±3.47	-4.50±4.78	-5.35±5.70	
	Jittery	Placebo	18.7±4.22	3.95±2.98	5.45±3.19	5.80±4.19	4.30±4.93	F(3, 171)<1
		2.5 mg	12.4±3.18	5.95±3.06	6.30±2.43	8.05±3.86	3.80±3.13	
		5 mg	15.8±4.17	7.75±3.35	1.30±2.09	3.50±3.28	3.65±2.92	
		10 mg	22.4±5.26	0.30±3.26	5.50±2.07	2.25±2.62	2.60±2.82	
	Tired	Placebo	48.4±4.97	-1.35±3.34	3.05±5.82	5.90±6.83	1.60±5.84	**F(3, 171) =3.56 p=0.02**
		2.5 mg	50.2±4.08	-2.55±3.94	-6.05±4.54	7.10±6.01	2.15±4.22	
		5 mg	44.8±6.08	2.80±4.97	3.75±4.36	20.9±6.17	17.3±6.29	
		10 mg	50.2±6.53	-0.55±4.68	0.90±5.72	7.05±5.59	12.2±7.70	
	Tense	Placebo	22.9±4.54	1.95±2.47	3.95±4.10	8.80±4.73	11.2±5.57	F(3, 171)<1
		2.5 mg	20.9±4.79	4.30±2.50	4.55±3.18	8.05±3.44	5.75±4.88	
		5 mg	20.0±3.95	5.20±4.59	8.20±4.09	6.75±4.72	3.55±4.83	
		10 mg	26.2±4.94	4.20±3.89	7.05±3.51	3.45±3.55	9.55±4.79	
	Headache	Placebo	11.7±3.58	-4.75±3.15	-1.30±4.22	-0.80±3.66	7.40±5.23	F(3, 171) =1.81 p>0.1
		2.5 mg	12.4±4.80	2.65±2.94	4.80±2.16	2.55±2.99	6.85±4.19	
		5 mg	14.6±4.68	-3.60±3.69	3.60±3.83	5.40±5.30	7.75±5.15	
		10 mg	14.3±4.89	3.70±1.60	3.60±5.69	8.50±6.28	9.90±6.92	
	Overall mood	Placebo	63.3±4.21	-2.70±2.61	1.05±2.45	-2.10±2.67	3.75±3.85	F(3, 171) =2.45 p=0.06
		2.5 mg	70.2±3.17	-2.55±1.78	-1.75±2.18	-4.10±2.44	-3.60±3.63	
		5 mg	71.4±2.93	-3.45±3.35	-3.50±2.12	-9.60±3.27	-3.45±2.49	
		10 mg	64.8±4.02	-1.25±1.96	-1.70±2.91	-4.65±3.18	-1.60±3.26	
	Mental fatigue	Placebo	35.9±5.05	7.55±3.34	9.45±4.33	15.3±5.46	12.6±5.10	F(3, 171) =1.39 p>0.1
		2.5 mg	34.7±4.78	4.40±3.86	5.85±5.02	11.9±5.52	4.65±5.49	
		5 mg	44.0±5.83	0.85±4.10	3.45±3.43	14.4±5.51	7.30±6.55	
		10 mg	40.5±5.32	8.30±4.70	8.05±4.56	10.7±5.19	15.6±4.82	
Caffeine research visual analogue scales factors	Alertness	Placebo	52.4±5.05	-1.58±2.50	-3.25±4.78	-3.30±5.84	-4.00±4.63	F(3, 171)<1
		2.5 mg	51.5±3.96	1.30±3.59	2.78±3.47	-11.1±4.80	-4.00±3.35	
		5 mg	51.9±4.94	0.70±2.90	-2.68±2.83	-15.25±4.76	-9.03±5.21	
		10 mg	50.3±5.64	-2.23±4.09	-0.70±4.14	-5.78±4.53	-8.78±5.86	
	Tension	Placebo	30.2±4.91	1.10±2.94	4.40±3.84	7.60±4.23	7.15±4.51	F(3, 171)<1
		2.5 mg	27.6±3.35	6.40±2.87	5.60±4.09	6.23±4.05	3.93±4.85	
		5 mg	25.7±3.47	7.63±4.26	10.0±3.72	9.95±4.60	4.23±4.24	
		10 mg	31.2±4.59	3.70±2.92	8.45±3.85	3.05±4.23	11.2±5.54	

7.3.5.1 Bond-Lader 'Alert' factor

Ratings on the Bond-Lader visual analogue scales 'alert' factor were significantly impaired at 3 hrs [t(171)=2.34, p=0.02], and 6 hrs [t(171)=3.69, p=0.0003] following 5 mg, and at 6 hrs following 2.5 mg [t(171)=2.08, p=0.04] and 10 mg [t(171)=2.12, p=0.04], see Fig 7.3a.

7.3.5.2 Bond-Lader 'Content' factor

'Content' ratings were significantly reduced at 9 hrs [t(171)=2.23, p=0.03] following 2.5 mg and at 6 hrs [t(171)=3.67, p=0.0003], and 9 hrs[t(171)=2.30, p=0.02] following 5 mg, see Fig 7.3b.

7.3.5.3 'Tired'

'Tired' ratings were significantly increased at 9 hrs [t(171)=1.99, p=0.049] following 10 mg and at 6 hrs [t(171)=3.67, p=0.006], and 9 hrs[t(171)=2.30, p=0.004] following 5 mg, see Fig 7.3c.

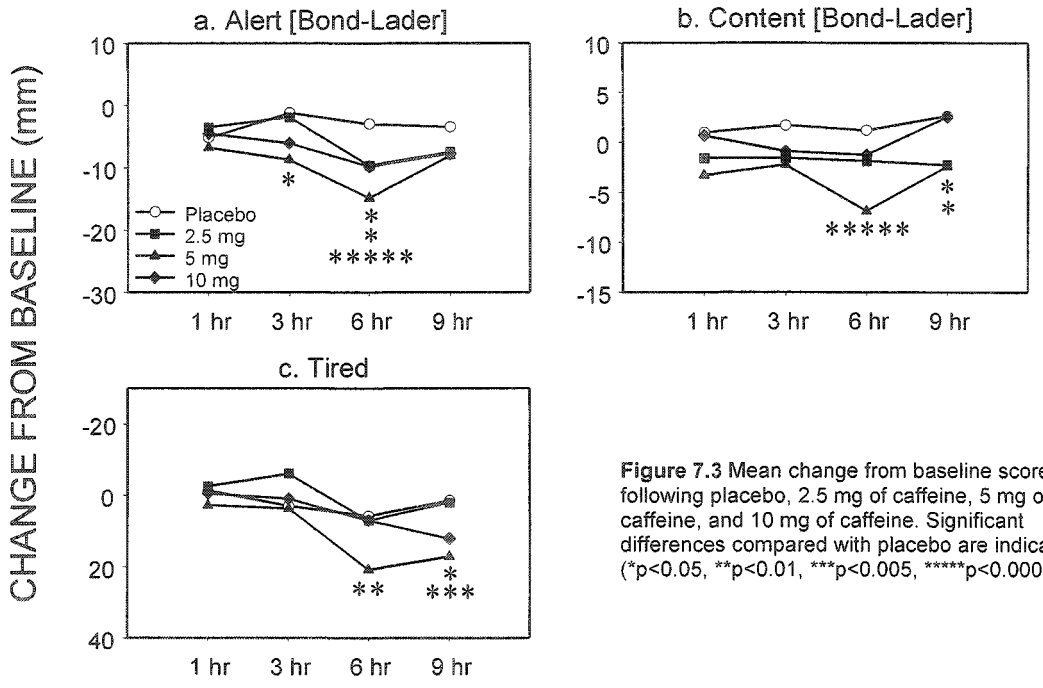

Figure 7.3 Mean change from baseline scores following placebo, 2.5 mg of caffeine, 5 mg of caffeine, and 10 mg of caffeine. Significant differences compared with placebo are indicated (*p<0.05, **p<0.01, ***p<0.005, *****p<0.0005).

7.3.6 Autonomic measures

Due to a data capture error with one dataset this analysis includes data from only 19 participants.

7.3.6.1 Diastolic blood pressure

Diastolic blood pressure was significantly reduced following 2.5 mg of caffeine at 1 hr post-treatment [t(162)=2.46, p=0.01], see Fig 7.4a.

7.3.6.2 Heart rate

Heart rate was significantly increased at 3 hrs following 2.5 mg of caffeine [t(162)=2.09, p=0.04] and at 9 hrs following 10 mg [t(162)=2.25, p=0.03]. 5 mg significantly decreased heart rate at 1 hr post-treatment [t(162)=2.12, p=0.04], see Fig 7.4b.

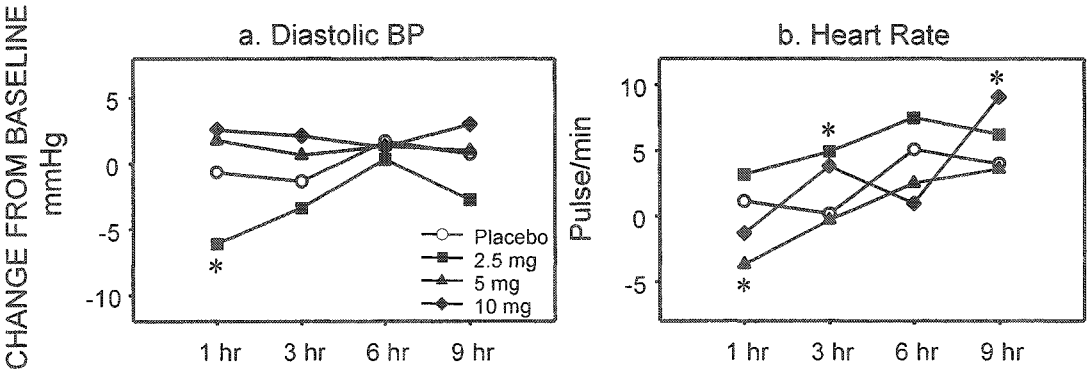

Figure 7.4 Mean change from baseline readings on autonomic measures following placebo, 2.5 mg of caffeine, 5 mg of caffeine, and 10 mg of caffeine. Significant differences compared with placebo are indicated (*p<0.05).

7.4 Discussion

The most striking finding from the current study was the pattern of negative effects following the 2.5 mg of caffeine dose. The effects of 5 mg of caffeine were mixed and, whilst the pattern of results following 10 mg were modest, the only consistent effect was positive, providing support for previous findings of psychoactive properties of 9 mg of caffeine (Chapter 6), and improvements to cognition following guaraná containing 9 mg of caffeine (Chapters 4 and 5).

The lowest dose produced more RVIP false alarms and slower speed of picture recognition. Several other effects were only seen at a single time point. These included impairment to speed of logical reasoning, impaired accuracy of numeric working memory, reduced 'alert' ratings, and decreased 'content' ratings. A single improvement to delayed word recall was also observed. Five mg of caffeine led to impairment to delayed word recall, slower speed of word recognition, and slower speed of picture recognition. 'Alert' and 'content' ratings were also decreased and 'tired' ratings were increased. However, the 5 mg dose was associated with faster responding during sentence verification. With regards the 10 mg dose, the current findings demonstrate improvement to accuracy of numeric working memory and a single time point improvement to reaction time during performance of the logical reasoning task. Some impairment was also seen in the form of increased RVIP false alarms and slower speed of picture recognition. 'Alert' ratings were also decreased and 'tired' ratings increased. This is in contrast to an improvement in 'mental fatigue' seen in Chapter 6 following 9 mg. However, it should be noted that these impairments were only apparent at a single time point.

The effects seen here with 2.5 and 5 mg of caffeine are particularly important because these levels are consistent with those found in an average cup of decaffeinated coffee (3.8 mg – Gray 1998). Although the level of caffeine typically present in decaffeinated coffee is often assumed to be below psychoactive levels, the behavioural effects of this dose of caffeine have not previously been investigated. These results suggest that such doses may be psychoactive. This finding is particularly relevant to studies of caffeine that have used decaffeinated coffee as a placebo (e.g. Smith et al. 2005; Tieges et al. 2004). The profile of results seen here would suggest that any effects seen when comparing caffeinated to decaffeinated drinks may be prone to an exaggeration of effects due to impairment in the 'placebo' condition.

Based upon the findings from Chapter 6 of psychoactive effects of 9 mg of caffeine it was deemed possible that 5 mg of caffeine may elicit behavioural responses. However, the finding of effects with the lowest (2.5 mg) dose was unexpected. It was wrongly assumed that this dose would be inactive and its inclusion was intended to

help to establish an effect threshold. Findings of predominantly detrimental effects following 2.5 mg are contrary to the literature suggesting that low levels of caffeine are inactive while high levels lead to impairment (Watters et al. 1997). The results presented here suggest that rather than being an inverted-U, the dose response may follow a sine wave (see Figure 7.5). According to this model, very low doses of caffeine (2.5 – 5 mg) impair, whilst higher doses, typically found in caffeinated beverages, have beneficial effects on mood and cognition. As with most drugs, very high doses have negative and even toxic consequences. Findings from previous studies support this notion and also suggest that this dose response may be task specific (Lieberman et al. 1987; Smit and Rogers 2000).

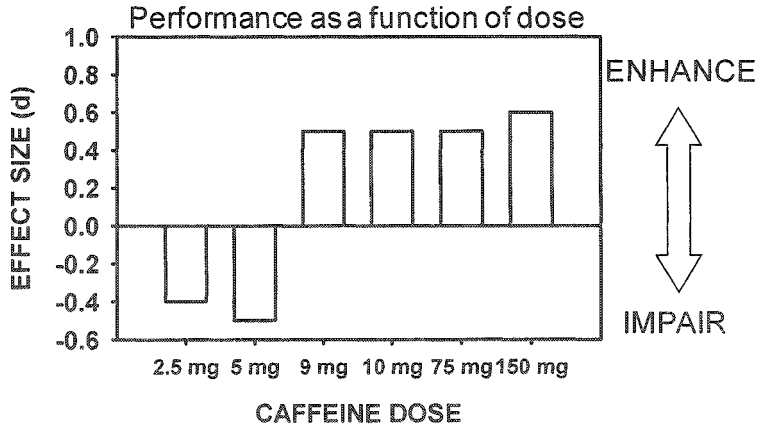

Figure 7.5 Graphical representation of proposed dose response to caffeine in relation to performance on the basis of effect sizes presented in this thesis.

One possible explanation for these effects is that ingested caffeine, even at very low doses, is detected and primes the body to prepare to respond to the caffeine with a drug opposite effects i.e. behavioural impairment. A possible mechanism for this response is an adenosine receptor-mediated effect on noradrenaline, which has been shown to be involved in some of the effects of caffeine (Smith et al. 2003). This suggestion of an immediate response to caffeine is supported by the finding that the effects of caffeine were detected on a pattern recognition task, which began immediately post-caffeine ingestion and lasted less than four minutes, despite plasma caffeine levels peaking only at around 50 minutes post-ingestion (Durlach 1998).

The findings here regarding autonomic activity are also interesting. The lowest dose led to increased heart rate and decreased blood pressure - the opposite effects to the expected pattern. Again these findings provide support for an anticipatory, antagonistic response to caffeine. Similar effects have been reported previously but only in response to decaffeinated coffee. For example, a caffeine challenge will reliably increase salivation, but when decaffeinated coffee was administered to habitual

caffeine consumers, decreased salivation was observed (Rozin et al. 1984). The startle response (latency of eye-blink reflex) has also been shown to be delayed following decaffeinated coffee when compared with caffeinated coffee, a novel caffeine beverage, or the same novel beverage without caffeine (Andrews et al. 1998). These data are presented as evidence of conditioned responses to caffeine-associated stimuli. However, it is unlikely that the decaffeinated coffee employed was completely caffeine-free so it is possible, as here, that the effects were responses to very low caffeine levels. Clearly this conjecture requires further investigation. One finding that it is difficult to explain using this theory is that relating to the time course of the effects. An antagonistic response would be expected to be observed at the first post-treatment assessment and, whilst it is not impossible that any effects may not be demonstrated until later, it is difficult to explain why an effect may be apparent at 1 hour post-treatment and then reappear at 9 hours. It is possible that the consumption of lunch may be relevant to any effects, or lack of, at 3 and/or 6 hours. It is also possible that withdrawal has some influence over these results and future work in this area would benefit by examining the effects in large groups of both habitual consumers and non-consumers of caffeine. However, any role for withdrawal in these effects is also complicated as effects of withdrawal would be expected to be greatest in the placebo condition.

A 9 hour post-treatment testing session was included in order to establish a time-course threshold for any effects of caffeine, assuming that any effects for the doses used here would have decayed to baseline by this time. Again, unexpectedly a number of effects were still apparent 9 hours post-treatment. These data have important ramifications. Participation in caffeine challenge studies often requires overnight caffeine abstention (usually equating to around 9 to 12 hours abstention). The findings from the current study suggest that moderate to heavy caffeine consumers taking part in acute studies of caffeine may already have psychoactive levels of circulating caffeine at the time of baseline testing. Such amounts may not be detectable in saliva, at least using typical immunoassays. Given that habitual caffeine consumers tend to have higher circulating levels of caffeine than non-consumers, even following abstention (Lieberman et al. 1987), this finding may help to explain the differences that are sometimes found between habitual consumers and habitual non-consumers at baseline. Past research has shown baseline mood differences between the two groups in favour of non-consumers (e.g. Richardson et al. 1995), baseline cognitive differences in favour of consumers (e.g. Chapter 2) and no differences between the two groups (e.g. Rogers et al. 2003, study 2). One controversial possibility that emerges from the current study is that negative behavioural effects in regular

caffeine consumers, which have previously been attributed to 'overnight withdrawal', may in fact be attributable to negative effects of residual caffeine levels. Clearly such a possibility may explain differences in studies into baseline performance of consumers and non-consumers of caffeine following overnight abstention. Moreover the idea could easily be subjected to empirical investigation and our laboratory is actively pursuing these possibilities.

This finding may also be important for studies of chronic caffeine withdrawal, as findings of improvements in participants who are one-week abstinent over those who are overnight abstinent (James 1998) may represent negative effects of low levels of residual caffeine, rather than withdrawal.

It is clear from the results of the current study that caffeine is capable of producing effects at far lower doses than previously thought and that these effects are apparent for longer than expected. However, until replication of these findings is achieved some caution should be employed when making conclusions regarding these data. This is particularly true where differences at baseline (although not significant) may represent a confounding factor. Clearly, a great deal more work is needed in order to fully understand the effects of low levels of caffeine and replication of these results is essential. The time course of effects of caffeine also requires further investigation in order to establish at what point post-ingestion the effects of caffeine begin to appear as well as how long these effects last.

CHAPTER 8. DISCUSSION

8.1 Summary of the objectives of the thesis

The aim of this thesis is to address some of the issues related to caffeine research that have been overlooked or that have not received sufficient attention. One such issue is the suggestion that habitual caffeine consumption may lead to withdrawal when the individual is deprived of caffeine, and that any effects of caffeine are merely the result of a reversal of this withdrawal. In order to study this, Chapter 2 of this thesis compared the behavioural responses to both deprivation and administration of caffeine in habitual consumers and habitual non-consumers of caffeine. This was also explored further by examining the responses of consumers and non-consumers separately. Significant improvements in habitual non-consumers of caffeine would seem to provide evidence of net benefits of caffeine.

Another important point relating to caffeine consumption is that it is rarely consumed in isolation. Despite this, the effects of caffeine in dietary form have been largely neglected in the literature. Clearly, it is important to understand these effects and studies within this thesis attempted to address this issue. In Chapter 3, the effects of combining caffeine with L-theanine (a component found alongside caffeine in tea) were examined. Chapters 4, 5 and 6 then explored this further by examining the effects of guaraná (a whole plant seed extract containing caffeine).

Examination of the literature regarding low doses of caffeine revealed that the lower threshold for psychoactive properties of caffeine had not been identified. This is clearly of fundamental importance when understanding the pharmacology of any substance. Another important issue raised by the examination of the literature relates to the time course of the effects of caffeine. The majority of behavioural research on caffeine focuses upon the effects evinced between 30 and 90 minutes after administration. However, the time course of any behavioural effects, in terms of onset or extinction, has not been established. As with the threshold regarding the lower active dose, this is a fundamental aspect of the psychopharmacology of caffeine, which it is important to explore. In order to address these gaps in knowledge, the final experimental chapter of this thesis explored the effects of doses of caffeine as low as those found in decaffeinated beverages, with these effects being examined up to 9 hours after administration.

8.2 General summary of the findings

The results of the collection of studies making up this thesis provide evidence that caffeine is able to modulate mood and cognition in healthy young volunteers. This

Table 8.1 Significant effects of the different doses of caffeine and any dose-matched caffeine combinations employed on cognitive measures.

	Measure	2.5 mg	5 mg	9 mg	10 mg	75 mg	150 mg	75 mg guaraná	150 mg+250 mg L-theanine
Attention	Simple Reaction Time			↑		⬆	⬆ Ch 3	↓ Ch 6	⬆
	Digit Vigilance Accuracy			↓			⬆ Ch 2*	⇧ Ch 5 ⇩ Ch 6	
	Digit Vigilance RT			↓		⬆	⬆		⇧
	Choice Reaction Accuracy							⇩ Ch 5	
	Choice Reaction Time						⬆ Ch 3	⇧ Ch 5	⇩
	RVIP Accuracy					⬆ **	⬆ Ch 3		⬆
	RVIP RT								⬆
	RVIP False Alarms	⇩			↓			↓ Ch 4	
Working Memory	Numeric Working Memory Accuracy				⇧				
	Numeric Working Memory RT	↓		⇧			⬆	↑ Ch 6	⇧
Secondary Memory	Word Recognition RT		⇩					⇧ Ch 4	⬆
	Picture Recognition Accuracy							⇧ Ch 5 & 6	
	Picture Recognition RT	⇩	⬇		↓		⬇ Ch 2**	⇧ Ch 5	
	Sentence Verification Accuracy					⬆	⬆ Ch 2		
	Sentence Verification RT		⇧	⬆				⇧ Ch 5 ↑ Ch 6	
Serial Subtractions	Serial Threes Correct							⇧ Ch 4 & 5	↑
	Serial Threes Errors							⇧ Ch 5 & 6	
	Serial Sevens Correct							⇧ Ch 5	
	Serial Sevens Errors							↓ Ch 4	

Where: ⬆/⬇ = improvement/impairment at all time points tested; ⇧/⇩ = improvement /impairment at more than one but not all time points tested; ↑/↓ = improvement/impairment at only one time, when tested at more than one time point; * = effect in consumers only (secondary analysis); ** = effect in non-consumers only (secondary analysis).

Table 8.2 Significant effects of the different doses of caffeine and any dose-matched caffeine combinations employed on subjective measures.

	Measure	2.5 mg	5 mg	9 mg	10 mg	75 mg	150 mg	75 mg guaraná	150 mg+250 mg L-theanine
Energetic Arousal	Alert [Bond-Lader]	↓	⇩		↓				⬆
	Tired		⇧		↑			↓ Ch 5	
	Mental Fatigue			↓		⬇	⬇	↓ Ch 6	⬇
	Alertness						⬆ Ch 2 ↑ Ch 3	⇧ Ch 4	⬆
Tense Arousal	Calm [Bond-Lader]								↓
	Relaxed							↓ Ch 5	
	Jittery						⬆ Ch 2**	↑ Ch 4	
Hedonic Tone	Content [Bond-Lader]							⇧ Ch 4	
	Overall Mood								⬆
	Headache						⬇ Ch 3		↓

Where: ⬆/⬇ = increase/decrease at all time points tested; ⇧/⇩ =increase/decrease at more than one but not all time points tested; ↑/↓ = increase/decrease at only one time, when tested at more than one time point; ** = effect in non-consumers only (secondary analysis).

Table 8.3 Significant effects of the different doses of caffeine and any dose-matched caffeine combinations employed on physiological measures.

	Measure	2.5 mg	5 mg	9 mg	10 mg	75 mg	150 mg	75 mg guaraná	150 mg+250 mg L-theanine
Physiological	Saliva			⬆	↑	⬆	⬆	Ch 4 & 5 ~	⬆
	Diastolic Blood Pressure	↓		~		~	~	~	~
	Heart Rate	↑	↓	~	↑	~	~	~	~

Where: ⬆/⬇ = increase/decrease at all time points tested; ↑/↓ = increase/decrease at only one time, when tested at more than one time point; ~ = not tested.

general finding is supportive of the previous literature regarding psychoactive effects of caffeine. The assertion that caffeine has no net effects on behaviour (James 1994; Rogers et al. 1995) was not supported, as the effects of caffeine were evinced in habitual non-consumers of caffeine (Chapter 2). Although not dependent upon consumer status, the effects of caffeine were shown to be modulated by other

components found concomitantly in caffeinated beverages (Chapters 3 - 6). Finally, Chapters 6 and 7 demonstrated that psychoactive effects of caffeine exist at lower doses than previously studied. The time-course of these effects was also shown to be longer than typically assumed. These findings are shown in Tables 8.1, 8.2 and 8.3, and are discussed in more detail below.

8.3 The effects of caffeine in isolation

Robust improvements were seen following typical doses of caffeine in isolation on the following measures: simple reaction time; digit vigilance reaction time; accuracy of Rapid Visual Information Processing (RVIP); numeric working memory reaction time; accuracy of sentence verification and subjective measures of arousal, most notably 'mental fatigue'. The findings relating to simple reaction time are supportive of a number of studies that have demonstrated the sensitivity of this measure to the effects of caffeine. Previous studies suggest that these effects may be less reliable at higher doses. For example, Jacobson and Edgley (1987) found that 300 mg of caffeine led to improvements to simple reaction time 45 mins after administration, but no such effects were found following 600 mg. In line with these findings, several studies have considered lower doses, which are more closely related to the level found in a typical serving. Richardson et al. (1995) found that 70, but not 250, mg of caffeine was capable of producing an acute improvement to simple reaction time 45 minutes following administration. Smit and Rogers (2000) also found that 100, 50, and 12.5 mg of caffeine were equally beneficial in reducing simple reaction time. However, no effect was found with 25 mg.

Reliable effects of caffeine on the digit vigilance task presented in this thesis are also supportive of previous studies. Although the effects here relate mainly to speed, with less reliable effects on accuracy, previous studies have shown effects on both measures. Smith et al. (1994a) found that 4 mg/kg (~ 280 mg) improved accuracy on this measure 1 hour after administration and led to faster reaction time at 2 hours. Kelemen and Creeley (2001) report similar effects of 4 mg/kg of caffeine on performance of a 12-minute visual vigilance task. Smith et al. (1994b) again found improvements on a repeated digits task (a measure of simple vigilance) following ingestion of caffeine - 3 mg/kg (~ 210 mg) improved hit rate at 30 minutes post-ingestion and also speeded response at 30 and 120 minutes. Lieberman et al. (1987) found that auditory vigilance hit rate, as measured by the 1 hour Wilkinson task, was also significantly improved by 32, 128, and 256 mg of caffeine and to a lesser, but significant, extent by 64 mg. Related to these effects are those upon RVIP. The findings with regards the performance effects of caffeine on this measure are fairly

reliable but have not always been demonstrated. Hasenfratz and Battig (1994) found that 1.5 (~ 105 mg) and 6 (~ 420 mg), but not 3 (~ 210 mg), mg/kg of caffeine added to decaffeinated coffee improved speed of response on a 20-minute RVIP task. Warburton (1995) found that 75 and 150 mg of caffeine added to decaffeinated coffee improved speed and accuracy on this task in a dose-related manner. Smit and Rogers (2000) also found that low doses of caffeine significantly improved RVIP performance. However, this effect was only seen in high consumers.

Consistent improvements to speed of numeric working memory were also demonstrated here. This task is essentially a Sternberg memory task and one study has shown that caffeine can positively affect speed on this specific task (Kerr et al. 1991), but other studies have shown no effect (Hindmarch et al. 1998; Hogervorst et al. 1998). The sentence verification task employed within this thesis also showed reliable effects of caffeine. This task could be described as measuring efficiency of retrieval of information from semantic memory. As with many aspects of memory, semantic memory has been under-researched with regards the effects of caffeine. However, a number of studies have shown positive effects using a similar task to that employed in this thesis (Smith et al. 1992; Smith et al. 1993; Smith et al. 1994b), and this effect has been demonstrated following a dose as low as of 40 mg of caffeine (Smith et al. 1999b). One study has also shown no effect on this measure, employing a dose of caffeine of 1.5 mg/kg (~ 105 mg). The findings presented here suggest that these two memory tasks require further investigation in relation to their susceptibility to the effects of caffeine.

The effects of caffeine on subjective mood presented here reflect the widely accepted view of caffeine as a 'stimulant'. The effects of caffeine on subjective arousal have been reported in numerous studies employing a variety of descriptive adjectives. Hasenfratz and Battig (1994) showed that 3 and 6, but not 1.5, mg/kg of caffeine were capable of increasing subjective ratings related to 'wakefulness'. Similarly, Smith et al. (1999b) found increased 'alertness' ratings following 40 mg of caffeine. Other studies have shown that, as well as increasing 'arousal', caffeine can produce reductions in ratings related to 'tiredness', which are reflected by decreased 'mental fatigue' ratings in the current thesis. Quinlan et al. (2000) found increases in 'energetic arousal' and decreased 'sedation' scores following 37.5, 75 and 150 mg of caffeine and, in a second study, 'sedation' ratings were decreased by all except a 50 mg dose, and 'energetic arousal' was increased following all doses (25, 50, 100 and 200 mg of caffeine). Richardson et al. (1995) also found decreases in 'tired' ratings following 70 and 250 mg of caffeine, and Smit and Rogers (2000) found that, although 100 mg of caffeine was

needed to increase 'energetic arousal', all doses (12.5, 25, 50, and 100 mg) attenuated an increase in 'bored' ratings seen in the placebo condition.

The only notable exclusion from these reliable effects is an improvement to choice reaction time. However, there was consistent improvement to this measure following 150 mg in Chapter 3. This is in keeping with the literature, which shows mixed effects, suggesting that the effects of caffeine on this measure are often moderated by other factors, such as fatigue, which create suboptimal conditions.

These findings regarding the effects of typical doses of caffeine in isolation provide evidence that the methodology employed was sufficiently sensitive to the effects of caffeine and, therefore, appropriate to examine the impact of habitual caffeine consumption, concomitant compounds, dose, and time tested on these effects. However, although the effects on these measures are fairly robust, there are some small differences between the findings presented in the different chapters of this thesis. Possible reasons for these differences are discussed below.

8.3.1 Possible explanations of differences

Although there are a number of measures that show robust effects of caffeine, even on these measures there is not always 100 % replication. For example, the findings relating to the effects of 150 mg of caffeine upon RVIP and simple reaction time presented in Chapter 3 were not observed in Chapter 2 and the effects upon sentence verification presented in Chapter 2 were not replicated in Chapter 3. These differences may merely be the result of subtle differences in cohorts, or subjective factors such as participants', conscious or unconscious, understanding of which tasks, or aspects of tasks should be prioritised. However, it is possible that the reason for a greater number of effects of 150 mg of caffeine in Chapter 3 than in Chapter 2 is that the extra testing session in Chapter 3 has induced fatigue or led to suboptimal performance in some other way. This seems unlikely as in Chapter 3 participants took part in a 30-minute testing session half an hour after treatment administration, were given a 30-minute break, then, tested again for 30 minutes. Therefore, the length of break between each post-dose assessment was of equal length, the tasks employed are fairly simple, and the participants only took part in 3 testing sessions over a 2.5 hour period. In addition, when the findings presented in Chapter 3 are explored, it is clear that the effects are apparent 30 minutes after administration as well as at 90 minutes. Another difference in procedure is the omission of the digit symbol substitution (DSST) and tracking tasks in Chapter 3, which were included in Chapter 2. Again this seems unlikely to have affected the results as, with the exception of sentence

verification, the relevant tasks are performed earlier in the battery than the tasks that were omitted in Chapter 3, and as single, brief motor tasks are unlikely to have induced mental fatigue.

Another factor that differed between Chapters 2 and 3 was the beverage that caffeine was administered in. The drink in Chapter 3 contained 100 ml more volume than that in Chapter 2 and also contained citrate; peach flavour; peach juice; malic acid; aspartame; acesulfame K; and ascorbic acid, whereas the drink in Chapter 2 contained apple (9 %) and blackcurrant (2 %) juice from concentrate; citric acid; anthocyanins; sodium citrate; aspartame; saccharin; potassium sorbate; and sodium metabisulphite. Although it seems unlikely, it is feasible, that these different components interacted with caffeine to produce different effects, or indeed had effects of their own. Another factor relating to the different beverages employed is the sensory differences between the two and the participants' familiarity with those sensory properties. The Robinsons Special R apple and blackcurrant drink used in Chapter 2 is likely to be familiar to some participants as an inert cordial drink. The Liptons Iced Tea employed in Chapter 3, although a commercially available drink, is less commonly consumed and participants may have attributed properties to this, which, when coupled with caffeine, led to greater effects. This idea could be explored by partial replication with a variety of beverages.

An additional and important difference between Chapters 2 and 3 relates to habitual caffeine use. This will be considered in the next section.

8.4 Caffeine and the role of withdrawal alleviation

8.4.1 Evidence against caffeine withdrawal effects

In order for acute improvements following caffeine challenge to be explained as the result of caffeine withdrawal alleviation, it is first necessary to demonstrate that caffeine deprivation leads to withdrawal effects. In Chapter 2, habitual consumers and habitual non-consumers of caffeine were asked to abstain from caffeine for 12 hours prior to testing (and this was confirmed by analysis of salivary caffeine levels). In this case, therefore, habitual consumers should be in a state of withdrawal and habitual non-consumers, who consumed less than 50 mg of caffeine per day, should not - according to Evans and Griffiths (1999), 100 mg/day is sufficient to produce withdrawal symptoms. However, a comparison of baseline responses showed no difference in favour of non-consumers on the measures employed in Chapter 2. This finding is further confirmed when comparing the two groups' responses to placebo. It may be expected that any effects of withdrawal in consumers would be more pronounced in the

placebo condition as this represents a further hour of caffeine withdrawal and the effects of withdrawal may be expected to be more pronounced with repeated testing and increased fatigue. Despite this, there were again no differences in favour of non-consumers in the absence of caffeine.

These findings are contradictory to several studies that have demonstrated differences when comparing baseline performance of different consumer groups. Richardson et al. (1995) found that overnight (13 – 15 hour) abstinence led to significantly increased 'headache' ratings in caffeine consumers as compared to non-consumers (<15 mg/day). However, these ratings in overnight abstinent consumers did not differ significantly from those of chronic and 90-minute abstinent consumers. 'Clearheaded' ratings were also significantly lower in the overnight group as well as the chronic group as compared to non-consumers and 90-minute abstinent consumers. Additionally, the overnight group were more 'angry', 'dejected', 'tired' and 'drowsy' than the other groups. Similarly, in the first of two studies Rogers et al. (2003) found that overnight (15 hour) abstinent consumers (>200 mg/day) were less 'alert' and more 'tense' than their non-caffeine consuming (~ <50 mg/week) counterparts. However, in a second study Rogers et al. (2003) failed to find this effect comparing similar populations to the previous study. There is also very little evidence of withdrawal effects on objective measures, with Richardson et al. (1995) failing to find any differences between consumer groups on simple reaction time, hand steadiness and tap performance, and Rogers et al. (2003) also finding no effects on simple reaction time. This suggests that the effects of withdrawal may be confounded by expectation and as such the analysis of response to placebo may be a clearer indicator of withdrawal effects. One exception to this is Rogers et al. (2005), who found that, as well as scoring more negatively on mood ratings (more 'tense', less 'clearheaded', and more 'light-headed'), overnight withdrawn consumers also gave higher ratings on a 'headache' scale, rated the tasks as more 'tiring', and performed significantly worse in terms of increased errors on a focus of attention task and providing less correct responses on a logical reasoning task than long-term (3 weeks) withdrawn consumers.

There is also evidence of support for the findings presented in this thesis. Smith et al. (2006) compared the performance and mood ratings of 25 non-consumers and 25 regular consumers (mean = 195 mg/kg) at baseline following 15.5 hours abstinence. The only significant difference between the two groups was the production of fewer false alarms on an RVIP task by consumers than non-consumers. This result is, therefore, suggestive of better performance in 'withdrawn' consumers than non-consumers. However, the authors assert that this is likely to be a chance effect. A similar assertion is made regarding the findings from a study comparing non-

consumers with high and low consumers. Hewlett and Smith (2006a) compared performance and mood in these three groups following overnight caffeine abstention and found that low consumers had lower 'alertness' scores prior to performance assessment and lower 'hedonic tone' post-performance assessment. However, low consumers were also more accurate than non-consumers on a semantic memory task whilst high consumers produced fewer false alarms than non-consumers on a recognition memory task. Attwood et al. (2007) also compared the mood and performance of moderate (<200 mg/per day) and high (>200 mg/day) caffeine consumers and found no differences between the two groups at baseline following 11 hours of abstinence.

The reason for these mixed findings with regards the effects of caffeine abstinence in consumers on mood and performance when compared to non-consumers (or long-term withdrawn consumers) is unclear. However, it is possible that the findings from Chapter 7 may shed some light on this issue. In Chapter 7 it was shown that 2.5 mg of caffeine was able to modulate performance but this was observed as impairment to RVIP false alarms and speed of picture recognition, with single time point impairment to delayed word recall, accuracy of numeric working memory, speed of logical reasoning and reductions in 'alert' and 'content' ratings (Bond-Lader). This suggests the possibility that at low levels caffeine can lead to impairment, furthermore this level was not detectable in saliva. The findings from Chapter 7 also demonstrate that psychoactive effects (including impairment) of caffeine can still be detected 9 hours after administration. Taken together these findings suggest that it is possible that the effects of previous caffeine consumption may still be apparent in overnight deprived caffeine consumers and this will not be detectable in saliva samples. Therefore, in studies that have found differences in favour of non-consumers at baseline, these effects may represent impairment due to residual low levels of caffeine in consumers.

Although there were no differences in baseline performance or placebo response in favour of non-consumers in Chapter 2, there were some differences between the two groups, which all favoured consumers. These were greater numbers of correct serial threes and serial sevens subtractions at baseline and a decrease in 'jittery' ratings in response to placebo. Similarly, correlation of average caffeine consumption level with average baseline performance demonstrated a positive relationship between serial subtraction performance (both threes and sevens) and the amount of habitual caffeine intake. Correlation of consumption level with placebo responses also showed a negative relationship between that and 'jittery' ratings and an additional positive correlation with accuracy of picture recognition. These findings could be related to those outlined above with regards residual effects of caffeine at low doses

(in this case representing positive effects of low levels of caffeine). They could also represent a significant benefit of chronic caffeine consumption. Some support for the latter assertion comes from the findings in Chapter 2 that show group differences between consumers and non-consumers, in favour of the former, in spatial working memory accuracy, and false alarms on the RVIP task. Few studies have examined the effects of chronic caffeine consumption but there is evidence that habitual caffeine use can be neuroprotective. van Gelder et al. (2007) recently examined the effects of coffee consumption in a 10-year prospective study of elderly men. It was found that men who consumed coffee exhibited significantly less cognitive decline than non-consumers as assessed by the Mini-Mental State Examination (MMSE). Cognitive decline was also related to the number of cups of coffee consumed with the least decline associated with 3 cups per day. Ritchie et al. (2007) also found that cognitive decline was inversely related to caffeine consumption, with those consuming more than 3 cups per day showing significantly less decline that those consuming less than one cup per day. This effect was only observed in women, however. Inference of neuroprotection also comes from Maia and Mendonca (2002) who carried out a retrospective study of caffeine consumption. They compared a sample of 54 patients with probable Alzheimer's disease (AD), with 54 matched control participants, and found that caffeine intake in the 20 years preceding diagnosis of AD was inversely related with AD. No such relationship was found for various other factors such as alcohol consumption, hypertension, diabetes, family history of AD, and education. Jarvis (1993) also found that habitual caffeine intake was positively correlated with performance of tasks of choice reaction time after controlling for sociodemographic, lifestyle, and health factors. This study has been criticised, as the results may be indicative of caffeine withdrawal (Rogers and Dernoncourt 1998). However, Hameleers et al. (2000) carried out a partial replication of this study, controlling for caffeine withdrawal, and found a relationship between habitual caffeine intake and choice reaction time tasks, as well as the Stroop task and delayed word recall.

8.4.2 The impact of withdrawal in acute caffeine challenge effects

The results presented in Chapter 2 not only show no evidence of caffeine withdrawal, they also demonstrate that caffeine can improve cognition and mood in habitual non-consumers of caffeine. Improvements following both 75 mg and 150 mg of caffeine were observed across both consumer groups. The only result that showed any differential effect, i.e. an interaction between the effects of caffeine and consumer status, was that of 'jittery' ratings. Consumers showed a significant decrease in 'jittery'

ratings in response to placebo as compared to non-consumers. The opposite was true in response to 150 mg of caffeine. This finding with regards 'jittery' ratings in response to 150 mg of caffeine is unexpected, as previous research has shown that, for instance, anxiety in response to caffeine is linked to A_{2a} receptor gene polymorphisms (Alsene et al. 2003). Similar polymorphisms have been related to habitual caffeine consumption, such that they are more common in those who consume less caffeine (Cornelis et al. 2007). Therefore, it would be expected that any differential effects in terms of an anxiety-response to caffeine would be greater in non-consumers. Previous comparisons of consumers' and non-consumers' responses to caffeine have produced mixed results. In two experiments Rogers et al. (2003) found that caffeine was capable of improving 'alertness' in consumers and non-consumers alike with no interaction effects on measures of 'tense/relaxed', 'jittery', 'overall mood', or 'headache', and in the second of these studies no interaction effect was observed on simple reaction time. Hewlett and Smith (2006a) considered the effects of 1 mg/kg of caffeine in low, high and non-consumers of caffeine and found that overall the responses to caffeine were the same for these groups. Smith et al. (2006) considered the effects of 2 mg/kg of caffeine in non-consumers and overnight withdrawn consumers and found few differences between the two groups' responses. However, the differences that were observed all demonstrated a greater response in non-consumers than withdrawn consumers - these were increased 'alertness' and 'anxiety' as well as fewer lapses of attention. Richardson et al. (1995) also found no significant differences in response to caffeine as a function of group on simple reaction time or hand steadiness in low/non caffeine consumers (<15 mg/day) and habitual consumers (>200 mg <1000 mg/day) who were either 90 minute, overnight, or chronic (7 - 19 days) abstinent. They also found significant effects of caffeine on 'headache', 'tired', and 'jittery' ratings irrespective of group.

These findings seem to suggest few differences in response to caffeine when comparing consumers with non-consumers. James and Rogers (2005) have suggested that comparison of these groups is invalid as they represent two distinct self-selected groups and as such may not be comparable populations. However, Hewlett and Smith (2006b) compared these two groups and found very little difference between them in terms of demographic variables or psychological characteristics. This is supported by findings from Brice and Smith (2001b), which show very little evidence that personality traits influence caffeine consumption levels. Nevertheless, other studies have compared the behavioural effects of caffeine in low and higher consumers (rather than non-consumers) and found similar responses. Findings from Hewlett and Smith (2006a) show that on the whole there are no significant differences in responses to 1

mg/kg of caffeine of low, high and non-consumers of caffeine. One exception is choice reaction time, which was significantly faster for high consumers than low consumers. Focused attention reaction time was also significantly faster for both high and non-consumers and significantly slower for low consumers. Attwood et al. (2007) also found no significant differences when comparing the mood effects of 400 mg of caffeine in moderate (<200 mg/day) and high (>200 mg/day) regular caffeine consumers. However, a lack of cognitive effects was found in moderate consumers. Another difference between the two consumer groups was found in post-session questionnaires – high consumers were more likely to perceive positive effects of caffeine such as increased 'cheerfulness', 'alertness', and 'well-being'. These studies seem to suggest that although behavioural effects of caffeine can be produced irrespective of withdrawal, level of habitual caffeine consumption may play a role in these effects. Again the findings relating to this are mixed.

Other studies have considered the effects of acute caffeine challenge in non-withdrawn consumers. Warburton (1995) found effects of caffeine on mood in consumers who had received a pre-load of 75 mg of caffeine 1 hour prior to a caffeine challenge with 75 or 150 mg of caffeine. Caffeine increased 'clearheadedness', 'calmness', and 'happiness' and decreased 'tension' even in those minimally abstinent. Improvements were also seen to RVIP, logical reasoning and delayed recall. It has been suggested that the preload within this study may not have been sufficient to alleviate withdrawal. However, Christopher et al. (2005) found that administration of 2 mg/kg of caffeine in the evening following a day of normal caffeine consumption led to increased subjective 'alertness' and faster reaction times on a repeated digits task. Similarly, Smith et al. (2005) examined the effects of repeated caffeine doses following a day of normal consumption. In alert participants the effects from Christopher et al. (2005) were replicated, with the addition of faster speed of encoding new information. With a repeated dose and repeated testing (whereby participants were fatigued) additional effects were also seen on categoric search and simple reaction time.

The finding of effects in non-withdrawn consumers and the lack of significant differences in response to caffeine of non-consumers and consumers suggests that the effects of caffeine are not necessarily dependent upon withdrawal. However, at least one previous study that has found no significant differences in the responses of these two groups has found that the effects in non-consumers were not apparent when analysed as a separate group. Rogers et al. (1995) found no significant consumer status x treatment interactions for mood ratings in response to 70 mg of caffeine of 24 low caffeine consumers (<120 mg; mean = 47 mg) and 25 moderate caffeine consumers (≥ 120 mg; mean = 205 mg). The authors suggested that this was due to

small, non-significant effects of caffeine in the low users and explored this by carrying out separate analyses of the two groups. This revealed significant effects of caffeine only in the moderate consumers with the decaffeinated drink reducing ratings of 'lively', 'clearheaded', 'cheerful' and 'energetic' and increasing 'tired' ratings. To examine this possibility in the current thesis, the effects of caffeine on consumers and non-consumers in Chapter 2 were analysed separately. The cognitive measures affected by caffeine in consumers were accuracy of RVIP and sentence verification. Although the effect on RVIP was not observed in non-consumers, additional improvements in non-consumers were observed to digit vigilance (speed and accuracy) and speed of numeric working memory. Impairment was also seen to delayed picture recognition. In terms of mood, greater effects were observed in consumers with caffeine resulting in greater 'alertness' ratings and reduced 'mental fatigue' as well as higher 'jittery' ratings. In non-consumers these effects were restricted to reduced 'mental fatigue' ratings. The findings from Chapter 2 demonstrate that not only do non-consumers show responses to caffeine but they also are more receptive to effects on cognition than consumers. The effect size is also greater overall in non-consumers than consumers. The results from Rogers et al. (1995) revealed no response to caffeine in non-consumers in terms of mood, whereas the results from Chapter 2 demonstrate that, although non-consumers show a lower mood response to caffeine, there is evidence of an effect. This difference in mood effects could explain why some people become habitual caffeine consumers and some people do not. A lowered sensitivity to the mood-enhancing effects of caffeine in certain individuals may result in lower reinforcing effects of caffeine consumption and, therefore, lower likelihood of consuming caffeine regularly. Alternatively, habitual caffeine consumption may simply increase sensitivity to the mood effects of caffeine. This suggests that there are differences between consumers and non-consumers in terms of response to caffeine but it is not possible to state whether these are due to differences existing prior to caffeine consumption or are the result of chronic caffeine consumption. One finding, albeit from a different area of research, that may be of interest here relates to the influence of glycaemic index (GI) of evening meal on the glycaemic response to breakfast the following morning (Wolever et al. 1988). It has been demonstrated that a low GI evening meal leads to a lower glycaemic response to breakfast the following morning, presumably as a result of the slower absorption of carbohydrate from the low GI evening meal. It is possible, therefore, that in studies of acute caffeine challenge employing different habitual consumer groups, any differential effects observed may reflect recent caffeine ingestion rather than habitual consumption. This is highly speculative but may be worthy of further investigation as a greater response to glucose in those who habitually

consume higher levels of glucose has not been related to glucose dependence or glucose withdrawal. Nonetheless, although habitual caffeine use may play a role in moderating the effects of acute caffeine challenge, the finding of effects in non-consumers when analysed separately, provides further support for net benefits of caffeine.

8.4.3 Dose effects of caffeine as evidence against the withdrawal alleviation hypothesis

The effects of 75 and 150 mg of caffeine presented in Chapter 2 suggest that, although the effects of 150 mg are more distinct, 75 mg produces a wider array of effects than 150 mg. Given that the withdrawal alleviation model suggests that caffeine has no effects beyond reversing impairment caused by withdrawal, then it would be expected, if anything, that greater and broader effects would be seen with the higher dose. Since, for some people, the lower dose may not be sufficient to alleviate withdrawal. This suggestion is contrary to the accepted view of an inverted-U dose-response curve for caffeine, which suggests that, at low doses caffeine has no effect, moderate doses have positive effects, and high doses have negative consequences (Watters et al. 1997). However, if caffeine merely reverses caffeine withdrawal there is no reason why higher doses would lead to negative effects. There is also no reason why repeated caffeine doses, following the alleviation of withdrawal, should have any effects. It is, therefore, interesting to note that people tend to decrease their caffeine consumption throughout the day. The argument for this in favour of caffeine withdrawal is that there is no reason to consume caffeine once the withdrawal has been alleviated. However, this does not explain why people avoid consuming caffeine prior to bedtime. There should be no reason that, for example, people could not consume a double espresso coffee at bedtime. Firstly, because they are most likely to have already alleviated any withdrawal earlier in the day, no effects would be expected. Secondly, even if they had not consumed caffeine recently and were in a state of withdrawal, the consumption of caffeine at bedtime should merely restore them to 'normal' levels, which following a normal day should be a state of tiredness and should not disrupt sleep. Related to this, it is also not clear why the alleviation of caffeine withdrawal should lead most consumers to intake more caffeine on weekdays, whilst at work, than at weekends (Sjaastaad and Bakketeig 2004). If discounting the withdrawal alleviation model, then the pattern of caffeine consumption, i.e. lower consumption later in the day and at weekends can be explained as resulting from a drop in workload demands and, therefore, a decreased need for increased alertness (both subjective and attentional)

and/or a desire to avoid over-stimulation when relaxing or attempting or sleep. The withdrawal alleviation model does not present any clear argument as to why caffeine consumption would be reduced at weekends as compared to weekdays.

The effects presented in this thesis relating to very low doses of caffeine also argue against withdrawal alleviation as an explanation of the effects of caffeine. In Chapters 6 and 7, it was shown that, 9 and 10 mg of caffeine respectively can induce cognitive and mood improvements. Further to this, in Chapter 7 it was also shown that 5 and 2.5 mg of caffeine are able to modulate behaviour, although largely in the form of impairment. It is unlikely that these very low doses of caffeine are able to reverse caffeine withdrawal and the role of withdrawal in the impairing effects of 2.5 mg of caffeine is even less clear. If the effects of caffeine are simply the result of withdrawal alleviation, there is no reason why doses of 2.5 mg should impair behaviour when compared to placebo. Examination of the effect sizes for these doses reveals that with the exception of 2.5 mg these effects are fairly similar to those seen with more typical doses. In both Chapters 2 and 3, 150 mg of caffeine led to an average effect size (*d*) of 0.6. In Chapter 2 it was observed that, when analysed separately, effects in both consumers and non-consumers produce effect sizes of 0.7. However, when combined this is reduced to 0.5, which is the result of the differing responses of consumers and non-consumers to specific tasks. Habitual consumer status was not taken into account in the remaining chapters of this thesis. Therefore, this could also explain the lower effect size in Chapter 3. The size of effects following 75 mg of caffeine in Chapter 2 was 0.5 and the same effect size was produced by 9 mg in Chapter 6 and by 10 and 5 mg in Chapter 7. 2.5 mg of caffeine produced an effect size of 0.4. This suggests a very flat dose-response to caffeine within the range of 5 – 75 mg.

8.5 The effects of caffeine in combination with concomitant compounds

In Chapters 3 – 6 the impact on behaviour of combining caffeine with its other concomitant compounds was considered. Caffeine is rarely consumed in isolation and any interactions between caffeine and other components, with which it is usually consumed, require investigation.

8.5.1 Dose-related effects of guaraná

In Chapters 4 - 6 the effects on cognition and mood of caffeine when consumed in the form of guaraná were considered. Guaraná contains other potential psychoactive components in the form of saponins, tannins, and catechins (Weckerle et al. 2003), as well as caffeine, and these chapters investigated the behavioural effects of caffeine

when combined in this form. Chapter 4 represented the first systematic investigation of guaraná and, as such, a range of doses were considered in order to ascertain the optimum dose. There were a number of improvements to mood and cognition following administration of guaraná. Several of these effects were apparent at each testing session, illustrating that the behavioural effects of guaraná were evident as early as 1 hour post-dose and remained until at least 6 hours after administration. In the range tested, 75 mg appeared to be the most beneficial dose, producing an average effect size of 0.6. Although the average effect size produced by 37.5 mg (0.7) was higher than that of the 75 mg dose, there were fewer effects. The highest dose (300 mg) also produced an average effect size of 0.6 and evinced a number of effects but these were mainly single time point effects, with the exception of content ratings, which were improved by all doses at more than one time point, 'alert' ratings (Bond-Lader), and correct serial threes subtractions. The 150 mg dose produced the fewest effects in general and also the lowest average effect size (0.5). Given that 75 mg of guaraná produced the most reliable effects, this dose was employed in Chapters 5 and 6. The effects of this dose of guaraná will be discussed in more detail in the following section.

8.5.2 Effects of 75 mg of guaraná

The most robust effects of guaraná were seen in terms of improved secondary memory performance and improved serial threes subtraction. Subjective measures relating to energetic arousal were also increased.

Seventy five milligrams of guaraná was shown to produce faster delayed recognition reaction time. In Chapter 4 this was evinced as an improvement to word recognition and in Chapter 5 this improvement was observed as faster delayed picture recognition. In addition, improvement to accuracy of delayed picture recognition was also observed in Chapters 4 and 5. Related to these improvements are the findings of faster sentence verification reaction time in Chapters 5 and 6. The number of correct serial threes subtractions was increased by 75 mg of guaraná in Chapters 4 and 5. In addition to this, an improvement in terms of a reduction in errors on this measure was also observed in Chapters 5 and 6.

'Arousal' ratings were increased by 75 mg of guaraná in Chapters 4, 5, and 6. In Chapter 4 this effect was observed as an increase in 'alert' ratings, whereas in Chapter 5 this effect was evinced as a decrease in 'tired' ratings, and in Chapters 6, a reduction in 'mental fatigue' ratings was observed. Partially related to this, are the findings in Chapter 4 of increased 'jittery' ratings, and in Chapter 5 of decreased 'relaxed' ratings.

Reference to Table 8.1, and to the literature pertaining to caffeine, suggests that these effects on cognition, particularly recognition memory, are not seen following caffeine alone (at any dose). Therefore, the cognitive tasks that were shown to be sensitive to guaraná are not those typically susceptible to the effects of caffeine. However, there is some overlap in the effects of caffeine and guaraná with the results from Chapter 2 showing improvements to accuracy of a sentence verification task following caffeine and the results of Chapter 5 showing improvements to this measure following guaraná, albeit in terms of speed rather than accuracy. Reference to Table 8.2 also suggests overlap between the effects of caffeine and guaraná with regards subjective ratings related to 'energetic arousal'.

Nevertheless, as referred to in Section 8.3, robust effects of caffeine are generally observed on measures relating to attention, vigilance and working memory with the effects on attention and vigilance being well accepted, and working memory effects being suggested but less well established (see Smith 2002 for review). However, there were no robust effects of guaraná on simple reaction time within this thesis, with the only observed effect taking the shape of impairment. The impact of 75 mg of guaraná on digit vigilance within this thesis was mixed but the findings are suggestive that this task is not susceptible to consistent improvement following guaraná. There were also no reliable effects of guaraná on RVIP or any measure of working memory employed.

These findings suggest that the effects of guaraná should not be solely attributed to its caffeine content. This suggestion is supported by the findings from Chapter 6, where a direct comparison of the effects of guaraná and caffeine was conducted. Surprisingly, these findings showed that the effects of caffeine appeared to be attenuated when combined with other components in the form of guaraná. It is possible that the other components within guaraná were modulating the effects of caffeine or that the other components were masking the effects of caffeine. In order to attempt to clarify the role of these other components within guaraná, a comparison was made between this and *Panax ginseng*, which contains saponins, a psychoactive component potentially responsible for the effects seen with guaraná. This will be discussed in the next section.

8.5.3 A comparison of the effects of guaraná with those of *Panax ginseng* and ginseng/guaraná combinations

As well as caffeine, guaraná contains a number of other potentially psychoactive components. These include saponins, which have been suggested as the component responsible for ginseng's effects on behaviour. Hence, a study of the

effects of guaraná and ginseng on tasks known to be susceptible to both plants would allow a comparison of their profiles to be carried out. A similarity in the profiles produced by these components would suggest a role for saponins in the behavioural effects of guaraná and would, therefore, add to knowledge with regards the role of caffeine in the effects of guaraná.

In Chapter 5, guaraná was shown to increase speed of digit vigilance with no interpretable effect of ginseng. Serial threes subtraction performance was improved by guaraná (in terms of a greater number of correct responses and reduced errors), with no effects of ginseng. Serial sevens performance was also improved following guaraná with no interpretable effects of ginseng. Ginseng led to faster choice reaction time with no interpretable effect of guaraná. Robust improvements to speed of numeric working memory and word recognition reaction time were observed following ginseng with no effect of guaraná. Robust improvements to speed and accuracy of picture recognition were evinced by guaraná with improvement to accuracy only following ginseng. Sentence verification was, however, significantly faster following both ginseng and guaraná. To summarise, these findings show similarity in the behavioural effects of guaraná and ginseng in terms of improved secondary memory performance, an effect not seen following caffeine administration. However, these findings are not supportive of saponins as the sole component responsible for the effects of guaraná due to the differing effects of ginseng and guaraná on digit vigilance, serial threes and sevens subtractions, choice reaction time, and numeric working memory reaction time described above.

Taken together the findings from this section and the previous one suggest that, what has now been shown to be a psychoactive level of caffeine, is having some effects, but at least one other component within guaraná is also exerting effects, which leads to an attenuation of the effects of caffeine on a number of measures but also has the capacity for potentiation on other tasks.

8.5.4 A comparison of the effects of caffeine with those of L-theanine and a caffeine/L-theanine combination

The effect of combining caffeine with other naturally concomitant components was also explored in Chapter 3. In this case caffeine was combined with only one other component (L-theanine), which is found in tea. The effects of this combination were compared to the effects of the component parts. This allowed for clarification of the effects of combining caffeine. The findings relating to the effects of an L-theanine-caffeine combination showed that a number of tasks were significantly affected by the

combination treatment but not by the individual components. These included speed of Rapid Visual Information Processing (RVIP), correct serial threes subtractions, 'overall mood', and, most dramatically, word recognition reaction time. Self-rated 'alert' (from the Bond-Lader scales) was also significantly improved by the caffeine-L-theanine combination but not by caffeine alone. In addition to these positive effects of the caffeine-L-theanine combination, there was also impairment in this condition – a reduction in 'calmness' 30 minutes after consumption. There was also evidence of effects of the combination on tasks that were affected by caffeine. In the case of simple reaction time, accuracy of RVIP, and 'alertness' ratings the combination led to a potentiation of the effects evinced by caffeine alone. Conversely, the effects on choice reaction time were attenuated by combining caffeine with L-theanine.

8.5.5 Implications of the findings relating to caffeine in combination

These findings indicate that the effects of caffeine can be modulated by the other components with which it is concomitantly consumed. In the case of combination with L-theanine, the effects are overwhelmingly positive but there is also some evidence for attenuation of the effects of caffeine. This mixed modulation of the effects of caffeine due to combination with L-theanine suggests that these effects are task-specific. These findings lend support to the notion that the effects of guaraná are the result of the effects of caffeine in combination with one or more concomitant phytonutrient. The findings presented here with regards modulation of the effects of caffeine are extremely important. They suggest that the effects of caffeine in isolation should not be generalised to caffeine-containing products. Given that consumption of caffeine in isolation is extremely rare as part of normal dietary consumption, this calls into question the ecological validity of studies that examine the effects of caffeine alone.

8.6 Effects of low doses of caffeine

8.6.1 Effects of 9 and 10 mg

In Chapters 6 and 7 of this thesis, the effects of very low doses of caffeine were considered (2.5 – 10 mg). As behavioural effects of caffeine at these doses have not previously been reported, it is difficult to draw direct comparisons with the literature, but the following section will discuss the findings relating to these doses, including consideration of how these findings relate to the effects of typical doses.

An improvement to simple reaction time following 9 mg of caffeine on this measure was observed in Chapter 6 but this effect was not replicated in Chapter 7

following 10 mg. The improvement observed in Chapter 6 supports the findings from Smit and Rogers (2000) showing improvements to simple reaction time following 12.5 mg of caffeine. Negative effects upon digit vigilance following 9 mg of caffeine in Chapter 6 were not replicated in Chapter 7 and are not supportive of the literature regarding the effects of typical doses of caffeine on this measure. However, as outlined in the relevant chapter, this finding is likely to be the result of spurious placebo effects and as such will not be considered further. Significant improvements to numeric working memory were observed in relation to reaction time following 9 mg of caffeine in Chapter 6. In Chapter 7, 10 mg was shown to improve accuracy on this measure. Despite the large difference in dose these findings are supportive of Kerr et al. (1991) who found improvement on this measure following 250 mg of caffeine. Although other studies have found no effect on this measure (Hindmarch et al. 1998; Hogervorst et al. 1998), these findings further support the assertion that this task requires further exploration with regards the effects of caffeine. Improvement to sentence verification reaction time was observed in Chapter 6 following 9 mg of caffeine. Increased speed on this measure was also demonstrated in Chapter 7 but only in response to 5 mg of caffeine. The findings on this measure are, therefore, mixed at these low doses. This is supportive of the literature relating to the effects of typical caffeine doses on this task, which has also presented mixed results. This may be indicative of the influence of other factors in the effects of caffeine on this measure.

Increases in 'mental fatigue' ratings were attenuated in Chapter 6 following 9 mg of caffeine. No effect was seen on this measure following 10 mg in Chapter 7 but partial support for this finding comes from a previous study that showed an attenuation of 'bored' ratings following 12.5 mg of caffeine (Smit and Rogers 2000). In the case of the findings presented in this thesis, the effects of 9 mg were only observed 6 hours after administration, which suggests that at this low dose these effects are more fragile and may be more apparent in suboptimal conditions, such as in cases of fatigue. Possible reasons for discrepancies between the results presented in Chapters 6 and 7 will be discussed below.

8.6.1.1 Possible explanations of differences

There are a number of discrepancies between the results presented in Chapter 6 and those presented in Chapter 7. The lack of replication of a number of the effects of lower doses of caffeine suggests that the effects at these levels are less robust and may be more susceptible to influence from other factors, including individual differences in response. The only reliable effect from Chapter 7 was an improvement to

accuracy of numeric working memory, whereas in Chapter 6, 9 mg produced improvements to simple reaction time, speed of numeric working memory, speed of sentence verification and 'mental fatigue' ratings. It is not clear why the effects in Chapter 7 are less widespread. The two chapters used slightly different doses of caffeine but given that more effects were evinced with the lower dose of 9 mg, this seems unlikely to have caused these differences. Chapter 7 also included an additional testing session to the schedule employed in Chapter 6. However, this additional time point came at the end of the day, at 9 hours after administration, and as such seems unlikely to have affected performance prior to that point.

Another important difference between Chapters 6 and 7 is the vehicle employed to administer caffeine. In Chapter 6 caffeine was administered as a capsule, whereas in Chapter 7 a blackcurrant and apple cordial drink was employed. The relevance of this difference is illustrated by the different salivary caffeine levels in the two chapters. In Chapter 6 the administration of a caffeine capsule lead to significantly elevated salivary levels from 1 hour, up to 6 hours. In the case of the caffeine administered in a drink (Chapter 7), these levels were significantly elevated at 1 hour after administration only. It has previously been shown that administration of caffeine in capsule form can slow absorption rates (Liquori et al. 1997) and it is possible, therefore, that the effects of 10 mg had dissipated by the 3 hour session. Interestingly, the effects observed on numeric working memory were evident at 3 hours and 6 hours following encapsulated caffeine (Chapter 6), whereas the effects of the drink (Chapter 7) were only apparent at 1 hour and 9 hours. The effect at 9 hours presumably being reflective of increased fatigue and, therefore, suboptimal performance at this point. In addition, with the exception of sentence verification performance, the improvements observed following encapsulated caffeine were not apparent until at least 3 hours post-dose. Nevertheless, the effect of vehicle described here does not explain the lack of effects of the 10 mg drink at 1 hour. The effects of this low dose of caffeine have not previously been reported so it is difficult to know whether they would have possibly dissipated by 1 hour. However, this seems unlikely given the effects seen with the 9 mg capsule up to 6 hours after administration and the effect of the 10 mg drink on numeric working memory at 9 hours.

8.6.2 Effects of caffeine doses lower than 9 mg

In Chapter 7, behavioural effects of caffeine were demonstrated following 2.5 and 5 mg. These doses have previously been assumed to be inactive and, given that typical 190 ml decaffeinated beverages contain almost 4 mg of caffeine (Gray 1998),

the finding of psychoactive properties has important ramifications with regards decaffeinated beverages. The effects were largely negative with 2.5 mg producing impairment to RVIP false alarms and speed of picture recognition. Five milligrams impaired secondary memory in the form of reduced accuracy of delayed word recall and slower reaction times on delayed word and picture recognition. Negative impact on mood was also evinced following 5 mg as reflected by decreased Bond-Lader 'content' and 'alert' ratings, and increased 'tired' ratings on the caffeine research visual analogue scales. One improvement was observed following 5 mg, as increase in sentence verification reaction time. These effects of 5 mg were apparent at each time point and effects of 2.5 mg were also observed up to 9 hours after administration.

The findings of predominantly detrimental effects following 2.5 and 5 mg of caffeine are contrary to the literature, which suggests an inverted-U dose response curve for caffeine, with low levels being inactive and high levels leading to impairment (Watters et al. 1997). Detrimental effects at these levels, rather than no effects, or weak effects, suggests that the dose-response is at least tri-phasic. The reason for this impairment at low doses is not clear, but it could be suggested that this may be the result of an antagonistic response to caffeine. A similar example of this would be the insulin response observed when glucose is ingested. In the case of very low doses of caffeine it may be that the caffeine is detected at ingestion and a counteractive response is initiated, but this low dose does not produce the expected effects, which leads to impaired performance.

As mentioned previously, the finding of any effects following these low doses has important implications, as these levels are found in decaffeinated beverages, chocolate bars, and chocolate drinks. It is a commonly held belief that decaffeinated products are inactive and, similarly, people are often unaware of any caffeine content within chocolate. If these findings are replicated, this would suggest that these substances are, in fact, psychoactive and as such should be avoided in those not wishing to expose themselves to such effects. This is particularly true as people often cease consumption of caffeinated beverages, in favour of decaffeinated ones, for health reasons or to avoid such psychoactive effects. It has previously been pointed out that consumers of decaffeinated products often consume multiple amounts throughout the day, which could cumulatively constitute an active dose (McCusker et al. 2006). However, the findings from Chapter 7 suggest that multiple doses may not be required and even a single decaffeinated beverage may produce psychoactive effects. The practice of consuming decaffeinated beverages may, therefore, not only fail to provide escape from psychoactive effects of caffeine, but may also have deleterious effects on cognition and mood, as well as possibly increasing heart rate.

The finding of impairment following caffeine doses equivalent to those present in a typical decaffeinated beverage also has implications for caffeine challenge studies that employ decaffeinated coffee as a placebo. These results suggest that this practice may lead to inflated positive effects of caffeine due to superimposition of these effects on negative effects of the 'placebo'. Substitution of caffeinated products with decaffeinated ones in studies of chronic or longer term studies of the effects of caffeine administration or caffeine abstention may also be confounded due to effects of the decaffeinated product.

8.7 Time-course of the effects

The findings presented in Chapter 7 of this thesis suggest that the effects of caffeine can be observed up to 9 hours after ingestion in the form of a drink. These effects were shown to be both positive (following 10 mg) and negative (following 2.5 and 5 mg). Given that positive effects at 9 hours tended to be evinced following doses that showed positive effects earlier in the study day and negative effects were evinced from those doses that demonstrated negative effects earlier in the study day, these findings suggest that the effects of caffeine can endure for 9 hours or more. This is surprising as the average half-life of caffeine is around 5 hours (Blanchard and Sawers 1983). There were also some effects that only became apparent at 9 hours, indicating the possibility that some effects of caffeine only emerge in the suboptimal conditions created by testing at 9 hours after caffeine ingestion.

The finding of effects at 9 hours is particularly important as it suggests that participants who are overnight caffeine abstinent when taking part in acute caffeine challenge studies could be affected by residual caffeine levels in their system. This may explain the differences found between studies that have examined the behavioural effects of withdrawal at baseline in caffeine consumers. Differences in the length of caffeine abstinence and the consumption patterns of the consumers would lead to differing effects following abstinence.

8.8 Potential methodological limitations

Although the studies which make up this thesis provide novel and compelling findings with regards caffeine and caffeine-containing products, it is also necessary to consider some potential methodological limitations.

One area offering potential for confounding the results is the use of change from baseline data. This introduces the possibility that baseline differences in performance may exert undue influence over post-dose change from baseline scores. In order to reduce this possibility an analysis of pre-dose baseline scores was

undertaken in each case and, where significant baseline differences existed, those baseline scores were included as a covariate in a repeated measures ANCOVA. Only those differences that retained significance following this inclusion of baseline score as a covariate were discussed within the thesis.

The chapters making up this thesis all utilised the same well-used approach to the analysis of multiple dose, multiple time point studies in accordance with Keppel (1991). Strictly planned comparisons assessing the limited number of questions of true relevance i.e. the effect of each treatment versus placebo at each discrete post-dose time point, were adopted. It is possible that this approach may have led to inflated Type I errors. However, Keppel (1991) suggests that the number of comparisons that should be made without any kind of correction is arbitrary and is dependant upon the nature of the investigation - providing the number of comparisons does not exceed K-1, as is the case here.

Another methodological issue that presents problems in comparing data across studies is the selection of assessment time points. It is unfortunate that in Chapters 6 and 7 the effects of caffeine were not assessed at 30 minutes and perhaps also 90 minutes after administration. This would have allowed a more direct comparison of the effects of caffeine at different doses. The reason for the selection of the first post-dose assessment at 1 hour was in order that the effects of guaraná in Chapter 6 could be compared with the previous 2 chapters, which employed the same dose. However, in hindsight it is clear that a more beneficial strategy would have been to examine the effects of guaraná 30 minutes after consumption also. However, the findings from Chapter 3 show that the effects presented following caffeine at 30 minutes are still apparent at 90 minutes suggesting that the effects of caffeine at 30 minutes and 60 minutes should be comparable. Also, given that peak saliva concentrations have been shown to occur between 35 - 45 minutes post-oral ingestion, a comparison of the effects of caffeine at 30 minutes with the effects at 60 minutes still seems valid. This is particularly true given that the absorption rate of caffeine has been shown to be slower when ingested in the form of capsule (as in Chapter 6) than in a drink (Liquori et al. 1997). This raises another potential methodological issue – the use of drinks to administer caffeine in Chapters 2, 3 and 7 and the use of capsules to administer guaraná and caffeine in the remaining chapters. The reason for the use of capsules to administer guaraná was that it was not possible to create a matched placebo in the form of a drink. Consequently, in Chapter 6, it was necessary to administer caffeine in the form of a capsule in order to compare its effects with those of guaraná. Liquori et al. (1997) found that peak salivary caffeine levels were reached almost 30 minutes later in capsule form (67 ± 7 min) than in cola (39 ± 5 min). Assessment at 60 minutes rather

than 30 minutes in Chapter 6 may have gone some way to counteracting this absorption issue, this is particularly true as Liquori et al. (1997) found that any differences in salivary caffeine levels between capsule and cola were eradicated by 1 hour after oral ingestion. This finding also further validates the choice of the first post-dose assessment at 1 hour in Chapter 7, as any differences in absorption between the capsule employed in Chapter 6 and the drink in Chapter 7 should not be an issue.

Also of relevance to the pharmacokinetics of caffeine is the use of females as well as males in the studies making up this thesis. The bulk of research in this area has focused upon the effects in males. This is partly because Callahan et al. (1983) found that $t_{1/2}$ is 20 - 40 % shorter in ovulating women as compared to men and Lane et al. (1992) showed $t_{1/2}$ to fluctuate throughout the menstrual cycle. The half-life of caffeine has also been shown to be affected by taking the contraceptive pill (Patwardhan et al. 1980) but this was also not considered an exclusion criterion within this thesis. The reason for this inclusion of men and women (including those taking the contraceptive pill) is partly one of generalisability. Studies have shown differences between men and women in their response to caffeine and for this reason it is not valid to simply recruit males and extend the findings to the whole population. The issue of fluctuations across the menstrual cycle is unlikely to have presented a problem with the counterbalancing of treatments and (with the exception of Chapter 2) the randomisation to treatment order. In addition, Kaplan et al. (1997) found no differences between men and women in the pharmacokinetics of caffeine after differences in body weight had been accounted for. Further support for the use of females as well as males in this thesis comes from analyses of sex differences in response to caffeine in Chapter 2, which showed no significant differences.

One final potential methodological problem is that of informing people of the nature of the study. With the exception of Chapter 3, participants were informed explicitly of the compound being tested. This has been criticised as a result of findings that show that caffeine-related information can influence the effects of caffeine (e.g. Mikalsen et al. 2001). Reference to a second, more controlled, experiment reported in Mikalsen et al. (2001) demonstrates that information about whether a drink contained caffeine was able to significantly affect subjective 'contentment' ratings following drink ingestion, with those told that a drink contained caffeine rating themselves as more content. The only other significant finding was that of increased startle reflex in response to caffeine-associated stimulus, which the authors suggest is a conditioned response. However, given that the associated stimulus was decaffeinated coffee and in light of the findings from Chapter 7 of this thesis, this response may be an actual response to the caffeine present within the decaffeinated coffee employed. Oei and

Hartley (2005) further explored the role of information and expectancy in the effects of caffeine. Participants were split into groups on the basis of whether they expected caffeine to stimulate them or not and were given messages regarding whether drinks contained caffeine or not. The findings showed that those participants who expected caffeine to stimulate them performed better on a signal detection task. There were no other significant findings of expectancy and the message given had no impact. It is feasible, however, that the participants' different beliefs with regards the effects of caffeine are based upon experience and that the effect on signal detection represents a greater effect of caffeine in those participants rather than expectancy. This is supported by the lack of interaction between expectancy and the information provided about the caffeine content of the drinks. Given the lack of strong evidence against informing participants of the nature of the compound being tested it was deemed more constructive to retain the ecological validity of participants being aware that they may be consuming caffeine (as is the case when consumed in dietary form). In addition, in Chapter 3 participants were merely told that they would receive a commercially available fruit drink containing active components (one of which may be caffeine) and the findings were comparable with those presented in Chapter 2 where participants were made fully aware of the nature of the study.

8.9 Future research

As well as addressing some of the potential limitations of this thesis, presented above, there are a number of issues that should be considered in future research. One of the main issues surrounding caffeine research remains to be that of the effects of habitual consumption. The findings presented in this thesis suggest that not only does caffeine abstinence not lead to impairment in habitual consumers as compared to non-consumers, but acute caffeine challenge also leads to improvement in habitual non-consumers when analysed separately. However, it has been suggested that comparisons between consumers and non-consumers may not be valid as these represent distinct, self-selected groups. In addition, it has been suggested that the effects observed in non-consumers would be subject to the development of tolerance (James and Rogers 2005). This suggests that, although caffeine may have positive effects as a novel substance, habitual use leads to dependence, which, when caffeine is deprived, results in impairment. One option for exploring this further is to track children from a very early age and ascertain whether these differences exist prior to long-term habitual caffeine use. The method is time-consuming and requires a substantial commitment from participants, it is also very difficult to control given the wide availability of caffeine. An alternative approach is to examine the effects of chronic

caffeine abstinence and chronic caffeine administration. For example, following baseline assessment, habitual caffeine consumers could be administered caffeine or placebo for 3 weeks, tested at 3-week baseline following overnight abstinence (in the caffeine group), then given an acute dose of either caffeine or placebo. Participants would then crossover to the other treatment for 3 weeks followed by a challenge day where they received the opposite treatment to challenge day 1. This would result in 4 groups, each group receiving chronic caffeine; chronic placebo; acute caffeine and acute placebo (in a balanced crossover). In light of the findings presented in Chapter 7, it would seem sensible to avoid substitution of caffeine with decaffeinated beverages and provide encapsulated caffeine and placebo instead. Although this is lacking in ecological validity, given that the majority of the population switch to decaffeinated products rather than giving up caffeine altogether, it does provide a clear starting point for this type of research, which is not confounded by the possible effects of low levels of caffeine. It also avoids the possibility of confounding effects of the other components within caffeinated products.

Further research is also needed examining the effects of caffeine in combination with those components that it is usually consumed with. In particular, the effects of tea have been largely neglected and there are a number of components, besides caffeine, with potentially psychoactive properties (for example, catechins, L-theanine etc). It is important to assess the impact of consuming caffeine in conjunction with these components given the high consumption level of this and other caffeinated products.

The findings presented in Chapter 7 are also extremely important and require further research. Firstly, these results require replication. If these effects are replicated then it will be necessary to test lower doses in order to establish the lower threshold of the psychoactive properties of caffeine. In order to further understand the mechanism of any such effects, comparison of the effects of low doses in consumers and non-consumers should be carried out. If the effects of the low doses are indeed due to anticipatory antagonistic effects then one would expect that the effects would not be evident in non-consumers of caffeine.

The findings from Chapter 7 also show effects of caffeine enduring for 9 hours. If this effect is replicated then it will be necessary to carry out further research in order to ascertain the cut-off for the time course of any effects. Related to this is the proposal that residual caffeine levels play a part in the effects seen at baseline in studies requiring overnight caffeine abstinence. This could be tested by giving various doses of caffeine and a placebo the night before a morning mood, cognitive and physiological assessment.

8.10 General conclusions

The principal aim of this thesis was to establish if caffeine has any net benefits to mood and/or cognition. This was specifically examined in Chapter 2 by comparing the responses to acute caffeine challenge of habitual caffeine consumers with those of habitual non-consumers. This method has been employed previously to test the withdrawal alleviation hypothesis with mixed results (e.g. Hewlett and Smith 2006a; Rogers et al. 2003; Smith et al. 2006; Smit and Rogers 2000). One possible explanation of discrepancies between previous studies relates to the reliability of the tasks employed. In order to overcome this problem a systematic assessment of the effects of caffeine was carried out in Chapter 2 with the use of an integrated computerised assessment battery employing a wide range of tasks. The findings offer some support to suggestions (e.g. Curtis-Prior et al. 1999; Vogler et al. 1999) that adequately objective and methodologically rigorous research, utilising computer based assessment tools, is a necessity in studies of food supplements. However, secondary analyses also revealed that some differences do exist between habitual consumers and habitual non-consumers in response to caffeine. These differences may be indicative of differences in sensitivity to the effects of caffeine, which determine whether people choose to become habitual consumers or may be the result of habitual exposure to caffeine.

In addition to the finding of effects of an acute caffeine challenge in habitual non-consumers, further support for net benefits of caffeine came from a lack of baseline differences in favour of habitual non-consumers in Chapter 2. As with caffeine challenge data, previous studies have produced mixed findings with regards baseline performance of habitual consumers and non-consumers. The majority of findings showing detrimental effects of abstinence in consumers have related to subjective effects and may, therefore, be indicative of expectancy effects, as consumers would normally be aware that they had been asked not to consume caffeine for the purposes of the research study. Another explanation for mixed findings in this area was offered by the findings from Chapter 7 of this thesis, which showed psychoactive effects of caffeine up to 9 hours after ingestion. This finding suggests that mixed results in studies of baseline differences as a function of consumer status may be confounded by the length of caffeine abstinence and by the level of caffeine consumption the day prior to testing. The findings from Chapter 7 also demonstrate that caffeine doses as low as 2.5 mg are psychoactive. It seems extremely unlikely that this level of caffeine would be sufficient to alleviate caffeine withdrawal and thus provides further evidence in favour of net effects of caffeine. The effects of this dose clearly require replication but

effects of 9 mg were observed in Chapter 6 and these were replicated with 10 mg in Chapter 7. Again this dose is unlikely to be sufficient to alleviate withdrawal. The findings with regards the effects of 2.5 mg of caffeine are also extremely important independent of the issue of withdrawal alleviation as this dose represents the level present in the majority of decaffeinated beverages and suggests that they should not be considered inactive.

A second aim of the current thesis was to assess the impact of combining caffeine with its concomitant compounds in order to assess the impact of caffeine as it is consumed within our diet. The findings from Chapters 3 – 6 suggest that the effects of caffeine can be modulated by combining it with other components. In the case of L-theanine the vast majority of effects appeared to be a potentiation of the effects of caffeine. However, in the case of guaraná, combining caffeine with the other components present appeared to attenuate any effects of caffeine. Nevertheless, the studies presented in Chapters 4 – 6 demonstrate that guaraná can increase subjective 'arousal' and improve cognition providing support for the anecdotal properties attributed to guaraná. The studies presented represent the first demonstration of such behavioural effects of guaraná in humans. These findings are important as they suggest that the effects of guaraná should not be assumed to be identical to caffeine. The findings presented in Chapter 3 are also novel in that they represent the first exploration of the effects of caffeine in combination with L-theanine in humans. These results are extremely important given that caffeine and L-theanine are consumed in tea and that tea is one of the most widely consumed beverages worldwide. The findings suggest that tea has different effects to other sources of caffeine such as coffee and are supportive of previous studies, which have demonstrated a difference in effects between tea and coffee (e.g. Hindmarch et al. 2000; Quinlan et al. 1997).

The findings presented within this thesis therefore include:

- Confirmation of net benefits to mood and cognitive performance following the acute administration of caffeine.
- Confirmation of a lack of negative consequences to mood and cognition following acute caffeine abstinence in habitual caffeine consumers.
- The first demonstration of cognitive and mood modulation in humans following the administration of a caffeine/L-theanine combination.
- The first demonstration of cognitive and mood modulation in humans following the administration of guaraná.

- Establishment of the lowest known psychoactive dose of caffeine.
- The first demonstration of effects of caffeine on cognition and mood up to 9 hours following administration.

Appendix I: Questionnaire assessing average caffeine consumption

Caffeine Consumption

Do you drink coffee?.....Yes ☐ **No** ☐

How often do you drink CAFFEINATED coffee? (Normal coffee)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink coffee regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink coffee occasionally (Some Days or Hardly Ever)

Quantity per week.....

How often do you drink DECAFFEINATED coffee?

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink decaf coffee regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink decaf coffee occasionally (Some Days or Hardly Ever)

Quantity per week.....

Do you drink tea?.....Yes ☐ **No** ☐

How often do you drink CAFFEINATED tea? (Normal tea)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink tea regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink tea occasionally (Some Days or Hardly Ever)

Quantity per week.....

How often do you drink DECAFFEINATED tea? (don't include Herbal Tea)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink decaf tea regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink decaf tea occasionally (Some Days or Hardly Ever)

Quantity per week.....

Do you drink soft drinks?.....Yes ☐ **No** ☐

How often do you drink CAFFEINATED soft drinks? (e.g. coke, pepsi, lucozade)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink caff. soft drinks regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink caff. soft drinks occasionally (Some Days or Hardly Ever)

Quantity per week.....

How often do you drink NON-CAFFEINATED soft drinks? (e.g. fruit juice, squash, lemonade, orangeade etc)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink non-caff. soft drinks regularly (Every Day or Most Days)

Quantity per day.....

Or if you only drink non-caff. soft drinks occasionally (Some Days or Hardly Ever)

Quantity per week.....

If you never drink caffeinated drinks is there any particular reason for this?..... Yes ☐ **No** ☐

If yes, please give details

_____

_____

_____

_____

_____

Appendix II: Caffeine research visual analogue scales

Please mark a cross on the following scales indicating how you feel RIGHT NOW:

Relaxed

Not at all _____ Extremely

Alert

Not at all _____ Extremely

Jittery

Not at all _____ Extremely

Tired

Not at all _____ Extremely

Tense

Not at all _____ Extremely

Headache

Not at all _____ Extremely

Overall Mood

Very bad _____ Very good

Mentally Fatigued

Not at all _____ Extremely

Appendix III: Examples of caffeine-containing products

You must not consume any alcohol or caffeine from 9pm the night before & throughout the testing day.

These are some examples of caffeine-containing products:

Hot drinks (including 'decaffeinated'):

- **coffee**
- **tea**
- **cocoa**

Soft drinks & energy drinks:

- **coke**
- **red bull**
- **pepsi**
- **lucozade**
- **irn bru**

Food:

- **chocolate**
- **coco pops**

Some medicines, such as certain cold and flu remedies

Appendix IV: List of journal publications resulting from research within this thesis

Haskell CF, Kennedy DO, Wesnes KA, Milne AL, Scholey AB (2008) The effects of L-theanine, caffeine and their combination on cognition and mood. Biological Psychology 77:113-22.

Haskell CF, Kennedy DO, Wesnes KA, Milne AL, Scholey AB (2007) A double-blind, placebo-controlled, multi-dose evaluation of the acute behavioural effects of Guaraná in humans. Journal of Psychopharmacology 21:65-70.

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