Impact of the Sensory and Postprandial Properties of Energy Drinks on Cognition

Christopher Mason, BSc.

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

ABSTRACT

The impact of energy drinks and their ingredients on cognitive functioning has been of considerable scientific interest in recent years; however studies investigating cognitive effects of energy drink consumption have centred on the postprandial impact, that is the influence of their ingredients once absorbed into the blood. It is possible however, that sensory perception of these drinks, or their ingredients can influence cognition.

The four studies outlined in this thesis aim to examine the influences of sensory perception of energy drinks in human volunteers and compare these with the effects observed in the postprandial period on a range of cognitive tasks.

Postprandially energy drink treatments were observed to reduce reaction times and improve accuracy compared with a placebo control in a saccadic peripheral conflict task when a 200ms gap was present between a pre-stimulus cue and the stimulus; however when this gap was absent accuracy decreased, suggesting treatment had affected information processing and decision making processes. Sensory perception of a non-carbonated energy drink was observed to improve reaction time and accuracy in a manual choice reaction time task irrespective of gap presence, however an artificially sweetened placebo energy drink had similar effects, but only when the pre-stimulus gap was present.

This thesis demonstrates that energy drinks can influence behavioural performance not only by increasing plasma glucose and caffeine levels in the postprandial period, but also through chemosensory perception, an effect elicited by the reward value of taste and flavour perception which is perhaps related to the calorific content of carbohydrates.

ACKNOWLEDGEMENTS

I would like to express the utmost gratitude to my supervisors Dr Joanne Hort and Prof Stephen Jackson in the aid and support they have provided in the four years it has taken to complete this thesis. I would particularly like to thank Joanne for the incredible effort she has put in to steer me through the most difficult times when I have struggled for motivation, without her this thesis would not exist.

The support of colleagues both in the Division of Food Sciences at Sutton Bonington and the School of Psychology at the University Park Campus throughout has been great. For this I would like to thank Dr Rodrigo Nova, Prof Andy Taylor, Dr Louise Hewson, Dr Ian Hardy, Helen Allen, Becki Clarke, May Ng, Rachael Abson, Dr Catherine Preston, Dr Se-Ho Nam, Dr Laura Condon, Dr Amy Parkinson, Jeyoung Jung, Lee Melton and Andy Smith.

The backing of my family and friends over the four years has been enormous and I would have struggled without them being there for me. I would like to thank my parents for supporting me through my education by encouraging me to learn at every opportunity, my brother Ronald whose competitiveness I share has driven me to this stage and my sisters Holly and Becky who have been there for support when I have needed them the most. My partner, Natalie Plau has endured two years of stress and anxiety brought on by this project and I can't thank her enough for her efforts in inspiring me to achieve my goals. In completing this research old friends and new have inspired me to carry on when I felt it was a lost cause, for this I should thank Natalie Plau, Rodrigo Nova, David Stewart, Chris Swatridge, David Jenkins and the warden-tutor team at Bonington Hall.

Finally I would like to thank the BBSRC and GlaxoSmithKline for their funding, which has made the research described in this thesis possible. The help and enthusiasm from Dr Henglong Hu, Lesley Kyle and Barry O'Neill at GSK has really helped push the project along.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

PREFACE

This thesis intends to explore the cognitive effects that energy drinks impart through both sensory perception and their ingredients (mainly glucose and caffeine) in the postprandial period. The project, a BBSRC case studentship in collaboration with GlaxoSmithKline aims to devise a series of cognitive/behavioural assessments that can be used to measure the effects of sensory perception of energy drinks on attention and alertness, and compare these effects with the well documented postprandial effects.

A review of the literature highlighted a well documented postprandial influence of energy drinks on cognition, particularly on tasks measuring aspects of attention; however very little research has examined the potential for sensory perception of taste and odour perception to influence the same measures. For this reason, chapter 1 begins by defining cognition and cognitive functions that have been observed to be influenced by energy drinks postprandially, before then discussing the observed effects and mechanisms responsible with regards to the full energy drinks and individual ingredients. The chapter then finishes by discussing behavioural effects of taste perception observed on performance in motor tasks and endurance time trials.

Chapter 2 discusses the rationale for the methodologies and procedures used in experimental chapters (chapters 3-6). Although this chapter is not strictly a 'materials and methods' chapter, it is structured in a similar manner and includes a discussion of the appropriate inclusion and exclusion criteria for human participants in experimental procedures; the appropriate energy drink and placebo treatments to be administered; cognitive assessments that are likely to be affected by chemosensory perception; and finally an explanation of experimental procedures and design, particularly how chemosensory perception can be elicited whilst limiting any postprandial impact.

Firstly, in order to compare the cognitive effects observed in the sensory period with those in the postprandial period, it was felt important to confirm the documented postprandial impact using the labs own methodologies. For this reason, chapter 3 investigates the postprandial effects of an energy drink on measures of attention, decision making and mood. This leads on to chapter 4 where the sensory and postprandial impacts of energy drink consumption on measures of attention and decision making were both investigated with a critical comparison of the effects of both.

The research outlined in chapter 5 aimed to determine the extent of the sensory impact of energy drinks observed in the previous chapter, using a task assessing sustained attention performance.

As the hypotheses regarding the mechanism surrounding the sensory impact of the energy drinks observed in chapter 4 involved the perception of glucose, chapter 6 discusses a study into the effects of unimodal taste solutions, sweet tasting glucose solutions compared with an artificially sweetened placebo solution on tasks found to be sensitive to the sensory inputs of energy drinks in chapter 4.

The final chapter then draws upon the observations of the previous four chapters and puts these into the context of the research aims and hypotheses. This section includes a critical analysis of some of the techniques used, discussing approaches that could be used to improve the experimental methodologies, before finally describing further research that could be used to determine the mechanisms behind the effects observed in this thesis.

CHAPTER 1: INTRODUCTION

Cognition is defined as "The mental activities involved in acquiring and processing information" (Colman 2009) with Eysenck (2006) stating that cognitive psychology is the study of cognitive processes such as attention, perception, learning, memory, thinking, problem solving and language and how one uses these to make decisions that will influence behaviour. Researchers utilise a range of techniques to study cognitive functions, such as lab-based experiments on healthy participants (experimental cognitive psychology), study of brain-damaged patients with cognitive impairments and how this influences behaviour (cognitive neuropsychology), and a number of brain imaging techniques (cognitive neuroscience); two or even all three of these techniques are often used in combination (Eysenck 2006).

It is perhaps most important to use experimental cognitive psychology as a means of investigating cognition, as there first must be a reasonable understanding of normal human cognition before the influences of brain damage or other (less serious) factors, such as fatigue, hydration or nutrition, can be investigated. Typically healthy volunteers who match strictly controlled inclusion criteria are asked to perform tasks that will assess the speed and/or accuracy of performance, allowing the researcher to make inferences regarding specific cognitive functions based on the behavioural data obtained.

In recent years the development of brain imaging techniques such as functional magnetic resonance imaging (fMRI) and magneto-encephalography (MEG) has allowed even greater exploration of cognition as brain regions involved in specific functions can be localised to a reasonable degree through non-invasive methods.

1.1 Cognitive functions

As stated above, cognition utilises a number of processes that allow an individual to perceive the outside world and make decisions or responses based on the

information perceived. These processes include attention, perception, learning, memory, language, problem solving, reasoning and thinking. The ability to pay attention plays a very important role in other cognitive processes; James (1890) states that the immediate effects of attention are to make us perceive-, conceive-, distinguish-, and remember- better than we could without paying attention. The greater ability to perceive and distinguish when attending to particular stimuli will lead to a shortened reaction time (RT) – e.g. the presence of a pre-stimulus cue in a visual reaction time task indicating the position of a stimulus increases accuracy and reaction time (Yeshurun and Carrasco 1999).

1.1.1 Attention

Raz (2004) defines attention as the mental ability to select stimuli, responses, memories and thoughts that are behaviourally relevant among a host of others that are behaviourally irrelevant, and also states that visual attention serves as a 'convenient lens' to examine the characteristics of attention, a view which appears to be widely held due to the enormous body of literature using visual perception as a means of investigating attention (Eysenck 2006). Many psychologists liken visual attention to a spotlight, where attention is focussed to a small section of the visual field and can be redirected onto any object of interest (e.g. Posner 1980). More recently focussed attention has been compared to a zoom lens, where the attentional field can be narrowed or widened, allowing an individual to attend to small visual fields in fine detail (i.e. reading/proof-reading) or to a larger visual field when necessary (i.e. driving) (Muller, Bartelt et al. 2003). Vigilance, or sustained attention, is described as the state of being alert and attentive and describes the ability to attend to rare events that require a response, as an air traffic controller or a driver on a long road with little traffic may have to (Colman 2009). Vigilance is studied in psychology using stimuli that require a fast response presented infrequently usually against a background of noise in the form of non-target stimuli or masking of the target. These tasks can often be analysed using a signal detection theory approach, where sensitivity in perception of difficult to perceive stimuli is measured (see chapter 4 for a more in depth explanation).

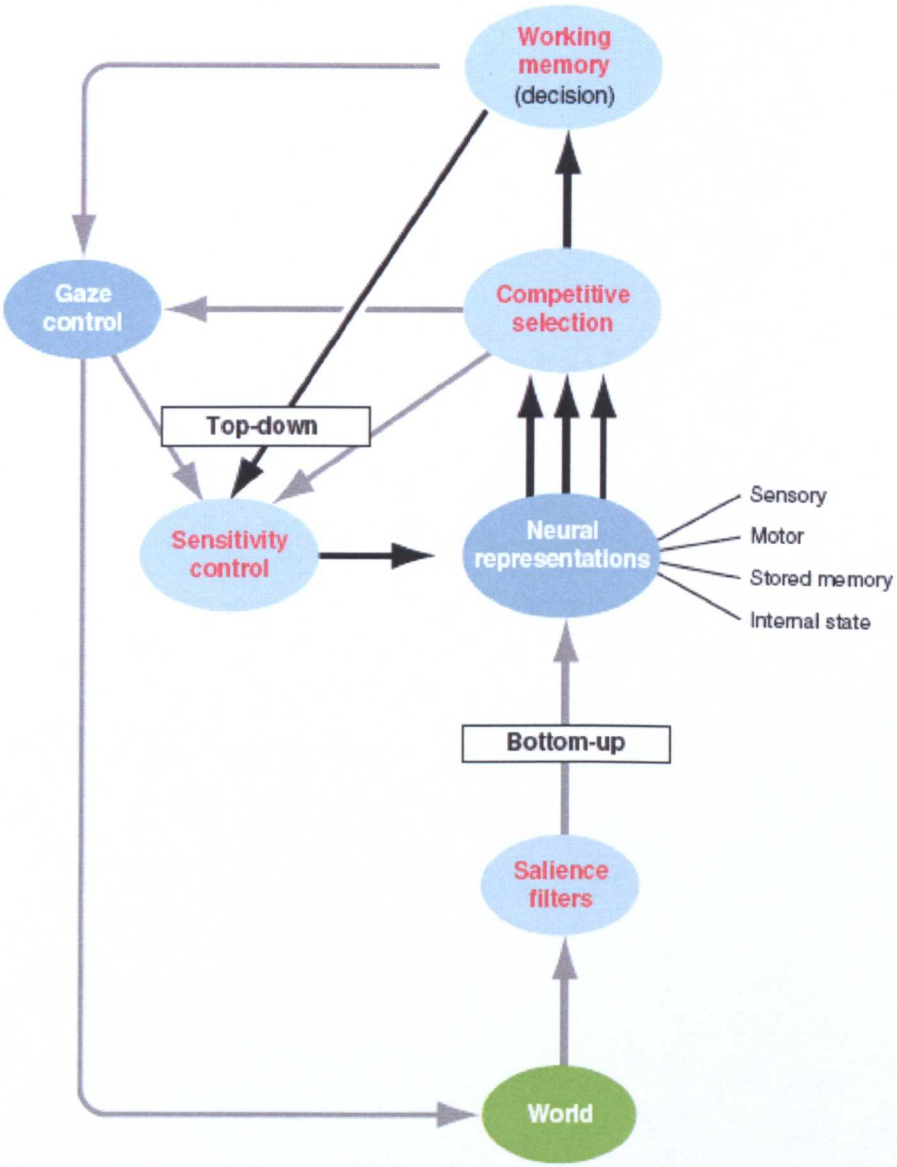

Figure 1.1: Functional components of attention (adapted from Knudsen 2007). Processes that contribute to attention are shown in red. Information about the world (*green ellipse*) is transduced by the nervous system and is processed by salience filters that respond differentially to infrequent or important stimuli (bottom-up). Neural representations in various hierarchies encode information about the world, movements, memories, the animal's emotional state, etc. A competitive process selects the representation with the highest signal strength for entry into the circuitry that underlies working memory. Working memory can direct top-down bias signals that modulate the sensitivity of representations that are being processed in working memory. The selection process can also direct top-down bias signals that reflect the result of the competitive selection. Working memory and competitive selection direct eye movements and other orienting behaviours that modify the effects of the world on the animal's nervous system. Corollary discharges associated with gaze control modulate sensitivity control. Voluntary attention involves working memory, top-down sensitivity control, and competitive selection operating as a recurrent loop (*dark arrows*).

Attention and readiness to respond are closely linked with physiological arousal; as arousal increases, so too does attentional ability up to a point where attention

levels drop off – this effect is known as the Yerkes-Dodson law and is described as an inverted U shaped relationship (Yerkes and Dodson 1908). Arousal is described as activation of the ascending reticular activating system in the brain (connecting the brainstem to the cortex) leading to alertness and readiness to respond, demonstrated by physiological signs such as increasing heart rate and blood pressure (Colman 2009).

1.1.1.1 Control of Attention

Corbetta and Shulman (2002) outline two different systems by which attention is controlled: top-down and bottom-up processing, outlined in figure 1.1. The former is goal-directed and influenced by expectation and knowledge, with the latter driven by the perception of salient or unexpected stimuli which had previously been unattended (i.e. looming stimuli, or flames under a door). This stimulus driven system has the ability to 'break' attention from its current focus to the more salient stimulus. The theory that two systems are linked and responsible for controlling what one attends seems to be generally accepted, with Kastner and Ungerleider (2000), Knudsen (2007), Raz (2004) and Eysenck (2006) all outlining both systems and how they interact.

One example of stimulus driven control has already been given, with a pre-stimulus warning having been observed to reduce RT (Yeshurun and Carrasco 1999). To investigate the interaction of the two attentional control systems, experimenters have used task switching methodologies where two cognitive tasks are completed by participants simultaneously (Rubinstein, Meyer et al. 2001; Sinha, Brown et al. 2006). Switching from one RT task protocol to another decreases the speed of response, however Sinha *et al.* (2006) monitored RT in switch trials (trials where the protocol differs from the previous trial) and repeat trials (trials where the protocol is the same as the previous trial), observing no difference in RT between repeat and control trials (where only one task protocol was used), however RT was considerably slower in switch trials, even though a pre-stimulus cue was presented. This suggests that top-down "goal-orientated" processes drive speedier RTs in

repeat trials however in order to respond to a switch trial successfully, stimulus driven processes must break attention from the previous focus, slowing reaction time considerably. Two theories that explain the processes by which task switching occurs in greater detail are the Attention to Action model (Norman and Shallice 1986) and the Frontal-Lobe Executive model (Duncan 1986), both of which involve a combination of top-down and bottom-up processing together with supervisory systems guiding behaviour and updating working memory.

1.1.1.2 Attention and Working Memory

Working memory is often likened to a processor in a computer, where items can be picked from the hard drive (long-term memory) or disc drives (sensory information), and information can be used to give output (i.e. a decision). Knudsen (2007) explains that working memory and attention are "inextricably inter-related", as once an animal perceives and attends to an object, information regarding the object enters the working memory. In fact, the characteristics of a visual stimulus can be maintained in working memory for a number of seconds without any loss in precision of visual information (Magnussen, Greenlee et al. 1991; Blake, Cepeda et al. 1997). In a task where participants are required to remember the location of a 'sample stimulus', and decide after a variable delay whether a 'match stimulus' has been presented in the same location, performance declined when a distracter stimulus was placed between the 'sample' and 'match' stimuli, indicating that disruption of attention will influence working memory performance (Awh and Jonides 2001).

1.1.1.3 Attention and Decision Making

Colman (2009) defines decision making as the process of choosing a preferred option or course of action from a set of alternatives, and states that it underpins all voluntary behaviour. In cognitive psychology, decision making is often investigated using information processing tasks where a decision to select a particular response must be made and reaction time and or accuracy is measured as a function of that decision (Rubinstein, Meyer et al. 2001). The decision making process is incredibly

complex, as is reflected by variation in reaction time recordings trial to trial even in simple tasks (Noorani and Carpenter 2011). Decision making processes are often investigated by monitoring saccadic initiation, the decision to shift one's gaze between ocular fixations, a decision made by humans every two to three seconds while awake (Sinha, Brown et al. 2006; Noorani and Carpenter 2011). The LATER model of decision making (Linear Approach to Threshold with Ergodic Rate) provides an explanation for the variation in reaction/decision times observed (Reddi, Asrress et al. 2003), as seen in figure 1.2. As a stimulus is perceived, sensory information increases the decision signal (S) for possible outcomes (at a rate of r) until the signal reaches a decision threshold (S_T), an example of two competing decision signals is displayed in figure 1.3.

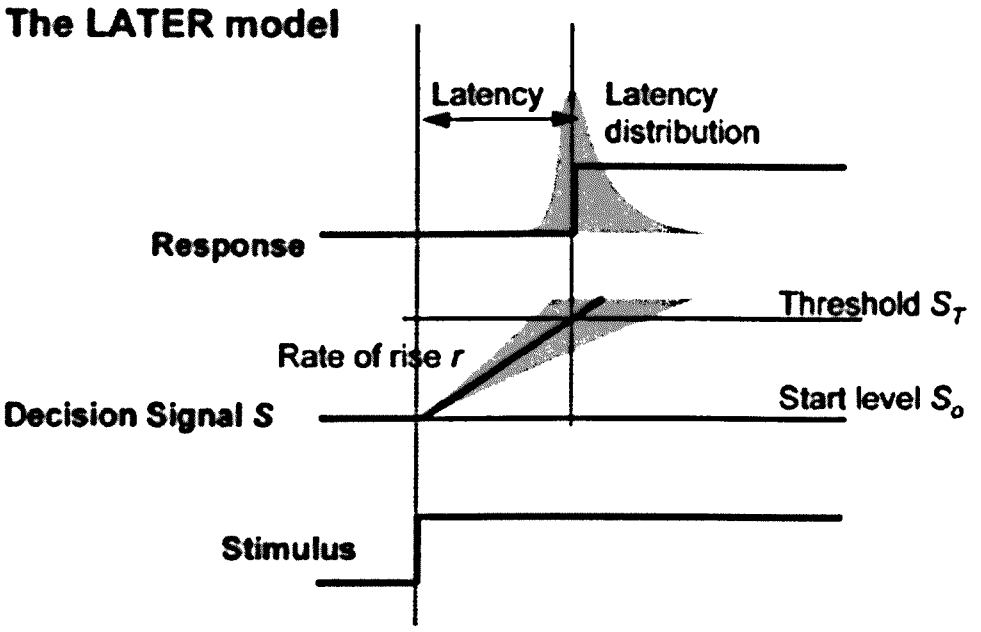

Figure 1.2: The LATER model of decision making (Reddi, Asrress et al. 2003). S indicates the strength of the decision signal (the neural drive to make a particular decision) starting at a baseline level (S_o) and increasing at a rate of r with the flow of sensory information following stimulus presentation. A decision is then made once the decision signal reaches its particular threshold, S_T). As the rate of information flow, r, varies randomly about a mean in a Gaussian manner, the latency distribution is skewed as shown in grey. Factors such as the quality of the stimulus and measures of attention/arousal will affect r, whilst S_o may be affected by preconceptions regarding the nature of the stimulus (i.e. noise during the night is more likely to be a pet than a burglar).

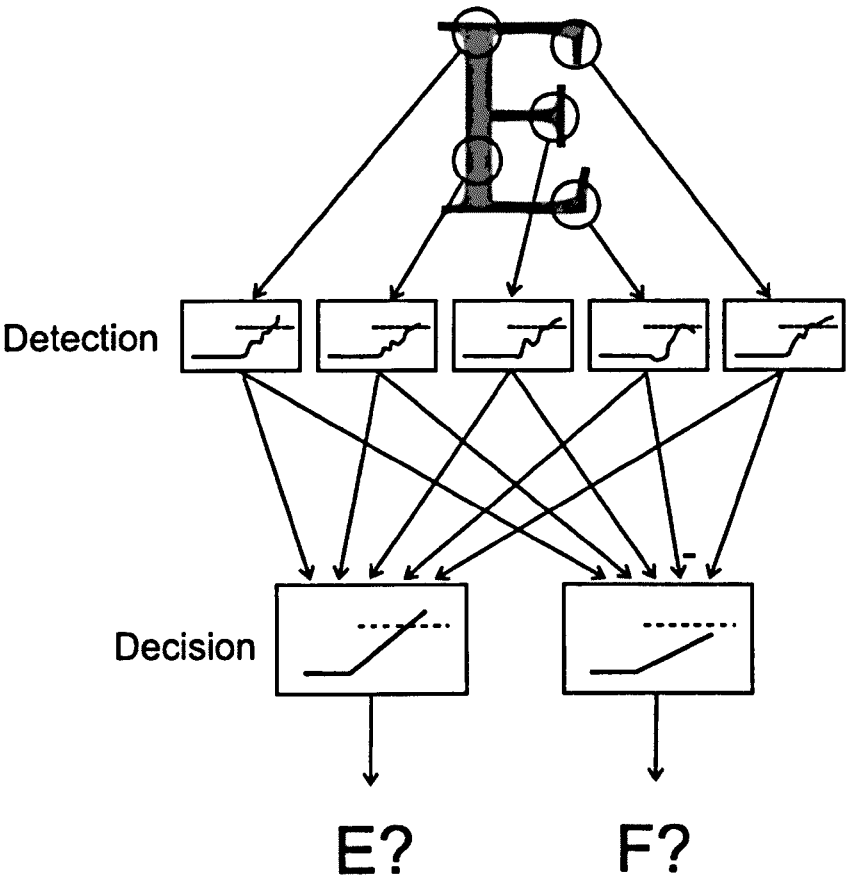

Figure 1.3: Competing individual decision signals levels following visual perception of a stimulus (Carpenter, Reddi et al. 2009). The stimulus provides a stream of information that is perceived and the working memory uses this information to arrive at a decision.

1.1.1.4 Brain imaging studies

In their review of the control of attention, Corbetta and Shulman (2002) identified brain regions active in the top-down and bottom-up systems, as well as those involved in attention during memory tasks using fMRI.

Corbetta *et al.* (2000) presented for 2s a cue indicating the location of a forthcoming stimulus, allowing participants to direct their attention to the correct location. When attending a stimulus, a short-lived activation in the occipital lobe is observed – attributed to the perception of the visual stimulus – whereas areas in the dorsal posterior parietal cortex and frontal eye field show a more protracted response, indicating that they are involved in controlling the location of attention. Other studies included in the review also found activation in the dorsal parietal

cortex along the intraparietal sulcus and the frontal eye field when paying attention to a range of visual stimuli (Shulman, Ollinger et al. 1999; Corbetta, Kincade et al. 2000; Hopfinger, Buonocore et al. 2000). In these studies participants have followed instructions and voluntarily paid attention to a cued location and stimulus that appeared covertly (that is without moving the eyes or head towards the cued location), and in doing so have utilised the goal-directed system of attentional control.

1.2 Alterations in cognitive functioning during the postprandial period

The potential impact of nutritional interventions on measures of cognitive performance has been a popular topic of study in recent years, with many studies investigating both short-term (immediate/postprandial) and more chronic/long-term effects (Reviews: Dye and Blundell 2002; Hoyland, Lawton et al. 2008). Hoyland *et al.* (2008) identified 31 studies where a total of 134 outcome measures of cognitive performance in response to oral macronutrient administrations were investigated.

Studies investigating the influence of breakfast habits stimulated research in this area, as it has been observed that breakfast consumption may improve cognitive functioning (Smith, Kendrick et al. 1994) as well as academic performance in children (Grantham-McGregor, Chang et al. 1998; Murphy, Pagano et al. 1998).

The vast majority of work in this area has centred on the impact on measures of short-term and working memory, however the influence of dietary interventions on measures of delayed memory, verbal fluency, attention/vigilance, psychomotor skill and problem solving/reasoning have also been investigated (Hoyland, Lawton et al. 2008). These studies often involve the measurement of performance at baseline, before intervention with a test meal/drink at either breakfast or lunch time, followed by repeating the measures of performance to determine the postprandial

effects (that is the effect observed in the period following meal consumption while the nutrients are being digested and absorbed into the bloodstream).

1.2.1 Cognitive effects of energy drinks and energy drink ingredients

1.2.1.1 Glucose

Brain function is dependent upon glucose as its major source of energy, with plasma glucose being its primary source as it has very limited stores. It has been hypothesised and observed that changes in plasma glucose levels affect brain function (Amiel 1994). For a long time now it has been understood that hypoglycaemia can lead to impaired brain function (Holmes, Hayford et al. 1983). The most studied cognitive effect of glucose is its memory-improving effect, with several reviews on the subject published (Gold 1995; Messier and Gagnon 1996; Benton 2001; Messier 2004). These reviews also describe the impact of glucose on other cognitive functions, such as attention and information processing, and relate the memory/learning improving effect to changes in attention and arousal.

Lapp (1981) first discovered the memory-improving effect of glucose in high school students. A 450g dose of carbohydrate over a 1 hour period was found to improve word learning. This discovery led to further investigation in the manner of animal studies. This early work (Messier and White 1984; Gold 1986) explained the effect as a result of reinforcement improving learning by strengthening associations (Thorndike 1933), i.e. behaviours followed a reward (in this case something sweet tasting) would be better associated with the experimental stimuli.

Animal studies investigating glucose and memory

Using rats, Messier and White (1984) hypothesised that sweet solutions (which the rats had a conditioned taste preference for) would act as a reward, retroactively enhancing retention of previously formed associations (memories). However a reinforcing effect was also observed when sucrose was injected, where taste stimuli were absent, suggesting that some physiological mechanism is most likely responsible for improvement of memory following sucrose or glucose ingestion.

Gold (1986; 1995) states that epinephrine enhances memory storage for several forms of learning, and that one action of epinephrine which may cause this enhancement is that it liberates hepatic glucose stores [and increases plasma glucose]. Gold (1986) concluded that peripheral glucose levels could have an important (beneficial) impact on memory storage in rats. Messier and White (1987) again found an improvement in memory performance with glucose injection, where there was no taste stimulation, but found the same effect with fructose injection, suggesting that improvement may be mediated either by the action of a sugar-sensitive absorption mechanism or by the intracellular metabolic action of various sugars, as fructose did not increase blood glucose levels. This also demonstrated that the memory improving effects of carbohydrate are not directly linked with blood glucose, but that a peripheral mechanism must be involved as fructose does not cross the blood brain barrier.

Stefurak and van der Kooy (1992) demonstrated that a post-training reward of 3.2% saccharin solution could retroactively enhance memory in rats, indicating that taste-stimulation may improve memory independently of blood glucose effects. In rats it has been observed that stimulation of taste receptors produces a small insulin secretion (Bellisle, Louissylvestre et al. 1983), which may explain any memory improvement (as it is very difficult to uncouple effects of glucose from effects of insulin; see figure 1.4). This effect has not been observed in humans, and Messier (2004) states that available evidence suggests that saccharin [i.e. taste-stimulation] may produce memory improvement in animals, but that this does not occur in humans.

The main difference between animal and human glucose intervention studies is that animal studies tend to use post-trial administration of glucose, while human studies use pre-trial administration. Manning *et al.* (1992), however demonstrated that both pre-and post-training had significant beneficial effect on memory (paragraph recall tasks) in healthy older individuals.

Glucose and cognitive effects in diabetic human patients

Early study into cognitive effects of glucose in humans involved diabetic patients. It was recognised in the 1920s that type I diabetes may affect brain function (Miles and Root 1922) and an increased risk of dementia in type II diabetic patients has since been observed (Stewart and Liolitsa 1999). Holmes *et al.* (1983) demonstrated differences in cognitive ability of type I diabetic patients under hypoglycaemic, euglycaemic, and hyperglycaemic conditions. In this study attention (measured by delayed reaction time) and fine motor skills were impaired by hypo- and hyper-glycaemia (compared with euglycaemia); however patients were less impaired (cognitively) during hyperglycaemia than hypoglycaemia. Hypoglycaemia was also found to increase the time taken for patients to perform simple addition problems. Holmes *et al.* showed in this study that, rather than high plasma glucose levels resulting in improved cognitive performance, low plasma glucose resulted in cognitive impairment.

Biessels *et al.* (2001) studied neurological and neuropsychological function in type II diabetic patients. Impairments were observed in learning and memory tasks but not in tests of attention or motor planning compared with healthy subjects. As task difficulty increased in attention tasks, type II diabetics' performance deteriorated, however this did not reach significance. Type II diabetic patients displayed negative mood and cognitive symptoms 1 hour following meal consumption when a rapid rise in pre- to post-meal blood glucose levels were observed (Cox, McCall et al. 2007). This suggests that rather than blood glucose levels, it may be glucose tolerance, or change in blood glucose levels that causes an impact on brain function.

Glucose tolerance and cognition

Donohoe and Benton (2000) demonstrated that poor fasting blood glucose levels, which were associated with poor glucose tolerance, impaired reaction time in a decision task in healthy participants. The study also demonstrated that those participants with a fast rate of falling blood glucose, following its post-ingestion peak (a sign of good glucose tolerance), had significantly faster reaction times.

Donohoe and Benton (1999) demonstrated that high baseline plasma glucose improved the time taken to solve critical problems in the Water Jars task (a problem solving task where participants complete a series of problems, and after solving many problems with the same solution, participants continue to apply the same solution to later 'critical problems' even when a simpler solution exists; the Einstellung effect (Luchins 1942)) in healthy participants, although no effect was observed in the glucose drink group compared with the taste matched placebo group. This suggests that even small differences in baseline plasma glucose affect cognitive functioning, as all plasma glucose levels recorded were within the normal range.

Glucose effects in healthy individuals

In a second experiment Donohoe and Benton (1999) demonstrated that a glucose drink improved verbal fluency and performance on the Porteus Maze Test (a non-verbal test of intelligence) at the more difficult levels, but not at easier levels. Another important finding of this study was the influence of changing plasma glucose levels. Participants who had falling plasma glucose levels performed better on difficult levels of the block design and porteus maze tasks compared with those who had rising plasma glucose levels. Memory function was improved by a 25g glucose dose under fasting conditions, and when administered 2 hours following meal consumption (Sunram-Lea, Foster et al. 2001). Kennedy and Scholey (2000) found that a 25g glucose supplement improved performance in a serial sevens tasks, but not a serial three task, indicating that the facilitation effect of glucose on information processing may only occur in difficult tasks, perhaps caused by a ceiling effect. Similar effects have been observed following 25g glucose consumption in a verbal recall task, with effects only observed when a secondary interference task was performed (Sunram-Lea, Foster et al. 2002).

It can be difficult to uncouple the effects of glucose administration from any possible effects that plasma insulin may have as plasma glucose and insulin curves follow each other very closely following any carbohydrate load (figure 1.4). In his review Messier (2004) explains that small doses of insulin have been shown to

reverse the amnesic effects of scopolamine (an anti-convulsive drug used to treat motion sickness that produces amnesic effects (Glick and Zimmerberg 1972)), but higher doses would lead to hypoglycaemia, and impaired cognitive function, without subsequent glucose administration.

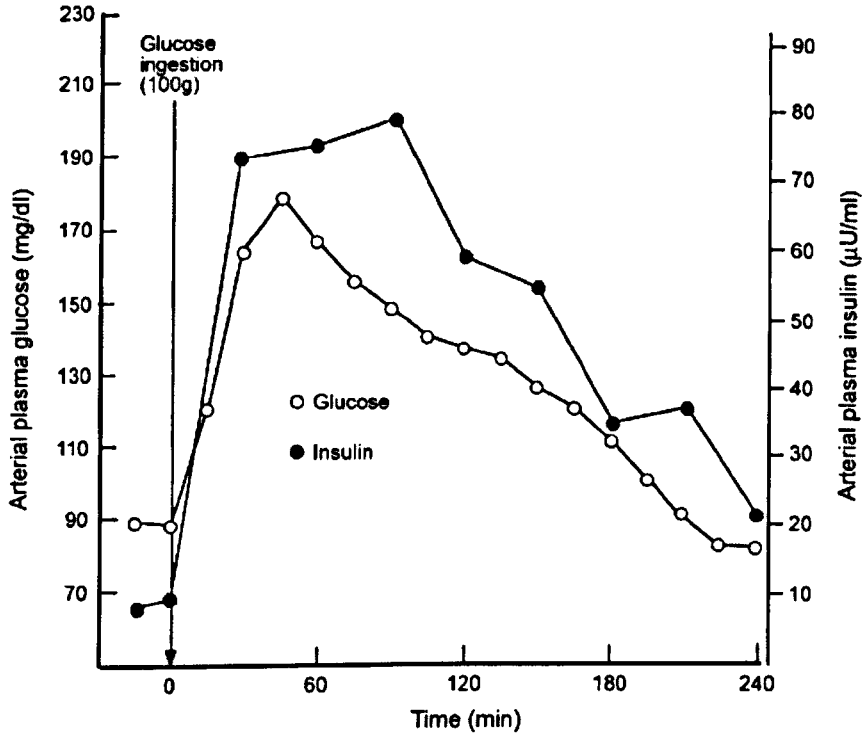

Figure 1.4: Glycaemic and insulinaemic response to ingestion of 100g of glucose (adapted from Messier 2004).

Mechanisms of Glucose impact on cognition

The simplest hypothesis of glucose' action on brain function is that an increase in energy supply to the brain will lead to an increase in brain function. This would suggest a linear dose response would be present, whereas it appears beneficial effects decline after an optimum dosage (an inverted U response curve). Other theories have been hypothesised describing the possible mechanisms of the observed effects.

The local extracellular glucose deficit

This hypothesis suggests that an increase in glucose uptake by active neurons in the brain would lead to decreased local extracellular levels, resulting in a decrease in

transfer of glucose into the active neurons. Rats performing a memory task rapidly experienced a 30% decrease in extracellular glucose levels on onset of the task, which returned to baseline values once the task was complete (McNay, Fries et al. 2000). When glucose was (250mg/kg) administered, extracellular glucose levels remained constant (and possibly at optimum level), and performance in the task was improved.

Glucose transporter deficit hypothesis

The results described in the previous section suggest there may be a deficit in glucose transport from the blood to the brain during task performance, and by raising plasma glucose this can be overcome. Messier (2004) explains that a continuous release of insulin results in an increase in the ratio of the GLUT1 glucose transporter on the vascular side of the blood-brain barrier compared to the brain extracellular fluid, resulting in greater uptake of glucose into the extracellular fluid, even in induced hypoglycaemia.

Interaction between glucose availability and acetylcholine levels

The important role of glucose in the brain, apart from energy production is as a precursor for several neurotransmitters, including gamma-aminobutyric-acid, glutamate and acetylcholine. Acetylcholine (ACh) is an excitatory neurotransmitter produced from choline and acetylcoenzyme A, the latter of which is a derivative of the Krebs' cycle, or glucose metabolism.

Dolezal and Tucek (1982) demonstrated that atropine induced reduction in acetylcholine content in the caudate nucleus (involved in learning and memory) was attenuated by a glucose injection. Similar observations in brain areas such as the striatum (planning of movement and cognitive control) and hippocampus (short term memory and spatial navigation) are outlined by Messier (2004).

Peripheral Mechanisms of Glucose action on cognition

Messier (2004) proposes that a peripheral mechanism may have an impact upon cognition, where a detection mechanism elsewhere in the body may send a neural signal to the brain, influencing physiological processes in the brain. Stimulation of

the vagus nerve has been observed to affect cognitive function under certain conditions, and it is possible that changes in the liver could result in such effects (Messier 2004). Glucose sensitive neurons have been identified in the hypothalamus (Anand, Singh et al. 1964). These neurons are thought to utilise an ATP sensitive K+ channel (K_{ATP}). When extracellular glucose increases, intracellular glucose increases, resulting in an increase in glucose metabolism and ATP production, which inactivates the K_{ATP} channel, increasing intracellular K+ rendering the cell more sensitive to depolarisation and neurotransmitter release.

1.2.1.2 Caffeine

Caffeine use

Due to its psychoactive properties, there has been much study into caffeine's use (Roberts and Barone 1983; Barone and Roberts 1996), and its impact on mental functioning (Brice and Smith 2002; Haskell, Kennedy et al. 2005; Childs and de Wit 2006). With caffeine occurring in coffee, tea, soft drinks (from the cola bean, guarana, or as an additive), the cocoa bean and some prescription medications (Smit and Rogers 2002), it has been estimated that 80% of the population of the USA regularly consume caffeine; in 1998 95% of the UK population, and in 1991 91% of the Danish population were reported to have consumed at least one caffeinated beverage during a 7-day survey (Barone and Roberts 1996).

The main mechanism by which caffeine exerts its stimulant effect is by competitive inhibition of adenosine (a retrograde neurotransmitter which inhibits pre-synaptic release of excitatory neurotransmitters, which is the final breakdown product of adenosine tri-phosphate, the 'cellular energy currency') at the A_1 and A_{2a} adenosine receptors, due to their similar structures (structures shown in figure 1.5). This results in alterations in the release and functional turn-over of neurotransmitters, most importantly dopamine, but also serotonin, acetylcholine, glutamate and GABA. Other theories of the mechanism of action for caffeine's stimulant effects are mobilisation of intracellular calcium in striated muscle, inhibition of phosphodiesterases and antagonism of benzodiazepine (Boulenger, Patel et al.

1982). Figure 1.6 shows the impact of caffeine on each of these mechanisms, showing that the only mechanism significantly affected by non-toxic levels of plasma caffeine is inhibition of adenosine, however the other mechanisms may play a minor role.

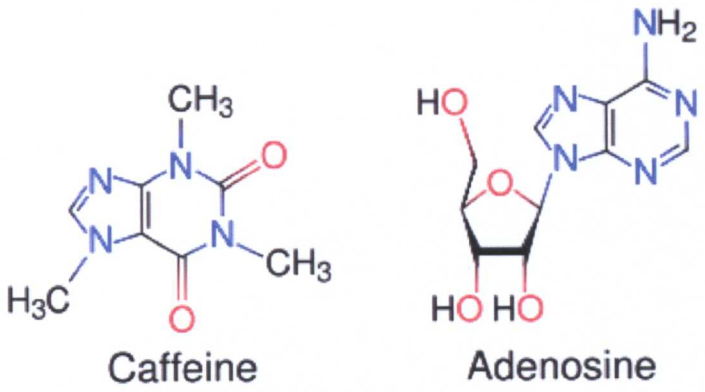

Figure 1.5: Structure of Caffeine and Adenosine

Pharmacology of caffeine

Caffeine is rapidly and almost completely (99%) absorbed from the gut into the bloodstream (Arnaud 1993). With doses of 250mg and 500mg Kaplan (1997) found that peak plasma caffeine concentrations were reached at 0.65 and 0.5 hours respectively following administration. Caffeine is distributed evenly throughout body water and even crosses the blood-brain and placental barriers. Its clearance has been observed to be increased by smoking and nicotine intake, and slowed in the second and third trimesters of pregnancy (Arnaud 1993).

Physiological effects of caffeine

Arousal Caffeine has been observed to exert an increase in both systolic and diastolic blood pressure and a decrease in heart rate at doses of 90mg, 250mg (Kourtidou-Papadeli, Papadelis et al. 2002), 150mg, 450mg (Childs and de Wit 2006), 2mg/kg body weight (Mikalsen, Bertelsen et al. 2001) and 5mg/kg body weight (Battram, Graham et al. 2005). An increase in noradrenaline release at sympathetic nerve terminals as a result of caffeine's inhibitive action at adenosine receptors explains the increase observed in systolic and diastolic blood pressure due to inhibition of vasodilatory effects of adenosine (Battram, Graham et al. 2005). One of three possible mechanisms could reduce heart rate: direct vagal

stimulation, baroreceptor reflex as a response to increased blood pressure or an effect on the sino-atrial node (Kourtidou-Papadeli, Papadelis et al. 2002). Mikalsen *et al.* (2001) also reported that participants' skin conductance had increased following caffeine consumption, consistent with results found by Davisdon (1991).

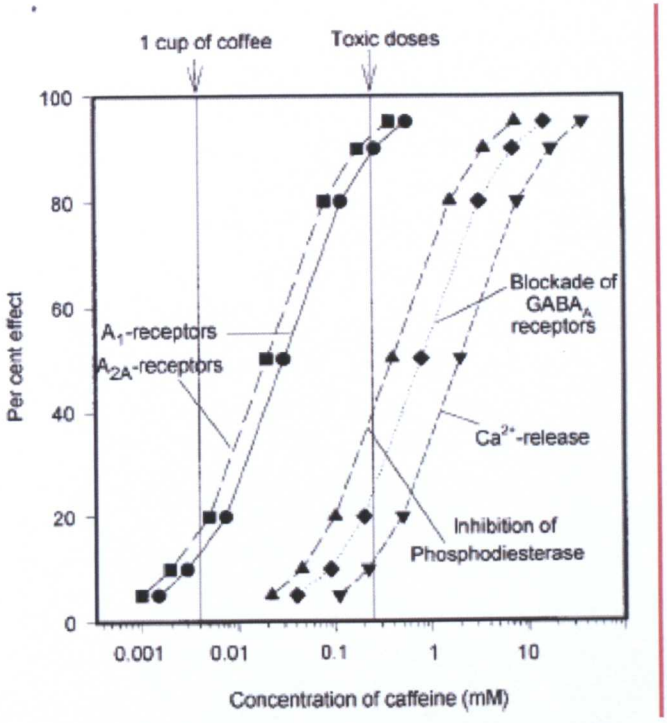

Figure 1.6: Effect of caffeine on different biochemical targets in relation to its levels in humans. Note that caffeine can significantly block effects of adenosine at low concentrations found in coffee (approx 75mg), but only affects other biochemical targets at toxic or close to toxic doses (adapted from Fredholm 1980).

Behavioural effects of caffeine

The raised action of excitatory neurotransmitters and physiological arousal would be expected to lead to improvements in performance of psycho-behavioural tasks. Reaction time is a very useful manner of assessing arousal and attention, and RT tasks have been used in many studies to assess caffeine's cognitive effects. At low doses, caffeine has been observed to decrease reaction time: a 200mg dose and 4x65mg doses (added to decaf coffee) over 5 hours both reduced SRT compared with decaffeinated coffee (Brice and Smith 2002); Haskell *et al.* (2005) found a main treatment effect with 75mg and 150mg doses and a significant decrease in SRT with the 75mg dose in a double-blind placebo controlled study; Smit *et al.* (2000)

reported a main treatment effect of caffeine and a significant effect at doses of 12.5mg, 50mg and 100mg compared with a placebo, which were most significant at 45 minutes post consumption. An interesting finding by Robelin and Rogers (1998) was no improvement in SRT with caffeine (one, two or three 1.2mg/kg doses), but a significant decline in SRT in the placebo group 45 minutes following meal consumption. Attwood *et al.* (2007) found no overall effect of a 400mg caffeine dose on SRT performance compared with a placebo, but habitual high caffeine consumers did perform better following supplementation, whereas low consumers performance declined following caffeine consumption.

Caffeinated drinks are used by many drivers to combat tiredness when driving (Anund, Kecklund et al. 2008), and for this reason the effects of caffeine consumption on driving performance in sleep deprived participants has been investigated. In the first such study participants were given either 200mg caffeine, or a placebo (both peppermint flavoured, delivered in a double blind, counterbalanced design), and asked to follow a car (in a driving simulator) closely for 90 minutes, before being given a repeat dose of the treatment and continuing to drive in the simulator for another 90 minutes (Regina, Smith et al. 1974). Performance was evaluated by response time to the front car decelerating, response time to the front car accelerating, response time to the presentation of a high-beam signal, and error in response to the high-beam signal. Caffeine supplementation improved response times in all response time measurements significantly, varying between being 12-32% quicker in each task compared with the placebo. Error rate in the high-beam task was reduced significantly following both the initial (30%) and repeat (50%) caffeine supplements when compared with placebo. Brice (2001) found a decrease in steering variability (fluctuations in steering travel from the centre in either direction, measured in degrees) in a driving task following consumption of 3mg/kg body weight caffeine added to decaffeinated coffee, compared with decaffeinated coffee with water added.

Effects of caffeine on Mood

Due to its effect on neurotransmitter activity (particularly serotonin), caffeine has been reported to have significant effects on mood and emotion, however this can be very difficult to investigate, due to the effects of caffeine withdrawal, which can lead to adverse effects on mood with short periods of abstinence (Silverman, Evans et al. 1992; James and Rogers 2005). Doses of caffeine around those found in around 0.5-3 cups of coffee (32mg-300mg) have consistently been shown to increase alertness, arousal and decrease mental fatigue and tiredness; other effects are an increase in happiness, calmness, jitteriness, anxiety and feeling tense and decreased thirst (Warburton 1995; Smit and Rogers 2000; Mikalsen, Bertelsen et al. 2001; Brice and Smith 2002; Haskell, Kennedy et al. 2005; Haskell, Kennedy et al. 2008). However some studies have found caffeine to have no effect on mood scores (Loke, Hinrichs et al. 1985; Lieberman, Wurtman et al. 1987). Hasenfratz (1994) demonstrated that these mood effects can be dose dependant, with low doses resulting in improved mood, and high doses (6mg/kg body weight) had negative mood effects, suggesting that the dose-response effect of caffeine consumption on arousal follow the inverted U theory of arousal. The idea that arousal is linked to task performance, and that there is an optimum level of arousal, above which performance will begin to decline is called the Yerkes-Dodson law (Yerkes and Dodson 1908).

Expectation effects can also influence the mood effects of caffeine. Mikalsen (2001) gave participants caffeinated or non-caffeinated drinks and told some from each trial group that they had received caffeinated drinks, and some that they had received non-caffeinated drinks. When participants were told they had been given a caffeinated drink, a significant decrease in calmness was observed. Silverman and Griffiths (1992) reported that participants showed no mood effects at caffeine levels they couldn't detect, but once they could identify a drink as containing caffeine correctly, they reported positive mood effects.

1.2.3 Cognitive effects of energy drink flavourings

Ginkgo biloba has been observed to influence neurotransmitter systems and cellular metabolism (Ramassamy, Girbe et al. 1995; White, Scates et al. 1996), improving speed of attention (as measured within a test battery) most significantly at doses of 360mg, 2.5 hours post-consumption (Kennedy, Scholey et al. 2000). A 400mg dose of *Panax ginseng* improved performance in secondary memory and quality of memory assessed by a computerised cognitive assessment battery at 4 hours following treatment. A 360 mg dose of *G. biloba* combined with 600mg *P. ginseng* improved secondary memory and quality of memory at 1 hour and 2.5 hours following treatment, as well as working memory at 1 hour and 6 hours post-treatment.

Kennedy *et al.* (2003) investigated the influence of *Melissa officinalis* (Lemon balm; a herbal medicine that has been traditionally attributed with memory enhancing properties and is often used as a sedative) on measures of mood and cognitive performance. A decrease in speed of memory was observed following administration of all three doses (600 mg, 1000 mg and 1600 mg) at 3 hours following treatment (compared with placebo). The 1000mg dose produced the most significant effect at this time, and at 6 hours post-treatment. Following the 1600mg dose, participants scored significantly better at 3 and 6 hours post-treatment than following administration of the placebo.

However, a study into the effects of EDs and individual components of the drinks found no effect of the herbal/flavouring extracts. This study concluded that herbal extracts would contribute very little to any overall cognitive effect of the full drink as they were administered at doses of 12.5mg *P. ginseng* and 2.004mg *G. biloba* (the approximate level found in typical EDs), approximately 1-3% of the doses administered in the studies above.

1.2.4 Energy Drinks and their Cognitive Effects

1.2.4.1 Energy Drink Consumption

In recent years there has been widespread growth in the use of energy drinks (EDs) to combat tiredness or raise alertness, particularly by students and shift workers (Malinauskas, Aeby et al. 2007; Hofmeister, Muilenburg et al. 2010). With evidence pointing to beneficial effects of both caffeine (at realistic consumption levels) and glucose consumption on cognitive task performance, an increasing amount of study is being carried out into the psychological impact of ED consumption. The postprandial effects of these drinks have been well documented (that is not to say the effects are clear); a recent review of these effects (Van den Eynde, Van Baelen et al. 2008) found 14 publications investigating the impact of energy drinks, 71 investigating the effects of caffeine and 79 looking at the effects of glucose on cognitive functioning.

1.2.4.2 Effects of Energy Drinks on Physiological Measures

ED consumption has been shown to have varying effects on heart rate. Alford (2001) found heart rate increased with ED consumption in one study, but no change in another. Scholey *et al.* (2004) found no effect of ED consumption on heart rate. This study did however find that a glucose treatment increased heart rate, and a caffeine treatment resulted in a decrease in heart rate (consistent with reviews of effects of glucose and caffeine in sections 1.2.1.1 and 1.2.1.2 respectively). These effects combined would result in no change in heart rate. ED consumption has been shown to increase systolic blood pressure (Alford, Cox et al. 2001; Rao, Hu et al. 2005), consistent with effects of caffeine.

Measuring of event related potentials (ERPs) allows the observation of speed of neuron activity. A slower RT and P3 latency have been observed following placebo treatment compared with combined caffeine, taurine and glucose supplementation (Seidl, Peyrl et al. 2000). Specterman *et al.* (2005) observed an increase in evoked potential area with caffeine, glucose and ED supplementation. The fact that

maximal ulnar nerve stimulation remained constant over time following consumption of an energy drink (Specterman, Bhuiya et al. 2005) suggests an attenuation of effects of fatigue, however this was not measured in any participants in the placebo group.

1.2.4.3 Cognitive effects of Energy Drink Consumption

Energy drink consumption and mood

ED consumption has been shown to have significant beneficial effects on energetic arousal, subjective alertness, mental fatigue and hedonic tone as assessed by mood questionnaire (Alford, Cox et al. 2001; Smit and Rogers 2002). An interesting finding was that presence of carbonation improved ratings of 'feeling awake' in the long term, but tended to decrease any immediate energising effects assessed by mood questionnaire (Smit, Cotton et al. 2004). A further experiment by Smit *et al.* (2004) demonstrated that a carbonated energy drink improved assertiveness up to 30 min post treatment, letter search task performance at 61 minutes post treatment, improved feeling cheerful and decreased feelings of tense, clearheaded and tiredness at 73 minutes post treatment compared with a non-carbonated energy drink, the author explaining that these effects may have been caused by the carbonation slowing the absorption of caffeine.

Energy Drink consumption and cognitive performance

EDs have been shown to affect a range of cognitive processes, most significantly focused attention (Mucignat-Caretta 1998; Alford, Cox et al. 2001) and sustained attention (Smit and Rogers 2002; Kennedy and Scholey 2004). Adan and Serra-Grabulosa (2010) observed significant positive effects of combined consumption of glucose and caffeine on RT in simple and systematic reaction time tasks, but not on choice reaction time. EDs have been observed to reduce RTs in several studies ranging from 30-60 minutes following consumption (Seidl, Peyrl et al. 2000; Alford, Cox et al. 2001; Smit and Rogers 2002; Smit, Cotton et al. 2004), however Anderson and Horne (2006) and Smit and Rogers (2002) found increased RT at 60-90 minutes

following ED consumption. Alford *et al.* (2001) found an improvement in choice reaction time following consumption of Red Bull.

Reyner and Horne (2002) investigated the influence of ED consumption on driving simulator performance in fatigued participants, and recorded 75% fewer driving 'incidents' (a driving incident occurred when one or more wheels crossed a lateral road marking) and lower ratings of perceived sleepiness in the group receiving the energy drink. Another study investigated the impact of ED consumption on driving performance and fatigue compared with a control drink (similar to the ED with the active ingredients removed) and a secondary manual dexterity/mastication task (MD/MT) and the time-of-day effects of these treatments when measurement was repeated in morning and evening sessions conducted on the same day (Gershon, Shinar et al. 2009). They found that after consuming the ED, participants were able to maintain their lane position better than when consuming the control drink or performing the MD/MT, however performance in this measure deteriorated over time with all treatments. In the morning session ED consumption also reduced the variability in steering compared with other groups, however no effect was observed in the evening session. Reaction time was reduced by ED consumption in both morning and evening sessions compared with the other treatments, however a time of day interaction was observed in the other groups, with lower reaction time observed in the evening session than in the morning. Subjective sleepiness and fatigue was measured using the Swedish Occupational Fatigue-20 Inventory, with the MD/MT resulting in lower ratings than both other treatments. ED treatment resulted in low ratings for sleepiness and fatigue in the morning, but not in the evening session, whereas ratings in the control treatment were high in the morning and low in the evening. These results suggest that whilst energy drinks do improve ability to pay attention in the short-term, in the long-term (i.e. having a second ED hours after the first one) ED consumption does not tend to improve driving ability and actually increases ratings of sleepiness and fatigue.

Energy drinks also appear to have an impact on memory performance. Scholey and Kennedy (2004) observed a positive improvement of quality of memory and secondary memory but not working memory. Alford (2001) found that those who had consumed Red Bull ED improved in an immediate recall task. Smit (2004) found no effect on immediate and delayed word recall tasks with ED supplementation compared with four placebos (one with no caffeine or carbohydrate, one with no caffeine, one with no carbohydrate and one with no carbonation).

Kennedy and Scholey (2004) investigated the influence of ED consumption on a repeated 10-minute battery of information processing tasks, and found that the drink attenuated the effects of mental fatigue, allowing participants to perform better at 30-69 minutes in a visual information processing task than if they had received a placebo drink (the placebo drink is not specified, but is described as a 'vehicle placebo' and likely contains the flavourings associated with an ED).

A study investigating the impact of EDs alone and in combination with alcohol found a non-significant tendency for reduced scores in visuospatial and language task performance following ED consumption, with performance in these tasks significantly poorer when the ED was consumed with alcohol compared with the control drink.

Specterman *et al.* (2005) used transcranial magnetic stimulation to produce motor-evoked potentials (MEP) in the thenar muscle in the right hand of participants who had consumed a 380 ml bottle of Lucozade Energy, finding that MEP area was increased at 30 and 60 minutes following drink consumption, and that MEP area was correlated with plasma glucose concentration. These results suggest greater corticospinal excitability following ED consumption, probably due to the influence of ED ingredients on neurotransmitter levels. Rao et al. (2005) measured event related potentials (ERPs) using electroencephalography (EEG) related to information processing in the brain in participants who were performing a task measuring selective attention. ED consumption improved measures of both RT and accuracy in task performance, as well as significantly affecting the ERP recordings.

Enhancement of early components of the ERP (C1/P1; reflecting visual cortical processing) as well as N1, N2 and P3 components, which appear to be involved in the decision making process (Di Russo, Martinez et al. 2002).

To date only one behavioural study investigating the combined effects of caffeine and glucose has incorporated brain imaging technology. Serra-Grabulosa, Adan *et al.* (2010) investigated the influence of glucose, caffeine and glucose + caffeine on performance in a sustained attention task. No impacts of treatments were observed on cognitive performance, but decreased activation was observed in the left prefrontal cortex and bilaterally in the parietal lobes, possibly indicating an increase in efficiency in attention and working memory processes, with the glucose + caffeine treatment.

1.3 Sensory impact of energy drinks

1.3.1 Taste and flavour perception and convergence of sensory pathways

The intake of food is the manner by which all animals acquire energy, and the perception of taste and smell (and also appearance and texture) allow the animal to decide whether or not to accept particular foodstuffs (Scott and Verhagen 2000). Foods containing energy often evoke pleasant sensations when perceived by the chemical senses, which rewards and encourages the animal to consume more of a particular foodstuff in order to acquire more energy (Li, Glaser et al. 2009). Mechanisms for the sensory perception of calorific sugars (leading to reward than non-calorific sweeteners, see below; Chambers, Bridge et al. 2009) and free fatty acids (Kock, Blaker et al. 1992; Herness and Gilbertson 1999; Rolls, Critchley et al. 1999; Mattes 2011) have been observed. In fact visual , auditory and olfactory sensory information also play a very important role in the digestion and absorption of nutrients prior to taste stimulation, with these cues preparing the gut and increasing saliva and production as observed by Pavlov in his classical study on conditioned response (Pavlov 1910; Smeets, Weijzen et al. 2011). Rolls (2007) reviewed evidence of sensory processing in the brain relating to food intake,

describing in detail the convergence of different sensory pathways (taste, olfaction, vision and touch) in brain regions such as the orbitofrontal cortex and amygdala, which have projections into areas responsible for behaviour including the striatum and anterior cingulate cortex, see figure 1.7. Chemosensory perception of food involves the perception of taste and trigeminal sensations in the oral cavity and of aromatic compounds in the nasal passage, with flavour perception often being described as the interaction between taste and smell (Small, Bender et al. 2007).

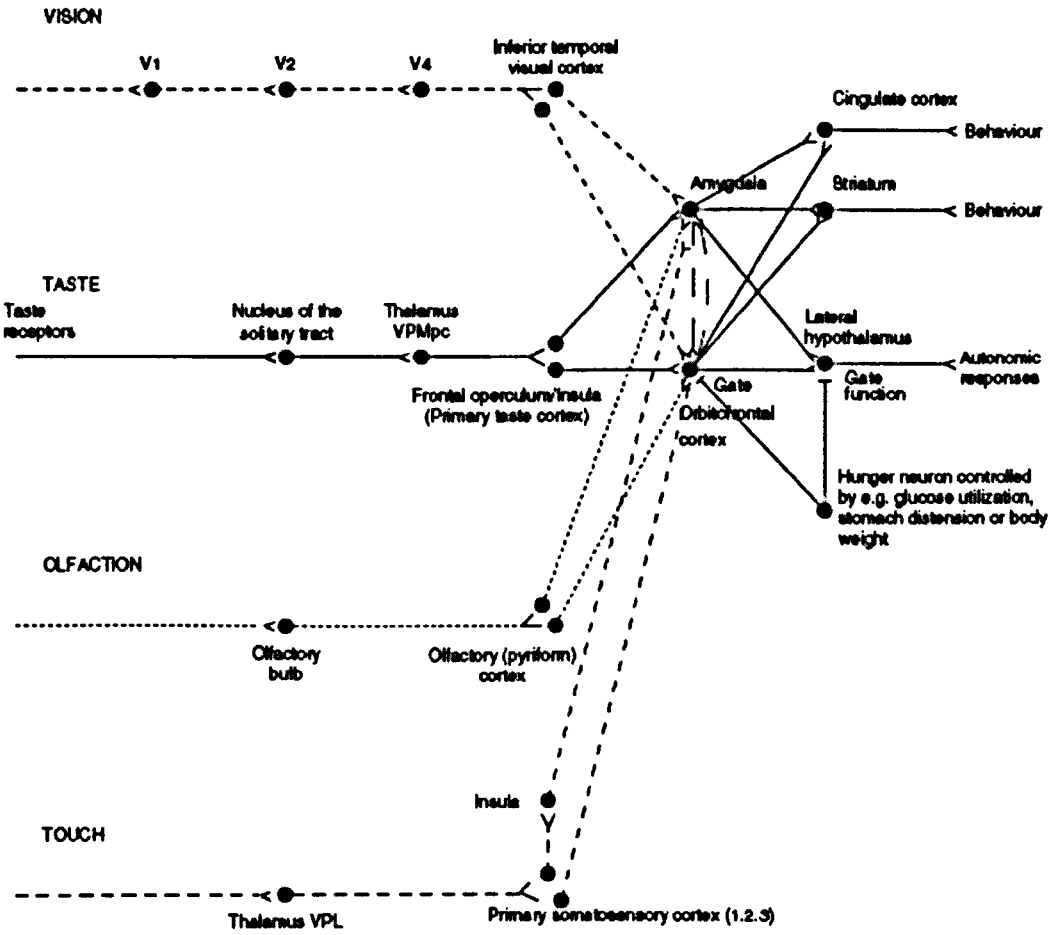

Figure 1.7: A schematic diagram of the taste and olfactory pathways in primates, including man, showing how they converge with each other and with visual pathways. Hunger modulates the responsiveness of the representations in the orbitofrontal cortex of the taste, smell, texture and sight of food (indicated by the gate function), and the orbitofrontal cortex is where the palatability and pleasantness of food is represented. V1, V2, V4, visual cortical areas; VPMpc, ventral posteromedial nucleus pars parvicellularis; VPL, ventral posterolateral nucleus.

Taste

Taste involves the detection of stimuli that are dissolved in water, oil or saliva, by receptors on the surface of the tongue. These receptors occur in clusters called

taste buds located on the surface of fungiform papillae, circumvallate papillae and the soft palate on the roof of the mouth. When innervated, these receptors communicate with the brain via the chorda tympani branches of the facial nerves (fungiform papillae), the glossopharyngeal nerves (rear of the tongue) and the vagus nerve (posterior areas of the tongue root). Although the classical view is that different regions of the tongue are responsible for perceiving different taste qualities, any of the five taste qualities (sweet, sour, salt, bitter and umami) can be perceived on any area of the tongue (Sato, Endo et al. 1995).

1.3.2 Glucose and sweet taste perception

Perception of sweet taste is mediated by the T1R2 and T1R3 receptors on the tongue (Damak, Rong et al. 2003; Zhao, Zhang et al. 2003) which respond to several naturally occurring sweet substances as well as a number of artificial sweeteners (Li, Glaser et al. 2009). Damak et al. (2003) found that knockout mice lacking the T1R3 receptor showed no preference for artificial sweeteners and actively avoided them at high concentrations, implying that the T1R3 receptor is responsible for perception of sweet taste in artificial sweeteners, but another receptor may mediate a bitter aftertaste in high concentrations. The knockout mice did however display behavioural and/or gustatory nerve responses to the sugars that were tested (sucrose, glucose and maltose), and although they were slightly diminished, this does suggest there is some other mechanism or receptor responsive to sugars, leading to preference of sugars.

Li et al. (2009) investigated the preference for twelve different sweet stimuli in six animal species using 2-bottle 24-hour preference tests, with one bottle containing the test compound in solution and the other containing tap water. Five of the six species (lesser panda, domestic ferret, haussa genet, meerkat and yellow mongoose) showed a strong preference for sucrose, maltose and glucose over water, with two of those (domestic ferret and yellow mongoose) also showing a preference for fructose. The lesser panda also had a preference for lactose, galactose, neotame, sucralose and aspartame, the only animal to display any

preference for artificial sweeteners. The sweet compounds that animals have a preference for must endow some rewarding factor encouraging uptake of the particular solution.

In a review, Hayes (2008) discusses the evidence supporting multiple mechanisms coding for sweet taste perception both in animals and humans, and two experiments published by Chambers *et al.* (2009) support this hypothesis. From an evolutionary perspective, it may be beneficial to have multiple sweet receptors that elicit different neural responses resulting in a different reward response (and therefore different behavioural response, i.e. raised arousal/attention or working memory allowing a hunter-gatherer to search for more of the food, and improved encoding to long-term memory to recognise the food in the future) for different sweet compounds, encouraging the consumption of those sugars which are calorific.

As discussed above, behavioural influences of sweet taste perception (saccharin) have been observed in rats (Stefurak and Vanderkooy 1992), Messier (2004) states that available evidence suggests this does not occur in humans with regards to memory, however taste perception of sweet stimuli has been observed to influence other behavioural constructs. Blass and Shah (1995) observed that sucrose significantly decreased pain perception in newborn babies having blood collected using the heel-lance procedure. These effects indicated that stimulation of gustatory afferent neurons could influence opioid-mediated efferent nerve conduction. These effects appear not to be limited to sucrose, as Barr, Pantel *et al.* (1999) observed similar effects with both sucrose and aspartame. Lewkowski *et al.* observed similar analgesic effects in response to sweet taste perception in adults, with increased pain tolerance, though no change in pain ratings during a cold pressor test (CPT), where participants were required to hold their hand in cold water (Lewkowski, Ditto et al. 2003; Lewkowski, Young et al. 2008).

Frank et al. (2008) studied the brain regions activated by sucrose and the artificial sweetener sucralose using fMRI, finding that whilst the primary and secondary taste

cortices were activated by both stimuli, perception of the sucrose stimuli also activated the anterior cingulated cortex, the dorsolateral prefrontal cortex and caudate nucleus, areas which are involved in the selection of appropriate response/behaviours and determining reward value of rewarding stimuli.

Carter *et al.* (2004), Rollo *et al.* (2008) and Chambers *et al.* (2009) have all found that rinsing a carbohydrate solution in the mouth has beneficial effects on exercise performance (1-hour cycle time trial, 30 minute treadmill run and 1-hour cycle time trial respectively) when the solution contained calorific sugar, with no effect when the solution contained artificial sweeteners, even when solutions were taste-matched.

Chambers *et al.* (2009) studied the impact of sweet taste perception not only on exercise performance but also on brain activation using fMRI. With evidence that intravenous infusion of glucose had no impact on performance in a cycling time-trial (Carter, Jeukendrup et al. 2004), and a large body of evidence suggesting that oral ingestion of glucose improves exercise performance, Chambers *et al.* hypothesised that a central neural response from an oral carbohydrate stimulus may have behavioural consequences. In their first experiment, Chambers *et al.* investigated the impact of a glucose (plus artificial sweetener) mouth rinse on performance in a cycling time trial compared with the impact of a mouth rinse containing only artificial sweeteners, finding a significant decrease in time taken to complete the trial when rinsing the mouth with a glucose solution compared with no treatment and artificial sweetener treatments. As the drink was rinsed but not swallowed, and participants were blind to what treatment they received, this effect must have been caused by perception of the drink in the mouth. This, combined with brain imaging data both from this study and previous studies by Frank *et al.* (2008), de Araujo *et al.* (2003) and O'Doherty (2001) that display greater brain activation in response to glucose/sucrose perception than for artificial sweeteners, suggests the presence of an oral nutrient receptor that is activated by the energy content of carbohydrates. This led Chambers *et al.* (2009) to run a second exercise performance study,

investigating the influence of a non-sweet but energy containing maltodextrin solution on cycling time trial performance. Again improved performance was observed, and brain imaging data showed similar brain activation to the glucose treatment, suggesting that perception of sweet taste is not necessarily important to the effect that the glucose mouth rinse had.

The explanation of this effect is that, as stated above calorific sugars activate sweet taste receptors in the mouth differently from artificial sweeteners (Damak, Rong et al. 2003) and also activate taste pathways in the brain differently (Frank, Oberndorfer et al. 2008), resulting in greater activation in the dorsolateral prefrontal cortex, anterior cingulate cortex and ventral striatum which are believed to mediate behavioural and autonomic responses to rewarding stimuli, or in other words the artificial sweetener does not fully satisfy the desire for ingestion of energy in the form of sweet sugars.

1.3.4 Impact of olfactory stimuli on performance

Olfactory stimuli reach the olfactory receptors located in the epithelium of the nasal cavity in one of two manners: through normal inspiration of air into the nasal cavity, and retronasally, when odours present in the mouth reach the nasal cavity during the consumption of food/drink (Pierce and Halpern 1996). Retronasal perception of odours mediates the perception of flavour, as taste and olfactory stimuli are normally perceived together. There are several million olfactory receptors, which are highly ciliated to allow such a large area for interaction with odourants, that when activated send information to the glomerular structures in the olfactory bulb. From here the olfactory nerves project to many different areas of the brain.

Castiello *et al.* have conducted a series of studies investigating the impact that perception of olfactory stimuli has on performance of reach-to-grab movements, with the hypothesis that interaction of different sensory modalities (olfaction and vision) would influence the task performance. One study examined the influence that six odourants (almond, garlic, strawberry, apple, orange and peach; the former three representing the odour of small objects, the latter three representing the

odour of large objects) had on the hand aperture of reach to grab movements when participants were asked to reach towards a visual stimuli which could be one of six plastic objects (each object matching one of the olfactory stimuli listed above), which either matched the olfactory stimulus with regards to size (congruent) or did not match (incongruent) (Castiello, Zucco et al. 2006). No impact of olfactory stimuli was observed in the congruent condition; hand aperture when stimuli were congruent was no different to that when no olfactory stimulus was presented, in the both visual stimulus conditions. In the incongruent trials, hand aperture matched more closely the size of the odourant and not the visual target, suggesting that cross-modal interference does indeed play a role in reach to grab movements, and perception of olfactory stimuli may evoke/influence behavioural responses to particular stimuli. A further study (Tubaldi, Ansuini et al. 2008) investigated the influence of four stimuli (apple, orange, strawberry and almond) on the duration of the grasping movement in the same task as above. This study demonstrated that reaching duration was shorter for large visual targets than for small visual targets, and that presence of an odour, irrespective of congruency, decreased reaching time. Reach duration was also shorter in the congruent trials than the incongruent trials, again indicating that olfactory information does have a specific influence on performance of the task, however the fact that even incongruent odours improved performance may suggest there is also an influence of odour perception on levels of alertness.

1.4 Aims of research

This thesis aims to examine and identify any potential sensory impact of taste/flavour perception, hypothesising that sensory perception of energy drinks can elicit behavioural effects, either through the presence of calorific sugars acting upon an oral nutrient receptor responsive to the energy content of carbohydrates or via the reward value of the sweet taste or pleasant flavour, and compare them with the well documented effects of energy drink consumption observed postprandially. In order to examine cognitive performance effects of energy drinks

sensorially and postprandially, the methods and techniques available were investigated thoroughly to develop protocols that would allow measurement of these potential effects, discussed in chapter 2. The thesis then aims to discuss the postprandial effects of energy drink consumption on measures of attention, decision making and mood (chapter 3) before a comparison of both the postprandial and sensory responses on similar measures of performance (chapters 4 and 5). As it was hypothesised that sweet taste mediated the observed alterations in cognition, chapter 6 aims to examine the influence of glucose perception on attention and decision making compared with perception of a combination of the non-calorific sweeteners aspartame and acesulfame K.

CHAPTER 2: CONSIDERATIONS FOR THE MATERIALS AND METHODS AND THE DEVELOPMENT OF PROTOCOLS TO BE USED IN THE PROJECT

Westenhoefer *et al.* (2004) and Schmitt *et al.* (2005) both reviewed the methodological considerations that must be accounted when designing a study seeking to assess the effects of nutritional interventions on cognitive performance, and Edgar, Pace-Schott *et al.* (2009) reviewed approaches to measuring the effects of 'wake-promoting drugs' on cognition; the findings of these reviews are discussed in this chapter with regards to the aims of the project as a whole and relating to the literature discussed in chapter 1. In this instance – investigating the impact of the sensory and postprandial properties of EDs on measures of cognitive function – factors requiring particular consideration are:

- Participant inclusion/exclusion criteria.
- Dietary restrictions and physiological (fed/fasted) state of participants on study days.
- Effects of caffeine withdrawal.
- Appropriate cognitive assessment measures
- Appropriate test treatments, including experimental and placebo/control treatments.
- The manner in which these test treatments are administered.

2.1 Participants

2.1.1 Inclusion/exclusion criteria

Westenhoefer *et al.* (2004) state in their conclusions that the target population is an important part of any claim that a food has functional properties. For example some effects may only be observed in particular populations, or some claims may only be relevant for certain populations. As stated in the introduction, energy drinks are consumed mainly by those who feel they are in need of energy or may need to avoid feeling fatigued, such as students (Miller 2008; Miller 2008; Reissig, Strain et al. 2009; Hofmeister, Muilenburg et al. 2010), shift workers (Gander,

Barnes et al. 1998) and drivers (van den Berg and Landstrom 2006), and these populations have been used regularly in studies into cognitive impact of these drinks.

Cognitive performance can be influenced by a number of factors, and in order to maintain validity of the studies, participants were recruited from the undergraduate and postgraduate population at the University of Nottingham, excluding those who were pregnant (it might be unethical to monitor the effects of caffeine supplementation in pregnant women; Food Standards Agency 2008), diabetic (as energy drinks contain high glucose levels), colour blind (some cognitive tasks require that different colours can be perceived in order to make a response) or smokers (nicotine improves performance in tasks of attention; Rycroft, Rusted et al. 2005). In addition to controlling for the population from which the participants originate, it is also important to consider the state they are in when they arrive to participate in the study, with particular regard to caffeine and food intake prior to arrival.

2.1.2 Dietary restrictions

2.1.2.1 Food

As discussed in chapter 1, the ingestion of macronutrients alters cognitive performance over the postprandial period (Bellisle, Blundell et al. 1998; Dye and Blundell 2002; Hoyland, Lawton et al. 2008), so the physiological state (fed/fasted) that participants arrive in for experimental sessions must be considered. Martin and Benton (1999) studied the impact of glucose drinks on the memory of subjects who had either fasted overnight or eaten breakfast. Facilitating effects were only observed in those who had not consumed breakfast. While investigating the impact of glucose on memory and learning task performance, Sunram-Lea *et al.* (2001) also looked at the effect of fast-duration, prior to attending and time of day effects. Improvements in both learning and memory performance were observed in groups who had fasted, or eaten a standard breakfast or a standard lunch 2 hours prior to attending. As these results were able to display a significant influence of glucose

only two hours following breakfast, it seems that participants in a study looking at effects of acute supplementation of foodstuffs can be asked to have a small meal made up of food they would normally eat in the morning, but not to eat for two hours prior to attendance without preventing alerting effects to be masked.

2.1.2.2 Caffeine

Reversal of withdrawal effects has been cited as the main cause of the cognitive benefits observed following caffeine consumption (James 1997; Heatherley, Hayward et al. 2005; James and Rogers 2005; Rogers, Heatherley et al. 2005; James and Keane 2007; Keane, James et al. 2007). A critical review of 57 studies into the effects of caffeine withdrawal found that its effects included headaches, tiredness/fatigue, decreased alertness, flu like symptoms, depressed mood, difficulty concentrating, irritability, decreased clearheaded feelings, yawning and confusion (Juliano and Griffiths 2004). Impaired behavioural/cognitive performance was observed with caffeine abstinence in 11 of the 23 studies where it was assessed, with reversal of these effects occurring within 30-60 minutes following administration of caffeine (Goldstein, Kaizer et al. 1969; cited by Juliano and Griffiths 2004). Positive effects of caffeine consumption have been observed in studies comparing the alerting effects of caffeine with and without caffeine abstinence (Warburton 1995) and in habitual consumers compared with non-habitual consumers (Haskell, Kennedy et al. 2005; Childs and de Wit 2006; Smith, Christopher et al. 2006).

For validity it is important that for all participants instructions are kept the same, so participants can either be asked to abstain from caffeine consumption overnight, or to consume habitual caffeinated products prior to arrival. The former might lead to false positive effects due to the reversal of caffeine withdrawal, however the latter may result in false negatives, with the Yerkes-Dodson law suggesting that increased arousal past an optimum point would not benefit cognitive performance and may in fact have negative effects (Yerkes and Dodson 1908). In the present studies it was decided that it was best to ask participants to abstain from caffeine consumption

overnight, but to monitor for effects of habitual caffeine consumption levels and their possible interactive influence on the effects of treatments.

2.2 Assessment of cognitive functions

Schmitt *et al.* (2005) discuss the importance of the sensitivity of the particular outcome measure(s) to alterations in cognitive functioning that may occur in response to interventions, with particular regard for floor/ceiling effects, the presence of which may mask any potential impact as the scope for improvement/decline in performance may be removed. For this reason task difficulty should be adjusted to the appropriate level for the study population. As discussed in chapter 1, many different tasks have been used to investigate the impact of energy drinks and their ingredients on cognitive performance throughout the literature. As there has been little study into the alerting effects that sensory perception of a foodstuff may have, it is difficult to hypothesise what tasks may be sensitive to these effects using the literature, however as this project is concerned with the potential impact of the sensory properties of EDs, it would make sense to consider tasks that have been shown to be affected by EDs postprandially, as well as the brain regions activated by perception of energy drink ingredients and what behavioural functions may be influenced as a result. Table 2.1 reviews cognitive tasks and functions that have been employed/assessed in 15 published studies investigating cognitive functioning in the postprandial period in response to ED treatment.[1]

2.2.1 Simple Reaction Time

Simple reaction time tasks (SRTT) ask participants to respond as quickly as possible to either one stimulus that is repeated or various stimuli with only one response (Mucignat-Caretta 1998; Horne and Reyner 2001; Smit and Rogers 2002; Smit, Grady et al. 2006; Adan and Serra-Grabulosa 2010). These tasks typically have very

[1] Many studies do not directly specify the ingredients used to create the energy drink or placebo treatments. Information regarding the glucose and caffeine content has been included where available. The abbreviation ED is used where full ingredients are not listed, or are stated to be a combination of glucose, caffeine and herbal extracts commonly found in energy drinks. The term placebo is used where soft drinks containing none of the active ingredients are used as a control.

high accuracy and the shortest reaction times due to the lack of information processing necessary (Donders 1869), meaning that ceiling effects may be observed as there is little scope for an improvement in performance. Beneficial effects of ED consumption have been observed on SRTT performance (see table 2.1), an effect that was thought to be mediated by the presence of caffeine in the blood in the postprandial period, however Adan and Serra-Grabulosa observed that the effect is only observed when caffeine is administered in combination with glucose.

2.2.2 Choice Reaction Time

Choice reaction time tasks (CRTT) require the participant to form one of multiple responses dependant on the stimuli presented, requiring as fast a response as possible – for example participants may be required to respond to the presentation of one shape by pressing a button with their right hand, and to another shape using their left hand. In the literature concerning ED consumption, tasks using between two and five choices of response have been utilised (Alford, Cox et al. 2001; Adan and Serra-Grabulosa 2010). As these tasks require a decision, a degree of information processing is required and slower reaction times and poorer accuracy are observed in comparison to SRTT (Donders 1869), suggesting that these may be more suitable for sensitivity to small alterations in cognition. As only two studies have investigated the impact of EDs on CRTT performance, one with positive results (Alford, Cox et al. 2001) and one showing no effect (Serra-Grabulosa, Adan et al. 2010) it is difficult to determine whether these tasks are suitable, however vigilance, go/no-go and decision making tasks are variations of CRTT where the choice is generally between making or inhibiting a response.

Table 2.1: List of cognitive tasks and functions used to assess cognitive responses to energy drink consumption in the postprandial period in the literature. Final column indicates whether findings support a beneficial cognitive effect of energy drink treatment (+), negative effect (-) or support the null hypothesis (N).

Task Type	Author	Findings	+/-/N
Simple Reaction Time	(Smit, Grady et al. 2006)	No effects observed.	N
	(Smit and Rogers 2002)	Reduced RT with with 150ml and 250ml ED compared with same volumes of water and no treatment (including effect at 5 min post treatment with 150ml drink).	+
	(Mucignat-Caretta 1998)	No effects observed.	N
	(Horne and Reyner 2001)	Trend for reduced RT at 0-120 minutes post consumption with 500ml ED compared with placebo, however difference only significant at 30-60 minutes post consumption.	+
	(Adan and Serra-Grabulosa 2010)	Poorer performance in group that received water compared with those receving glucose, caffeine or glucose and caffeine.	+
Choice Reaction Time	(Alford, Cox et al. 2001)	Reduced RT with Red Bull treatment compared with carbonated water and baseline measures.	+
	(Adan and Serra-Grabulosa 2010)	No effects observed.	N

Task Type	Author	Findings	+/-/N
Vigilance	(Serra-Grabulosa, Adan et al. 2010)	No effect of treatment observed on task performance; however **fMRI** showed facilitation effect of glucose+caffeine treatment compared with each of glucose, caffeine or water alone.	N/+
	(Adan and Serra-Grabulosa 2010)	Those who received water had slower responses than those who received glucose or caffeine and glucose.	+
	(Anderson and Horne 2006)	Increased RT and number of lapses with ED treatment compared with orange flavoured placebo drink at 60-90 minutes post treatment.	-
	(Jay, Petrilli et al. 2006)	No significant effects of ED on Psychomotor Vigilance Task.	N
	(Kennedy and Scholey 2004)	Improved accuracy in a rapid visual information processing task (RVIP) with two glucose+caffeine treatments attenuating the decrease observed with placebo.	+
	(Rao, Hu et al. 2005)	ED improved accuracy and speed of reactions compared with similar tasting placebo treatment.	+
Go/no-go	(Mucignat-Caretta 1998)	Improved RT in female participants only with ED compared with placebo.	+
	(Howard and Marczinski 2010)	Significantly faster RT in invalid (no-go) trials with 1.8ml/kg, 3.6 ml/kg and 5.4 ml/kg Red Bull compared with lemon flavoured placebo. Trend for faster RT for valid (go) trials with ED treatments.	+
Decision making	(Smit, Grady et al. 2006)	Reduced number of errors with original tasting ED and placebo drinks, increased error rate with novel tasting placebo compared with both original drinks and the novel tasting ED drink.	+
	(Smit, Grady et al. 2006)	Improved performance in a letter search task with the novel tasting ED drink compared with the novel tasting placebo drink.	+
	(Smit and Rogers 2002)	150ml ED reduced errors compared with no treatment but not compared with water.	+
Visual search	(Alford, Cox et al. 2001)	Improved performance with Red Bull treatment compared with 'dummy energy drink' placebo.	+
	(Smit, Cotton et al. 2004)	ED improved accuracy in a letter search task compared with placebo.	+

Task Type	Author	Findings	+/-/N
Wisconsin Card Sorting Test	(Adan and Serra-Grabulosa 2010)	No effects observed.	N
Immediate Recall	(Smit and Rogers 2002)	No effects observed.	N
	(Alford, Cox et al. 2001)	Increased number of words recalled with Red Bull compared with 'dummy energy drink' placebo.	+
	(Adan and Serra-Grabulosa 2010)	No effects observed.	N
Delayed Recall	(Smit and Rogers 2002)	No effects observed.	N
	(Adan and Serra-Grabulosa 2010)	Those who received glucose and caffeine remembered more words in the final two (of five) trials compared with water, glucose alone or caffeine alone.	+
Driving performance	(Horne and Reyner 2001)	500ml ED significantly reduced number of driving incidents (i.e. crossing a lateral lane marking) compared with 500ml ED minus active ingredients at 0-30, 30-60 and 60-90 minutes post consumption but not at 90-120 minutes.	+
	(Reyner and Horne 2002)	ED reduced driving incidents compared with placebo at 0-90 minutes post consumption but no difference at 90-120 minutes.	+
Physiological measures	(Alford, Cox et al. 2001)	No change in heart rate, systolic blood pressure or diastolic blood pressure in study 1, at rest. Increased heart rate in study 2 where aerobic endurance performance was also measured compared with carbonated water.	N/+
	(Specterman, Bhuiya et al. 2005)	Mean evoked potentials (initiated using transcranial magnetic stimulation (TMS)) and monitored using electromyographic recordings (EMG)) increased in size 30 minutes following Lucozade consumption compared with baseline.	+
Motor skills	(Adan and Serra-Grabulosa 2010)	Glucose alone improved Purdue Pegboard Assembly Task performance compared with placebo.	N

Task Type	Author	Findings	+/-/N
Mood	(Smit, Grady et al. 2006)	Increased feeling of energetic arousal with ED drinks compared with placebo but not baseline.	+
	(Smit and Rogers 2002)	Increased and sustained feelings of energetic arousal and overall mood in 150ml and 250ml EDs compared with same volumes of water and no treatment.	+
	(Alford, Cox et al. 2001)	Increased subjective alertness with Red Bull treatment compared with carbonated water.	+
	(Adan and Serra-Grabulosa 2010)	No effects observed.	N
	(Anderson and Horne 2006)	Trend for greater sleepiness scores with ED, however no significant effects.	-/N
	(Howard and Marczinski 2010)	Increased stimulation with 1.8ml/kg and 5.4ml/kg ED compared with baseline and lemon flavoured placebo treatment. Reduced mental fatigue with 1.84ml/kg, 3.64ml/kg and 5.4ml/kg compared with baseline.	+
	(Kennedy and Scholey 2004)	ED treatment Reduced and delayed onset of mental fatigue during a 1-hour test battery.	+
	(Reyner and Horne 2002)	ED significantly reduced and delayed an increase in subjective sleepiness compared with placebo.	+
	(Smit, Cotton et al. 2004)	Carbonated ED improved ratings of feeling awake, assertiveness and cheerful and decreased ratings of tense, clearheadedness and tiredness compared with a non-carbonated treatment.	+

2.2.3 Vigilance

As described in chapter 1, vigilance tasks involve the presentation of few target stimuli requiring a response amongst many non-target stimuli, requiring ability to sustain attention for a period of time while boredom, tiredness or response inhibition may slow response time or possibly reduce ability to detect the target. Table 2.1 shows that six studies have utilised vigilance tasks to investigate the effects of EDs with mixed results. Positive effects of ED consumption have been observed by most studies using this task type (Kennedy and Scholey 2004; Rao, Hu et al. 2005; Adan and Serra-Grabulosa 2010), however Anderson and Horne (2006) believe that arousal (and therefore alerting effects of EDs) should be assessed by a

measure of sleepiness which is highest in afternoons and lowest in the mornings when most studies into cognitive effects of nutrients are assessed. Both reaction time and accuracy measures were poorer with ED consumption than with placebo treatment, suggesting that no alerting effect is observed when participants are tired.

2.2.4 Go/No-go

Go/no-go tasks require a response to frequently occurring target stimuli amongst rare non-target stimuli, and it is the response to the non-target stimuli that generally provides information about cognition, specifically ability to inhibit a response. Howard and Marczinski (2010) observed that RT significantly decreased with ED consumption in non-target trials but not in target trials, suggesting that ED treatment affects response inhibition, however error rates did not differ between treatments.

2.2.5 Decision Making/Information Processing

Decision making tasks assessing the effects of EDs in the literature involve a combination of cognitive processes. Smit *et al.* (2006) used a letter search task where four target letters were presented for 10 seconds before the task started, then letters appeared for four minutes and participants were required to respond with 'true' or 'false' to indicate whether the stimulus was a target letter or not. This study examined the effects of four test drinks on performance, two functional EDs (one with a typical ED flavour (original) and one with a 'novel' flavour) and two placebo EDs (again one 'original' flavoured and one with a 'novel' flavour). When drinks had a novel flavour, ED treatment significantly improved RT compared with placebo in this task, which was essentially a CRTT with a working memory element. The same study also observed an effect on a Rapid Visial Information Processing (RVIP) task where the numbers 1-9 were presented in a random order and participants were required to respond if three odd or three even numbers appeared sequentially, utilising working memory, sustained attention and decision making processes. Both novel and original tasting EDs reduced RT and improved accuracy in response compared with a novel tasting placebo; however the original tasting

placebo also improved accuracy compared with the novel placebo. As decision making tasks are generally complex from a cognitive point of view (i.e. they incorporate multiple aspects of cognition) they can be sensitive to the effects of treatment but it might not be so obvious what mechanism has been affected.

2.2.6 Memory

As discussed in the first chapter, glucose has a significant influence on memory task performance; however the literature concerning memory effects of ED consumption listed in table 2.1 is varied, with three studies finding no effect and two finding positive effects, one on working memory and one on long term memory. It may be possible to use memory tasks to examine the postprandial cognitive effect of EDs, however they may not be as suitable for measuring sensory effects of the drinks due to the immediate nature of the drinks and the methodologies that would be required to study this impact (see below).

2.2.7 Driving Performance

Driving simulator tasks generally measure aspects of sustained attention and motor control, using lapses or driving incidents (crossing the lateral lane markings) as a measure of errors over a long period of time (up to two hours). Horne and Reyner (2001; Reyner and Horne 2002) have produced consistent results displaying a reduction in lapses at 0-90 minutes post drink consumption compared with placebo energy drinks. This contradicts results observed by the same author on a sustained attention task (Anderson and Horne 2006), however this difference is likely

2.2.8 Mood

It may also be important to consider mood as a potential outcome measure, as subjective mood has been observed to be influenced postprandially by ingestion of food and may also be influenced by taste/flavour perception (see above). In a similar vein to task difficulty/sensitivity with the cognitive tasks, baseline mood should be considered when measuring the impact of interventions on subjective mood (Westenhoefer, Bellisle et al. 2004). On the day of testing, a participant's initial (non-pathological) level of mood may be particularly high/low, leaving no

capacity for any increase or decrease. Beneficial effects have been observed in several studies (Alford, Cox et al. 2001; Smit and Rogers 2002; Kennedy and Scholey 2004; Smit, Cotton et al. 2004; Smit, Grady et al. 2006; Howard and Marczinski 2010), however mood questionnaires are answered by participants subjectively so could be prone to placebo effects in studies where there was a discernable difference between treatments (Alford, Cox et al. 2001; Smit and Rogers 2002; Smit, Cotton et al. 2004).

2.3 Treatments

2.3.1 Sensory perception of treatments and method of delivery

Cognitive tasks often last periods of 10 minutes or longer in order to find median/mean values for reaction time, accuracy or other measures that truly represent cognitive ability at that moment in time, however sensory perception of most food products is generally much shorter. Sensory perception of flavour incorporates taste and olfactory inputs of oral stimuli (de Araujo, Rolls et al. 2003), taste is perceived when sweet, sour, bitter, salty or umami stimuli dissolved in water or saliva innervate receptors on the tongue and odours are perceived orthonasally before and whilst food in present in the mouth and retronasally after the food is swallowed (Pierce and Halpern 1996). In order to determine the sensory impact of food samples on cognitive performance, repeated doses of stimuli must be administered throughout the testing period.

Holding test solutions in the mouth without swallowing (mouth-rinse) was used to determine the effects of sweet tasting stimuli on behavioural measures such as perception of fatigue (Rollo, Williams et al. 2008; Chambers, Bridge et al. 2009) and pain (Lewkowski, Ditto et al. 2003), however this method would not be valid for full sensory perception of flavour as this would not allow perception of odours retronasally, as considerable mouth movements or swallowing are required to deliver volatime stimuli (Pierce and Halpern 1996). Studies involving odours alone have used a 'computer controlled odour generator' (Villemure, Slotnick et al. 2003), presence of ambient odours (Millot, Brand et al. 2002) and masks containing pads

impregnated with odourants (Prescott and Wilkie 2007) to deliver stimuli to participants' noses, however delivering odours via these means again would not allow complete perception of the odour/flavour as odourants would not be perceived retronasally.

For these reasons, it was important that a methodology was developed that would elicit full sensory perception of test solutions, including retronasal perception of odours whilst avoiding any postprandial influence on performance. This means that repeated small doses of test solutions had to be swallowed, allowing perception of both taste and odours retronasally, whilst doses of alerting ingredients such as glucose and caffeine were kept to a minimum. The methods used to deliver repeated doses of test samples to participants' mouths also had to be considered, as asking participants to take regular breaks from the task would act as a distraction and affect performance. For this reason a peristaltic pump was used to deliver repeated doses of sample solutions to participants' mouths allowing them to continue performing cognitive tasks (see figure 2.1). Participants attended familiarisation sessions where they were allowed to become accustomed to performing tasks whilst receiving water samples from the pump through the tubing. Participants were also asked to hold the mouthpiece that delivered samples in their mouth during each completion of cognitive tasks, even when no drinks were being delivered in order to keep somatosensory cues consistent throughout trials.

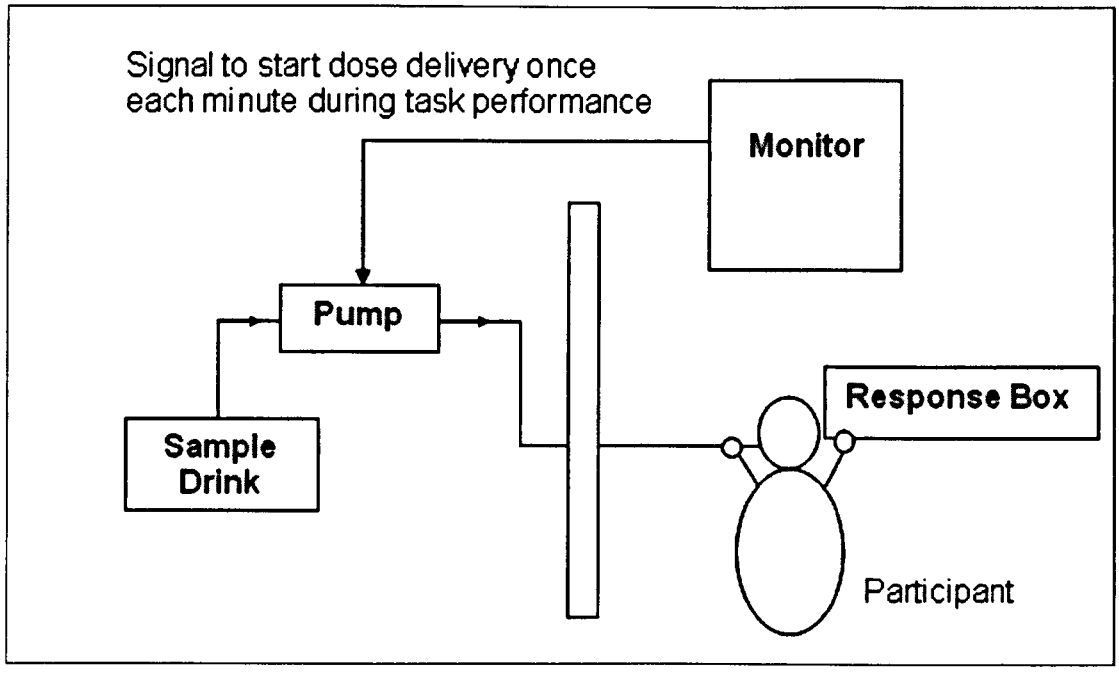

Figure 2.1: Diagram showing setup of experimental equipment. Small arrows indicate flow direction of the sample drink through the pump to the participant, with the participant holding the tube in their left hand, whilst performing the computer based cognitive tasks. The right hand is free to make the responses on the computer keyboard.

2.3.2 Choice of active and placebo treatments

As with any scientific study, the samples and placebos used are crucial to any scientific claim that can be made regarding the interventions. The aim of this thesis is to investigate the effects of EDs on cognition in response to sensory perception and in the postprandial period, therefore it is important to use a full functional ED as the main experimental treatment. One issue though, is that if a pump is being used to deliver a test sample and elicit sensory perception of the treatment, carbonated samples are difficult to handle as the pressure may cause carbon dioxide or the test sample itself to leak. As the main hypotheses consider glucose to be the main driver of any potential cognitive impact, and the combination of glucose and caffeine to produce postprandial effects, it was decided that a non-carbonated ED could be used. It is also important to consider that, fundamentally the thesis needs to examine sensory effects of taste perception on cognition, and in particular the effects of unimodal taste solutions as well as the more complex EDs. For this reason, the thesis investigates the effects of full EDs on performance before

attempting to identify what ingredients of the drinks drive any observed alterations in performance.

The placebos used in previous research into ED effects on cognitive function have been rather varied. Many studies have used a taste matched control, meaning there *should* be no sensory difference in the drinks, allowing delivery of treatments to blind participants. In two sets of experiments that tested the effects of EDs and different placebo types (taste-matched placebo, water or no treatment, Smit, Meikle *et al.* (2002) concluded that the choice of placebo is not 'particularly critical' in measuring the postprandial impact of EDs. The use of a placebo drink, however, that is perceived to be similar to the active intervention does allow participants (and even experimenters) to remain blind as to what treatment is being administered. Additionally, the possibility that an oral nutrient receptor responsive to the energy content of carbohydrates might drive behavioural effects of sensory perception of energy drinks (Kringelbach, de Araujo et al. 2004; Frank, Oberndorfer et al. 2008; Chambers, Bridge et al. 2009) suggests that taste-matched placebo treatments should be used. For this reason, placebo treatments were designed to be similar in taste to the functional energy drink treatments, without any of the active ingredients, glucose or caffeine. This allowed comparison of the sensory influences of energy drink perception with and without calorific carbohydrates on cognitive performance. It was also decided that still water and no treatment conditions were to be used as controls. Using a still water treatment allowed the effects of somatosensory perception on cognitive performance to be monitored, whilst the no treatment acted as a somatosensory control.

CHAPTER 3: THE POSTPRANDIAL IMPACT OF ENERGY DRINKS ON MOOD AND COGNITIVE FUNCTIONING

3.1 Introduction

The postprandial influence of energy drinks on measures of cognition has been well documented (see chapter 1), however this study intends to investigate and confirm these effects using methodologies that may integrate well with future work investigating potential sensory effects of energy drinks on the same or similar measures. It is hypothesised that tasks sensitive to the postprandial impact of ED consumption may also be sensitive to the perception of their sensory properties. For this reason, this study aims only to confirm that EDs do confer behavioural responses postprandially, as stated in the literature review, using methods that may also be used to measure the sensory impact of these drinks in future studies.

The main aims of this study were as follows:

- Measure the postprandial impact of drinks on cognitive performance and mood, in order to confirm effects of energy drinks observed in the literature using our own methodology.
- Determine at what time following ED consumption potential cognitive effects are likely to be strongest.
- Investigate any potential interactive effects of time of day or gender on cognitive effects of energy drink consumption.
- Investigate sensitivity/ability of saccadometer and mood questionnaire to detect small variations in cognitive ability that may be inferred by postprandial (and possibly sensory) impact of EDs.
- To develop methodology for use in future study concerning sensory impact of taste/flavour on cognitive functioning.

3.2 Methods

3.2.1 Participants

24 healthy participants (12 male, 12 female, mean age 24.7) were recruited (8 for each trial condition), and were awarded a monetary reward for their participation. Participants were excluded from the experiment if they were pregnant, diabetic, smokers or colour-blind. Participants were asked not to consume any caffeinated drinks on the day of the trial, and not to eat anything for 2 hours prior to arrival (and only to have a small breakfast if attending a morning session and a small lunch if attending an afternoon session). Participants were required to estimate their weekly habitual intake of caffeinated products and average daily caffeine intakes were calculated using figures given by the Food Standards Agency (2008). Information sheets given to participants and consent forms are displayed in appendices 1 and 2. Application for ethical approval was deemed unnecessary following a review by the project supervisors after considering that the tasks used were of a standard nature and the samples given to participants were commercially available brands of energy drink and bottled water.

3.2.2 Cognitive Assessment

3.2.2.1 UWIST Mood Adjective Checklist

The UWIST Mood Adjective Checklist (displayed in appendix 3; Matthews, Jones et al. 1990) consists of three main bipolar scales supported by item factor analysis - Energetic Arousal (EA), Tense Arousal (TA) and Hedonic Tone (HT) - plus an additional, monopolar Anger/Frustration scale (AF).

3.2.2.2 Saccadometer

The Saccadometer Advanced (Ober Consulting, Poznań, Poland) is a small device that is used to track eye-movements (as displayed in figure 3.1). It is strapped to the participant's forehead and allows the participant to complete eye-tracking tasks using four lasers (central green and red lasers, with two peripheral red lasers (one either side), 10 angular degrees from the central one). Eight calibration trials were completed prior to the start of each set of trials. In these calibration trials the

target would step from the centre to the left four times, then from the centre to the right four times. The Saccadometer Advanced contains a number of pre-programmed tasks that assess eye movements (saccades) towards peripheral targets following presentation of a central cue. In each trial, the time taken to start a saccade (referred to here as latency (ms; with 1 ms resolution)), saccade direction (used to calculate response accuracy), saccade amplitude (angular degrees (deg), accurate to 0.1 deg), saccade duration (ms) and saccadic peak velocity (deg/s) were recorded (Ober Consulting 2008; Ober Consulting 2008).

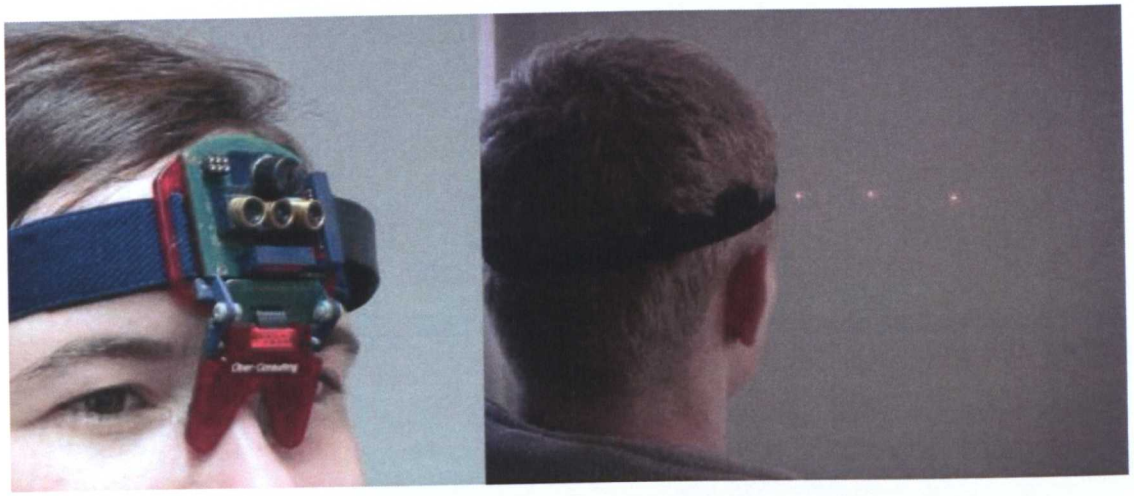

Figure 3.1: Photographs of the Saccadometer Advanced. Left picture shows the headpiece as it is worn during trials. Right picture shows the position of red central cue and the peripheral stimuli on the wall (note that the lights would not appear in this combination in the tasks used in this study). Photographs used with permission (Advanced Clinical Instrumentation 2008).

Latency (LAT) Task

A diagram showing the task stimuli and appropriate response (saccade direction) is shown in figure 3.2. The LAT task is a type of choice reaction time task where the choice is between making an eye movement towards a peripheral target which appears either 10° to the right or left of a central cue. This task was chosen as it is a direct measure of alertness with resulting in reasonably fast reactions and high accuracy due to little need to process information. The subject was to fixate on the central fixation point, which then switched off and a peripheral fixation point switched on. Participants completed 50 trials of this task in each block (approx. 3 minutes).

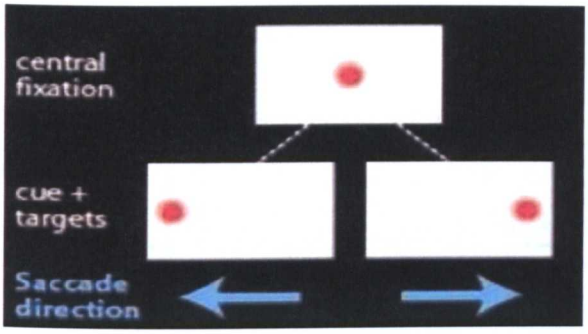

Figure 3.2: Schematic diagram outlining one trial in the Latency Task, displaying the central fixation point (appearing directly in front of the participant), cue/targets and the appropriate saccade direction for each target.

Peripheral Conflict with Gap Condition (PCG) task

A diagram showing the task stimuli and appropriate response (saccade direction) is shown in figure 3.3). At the start of each trial, both central fixation points (red and green) were on. These disappeared for 200ms before one (either red or green) reappeared, along with a peripheral target. For trials where the central cue was green, the correct response was to make a saccade towards the peripheral target (a prosaccade), and when the central cue was red, the correct response was to look away from the peripheral target (an antisaccade). This task assesses attention and decision making processes by demanding as fast a response as possible under conditions that are uncertain, that is whether the participant is required to make a prosaccade or an antisaccade and in which direction the peripheral target will appear. Antisaccade trials in particular assess inhibition of response towards the peripheral target, the peripheral conflict. Participants completed 50 trials of this task in each block (approx. 4 minutes).

Peripheral Conflict with Overlap Condition (PCO) task

A diagram showing the task stimuli and appropriate response (saccade direction) is shown in figure 3.4. This task was performed in the same manner as the PCG task, except no gap was present between central fixation and onset of stimulus. As in the PCG task attention, decision making and response inhibition is involved, however the overlap condition slows reaction times due to the need to disengage attention from the pre-stimulus cue and re-engage attention to the stimulus before making a

decision. Participants completed 50 trials of this task in each block (approx. 4 minutes).

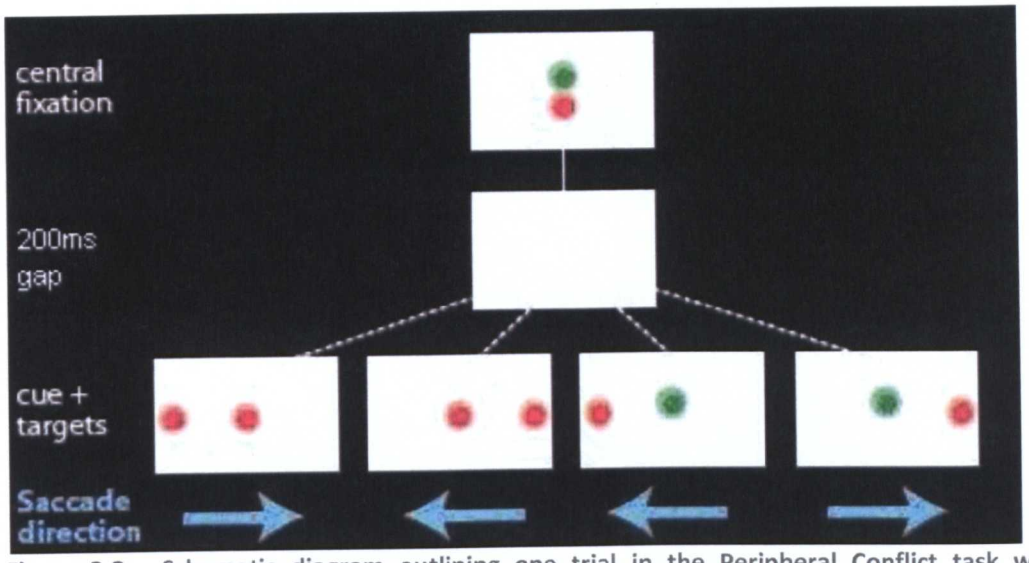

Figure 3.3: Schematic diagram outlining one trial in the Peripheral Conflict task with Gap paradigm, displaying the central fixation point (appearing directly in front of the participant), the 200ms delay where no fixation/cue/targets are present, cue/targets and the appropriate saccade direction for each cue-target pair.

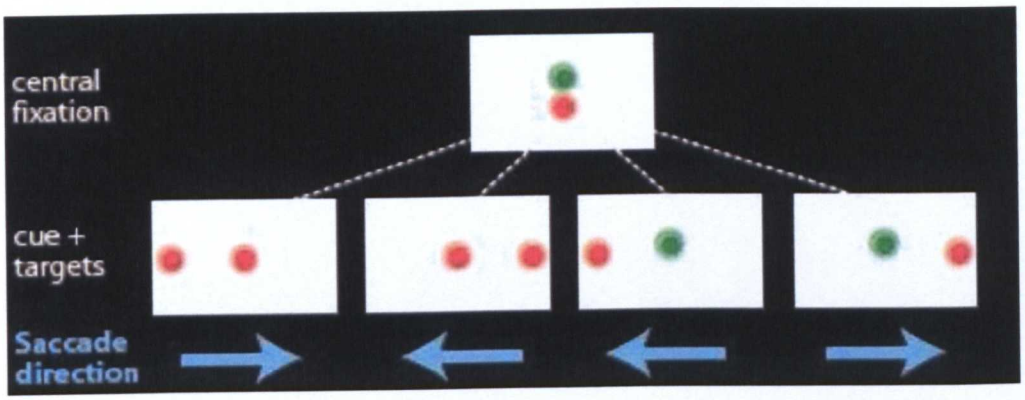

Figure 3.4: Schematic diagram outlining one trial in the Peripheral Conflict task with Overlap paradigm, displaying the central fixation point (appearing directly in front of the participant), cue/targets and the appropriate saccade direction for each cue-target pair.

3.2.3 Experimental Design and Treatments

Using a balanced and randomised 3x2x2 design (treatment x time of day x gender), participants arrived at 10.00am (n=12) or 2.00pm (n=12) and gave written informed consent for their participation. A schedule of the full experimental session is shown in table 3.1. To familiarise themselves with procedures participants completed the mood checklist and were asked to complete 50 trials of each of the saccadometer

tasks in order to become familiarised and results were not recorded. To gain a baseline measurement of performance participants were then asked to complete a second mood checklist, and repeat each of the saccadometer tasks (again, 50 trials of each) in the same order, and the results were recorded as baseline assessment. Participants were then randomly assigned to one of three treatment groups in a between subjects design:

- Energy drink (ED) condition (380ml of a commercially available energy drink containing 46mg caffeine and 66g carbohydrate served in the bottle);
- Placebo (SW) condition (380ml Evian still mineral water[2] (Danone, Ltd) served in a glass[3]);
- No treatment (NT) condition as a control.

As soon as the drink was finished the participants were asked to complete another mood checklist and the timer was started. Starting at 10 minutes post-treatment (to allow time for glucose and caffeine absorption from the gut to begin), the saccadometer tasks were repeated in the same order as at baseline, followed by a mood checklist. This 14-minute long task battery was repeated at 26 minutes and 42 minutes post-treatment, with the experiment finishing at 58 minutes post-treatment.

3.2.4 Data Analysis

3.2.4.1 Saccadometer Data

Trials where participants responded before 100 ms or after 1000 ms following stimulus presentation, or responded with the incorrect saccade direction were excluded from the analysis. For each 3-4 minute group of 50 trials, median latency

[2] Still mineral water was used as Meikle *et al.* (2002) concluded that the choice of placebo was not particularly critical in measuring the cognitive impact of energy drinks. Evidence to the contrary (e.g. Chambers *et al.* 2009) was only discovered by this research group after this study had been concluded.

[3] Placebo treatment was served in a glass as at the time this was the only means available, and it has been noted that perhaps the energy drink should have been delivered in the same manner. It is therefore possible, however unlikely, that any effects of energy drink treatment could be caused by the method of delivery.

was calculated. Mean change in median latency scores (Δ latency) between baseline and each post-treatment measurement were also calculated.

Table 3.1: Schedule of Experimental Protocol – outlining the order of tasks/breaks over the experimental session.

	Duration (min)	Start time (min post treatment)	
Mood Checklist	4	-42	Familiarisation (practice)
Saccadometer LAT trials	4	-38	
Saccadometer PCG trials	4	-34	
Saccadometer PCO trials	4	-30	
Mood Checklist	4	-26	Baseline measurement
Saccadometer LAT trials	4	-22	
Saccadometer PCG trials	4	-18	
Saccadometer PCO trials	4	-14	
Participant consumes test drink	10	-10	
Mood Checklist & Break	10	0	
Saccadometer LAT trials	4	10	
Saccadometer PCG trials	4	14	
Saccadometer PCO trials	4	18	
Mood Checklist	4	22	
Saccadometer LAT trials	4	26	
Saccadometer PCG trials	4	30	
Saccadometer PCO trials	4	34	
Mood Checklist	4	38	
Saccadometer LAT trials	4	42	
Saccadometer PCG trials	4	46	
Saccadometer PCO trials	4	50	
Mood Checklist	4	54	
End		58	
Total	100		

Trials where participants responded before 100 ms or after 1000 ms following stimulus presentation were excluded from the analysis. Accuracy data from the PCG and PCO tasks only were analysed, accuracy data in the LAT task was ignored due to almost 100% accuracy. For each 4 minute group of 50 trials, the percentage of correct responses was calculated (accuracy). Mean change in percentage correct responses between baseline and each post treatment measurement were also calculated (Δ accuracy).

Trials where participants responded before 100 ms or after 1000 ms following stimulus presentation, or responded with the incorrect saccade direction were excluded from the analysis. For each 3-4 minute group of 50 trials, mean duration,

amplitude and peak velocity were calculated. Mean change in scores between baseline and each post-treatment measurement were also calculated (Δ duration, Δ amplitude and Δ peak velocity).

Between treatment effects on group mean of individual median latency, mean accuracy, mean duration, mean peak velocity and mean change in each of these scores were analysed using GLM Multivariate ANOVA and *post hoc* LSD test (SPSS 16, IBM). Within treatment effects were monitored using a separate ANOVA where measurement (e.g. baseline or time of post-treatment measurement) was the only fixed factor. *Post hoc* Dunnett Tests were used to compare mean median baseline scores with each post treatment score.

3.2.4.2 UWIST Mood Adjective Checklist Data

Scores for EA, TA, HT and AF were calculated using the formulae outlined in appendix 4 using Microsoft Excel. Mean change in scores from baseline and each post treatment mood assessment were calculated (ΔEA, ΔTA, ΔHT and ΔAF). Between treatment effects on mean scores and mean change in scores were analysed using ANOVA and *post hoc* LSD test. Within treatment effects were monitored using a separate ANOVA where measurement (e.g. baseline or time of post-treatment measurement) was the only fixed factor, and *post hoc* Dunnett Tests were used to compare mean median baseline scores with each post treatment score.

3.2.4.5 Effects of Treatment, Time of Day, Gender and Habitual Caffeine Intake

As the experimental design was balanced across treatment, gender and time of day, these factors were added as fixed factors into the GLM Multivariate ANOVA. Median habitual caffeine intake (HCI) was calculated using the information provided by participants, and participants with habitual intakes lower than the median were classed as having low HCIs and participants with intakes above the median were classed as having high HCIs. Age of participants was also taken into account, with the median splitting 'older' and 'younger' participants into two groups. Age and HCI

were also included as fixed factors in the ANOVA. The experimental question of whether ED, SW or NT conditions affect cognition is best answered using 'difference inferential statistics' (such as ANOVA), and as this is the main purpose of the experiment, other factors (age, gender, HCI and TOD) were included in the same statistical analysis. Although associational statistics could be used (correlation or regression) to determine the effects of continuous variables (age and HCI), this is not the principle aim of the study and these factors were being investigated to monitor any influence they may exert on effects of treatment (Morgan, Leech et al. 2011). It was decided that although these continuous variables could be included in the analysis as covariates, the main function of the study was to investigate the influence of treatments on performance, and including covariates in the ANOVA prevents the use of *post hoc* tests in SPSS. For this reason, the median was used to split participants into two groups as described above.

3.3 Results

The results from the 24 participants are featured in this section. The results from the anti-saccade trials of the PCG and PCO tasks for one participant have been removed from the analysis due to such poor accuracy which made it impossible to analyse the data if included. This participant's performance in the LAT task and pro-saccade trials of the PCG and PCO tasks were normal, as was the mood data, and this data was included in the analysis.

3.3.1 Saccadometer Tasks

3.3.1.2 Effects of Treatment

Latency Task

Table 3.2 outlines the results observed in the Latency Task, showing mean group baseline scores for saccadic latency, duration, amplitude and peak velocity and corresponding standard error mean (SEM) measured in the postprandial period.

Within treatment analysis (ANOVA and *post hoc* Dunnett test) found no significant differences between baseline measurements and any post treatment

measurements in the LAT task. No between treatment effects of treatment were observed on latency or peak velocity data. Figure 3.5 shows the mean Δ saccade duration from baseline in each of the LAT tasks carried out in the postprandial period, displaying significantly lower mean Δ saccade duration in the NT group at 42-46 minutes post consumption than observed with ED treatment (p=0.014, ANOVA; p=0.010, LSD) and SW treatment (p=0.020).

Table 3.2: Results from LAT task. Table presents mean and standard error mean (SEM) values for latency, accuracy, duration, amplitude and peak velocity data for energy drink (ED), still water (SW) and no treatment (NT) conditions. * indicates significant difference from ED and SW treatments (P<0.05, ANOVA and *post hoc* LSD)

Measure	Treatment	Baseline		10-14 min post cons.		26-30 min post cons.		42-46 min post cons.	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Latency (ms)	ED	185.25	6.85	189.19	10.34	189.19	9.75	185.00	10.68
	SW	178.69	10.28	193.44	11.25	188.63	10.18	185.44	8.72
	NT	178.50	7.46	180.06	7.10	182.06	6.14	178.31	7.25
Duration (ms)	ED	43.85	1.58	45.86	0.92	44.82	1.33	47.03	1.23
	SW	50.17	2.83	48.85	2.32	50.15	2.61	53.26	3.57
	NT	45.79	1.25	43.84	1.74	45.03	2.36	43.17	2.08
Amplitude (deg)	ED	10.00	0.22	10.60	0.33	10.52	0.24	10.14	0.34
	SW	9.26	0.25	10.00	0.63	9.85	0.58	10.82	0.51
	NT	11.20	1.31	9.81	0.18	9.77*	0.33	9.55*	0.48
Peak Velocity (deg/s)	ED	473.20	26.71	490.21	10.13	491.58	17.80	454.36	16.76
	SW	399.18	32.12	430.77	31.85	419.40	31.15	466.58	47.05
	NT	525.76	46.16	450.34	20.00	500.10	29.19	476.03	23.71

Table 3.2 shows that saccadic amplitude was significantly lower in the NT group than both ED and SW groups at 26-30 (ANOVA: P=0.013; LSD: ED, p=0.022; SW, p=0.032) and 42-46 (ANOVA: P=0.019; LSD: ED, p=0.044; SW, p=0.010) minutes post drink consumption. Figure 3.6 displays the mean Δ amplitude from baseline at each postprandial measurement; ANOVA found significance of treatment on mean Δ amplitude at 26-30 minutes (p=0.025) and 42-46 minutes (p=0.013). *Post hoc* LSD indicated significant differences at 26-30 minutes between ED and NT groups (p=0.025) and SW and NT groups (p=0.010). LSD also found significant differences

between ED and SW (p=0.008), ED and NT (p=0.032) and SW and NT (p=0.004) at 42-46 minutes.

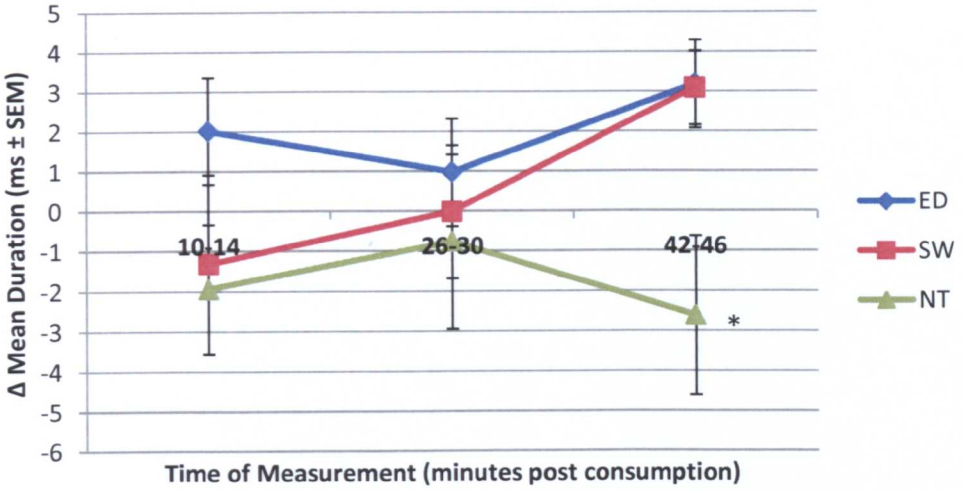

Figure 3.5: Δ Mean Saccadic Duration in the LAT task for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from all other treatments ($p<0.05$, ANOVA and LSD).

ANOVA found a borderline significant effect of treatment on peak velocity at 42-46 minutes post consumption (p=0.067). Figure 3.7 shows that change in peak saccadic velocity increased in the SW group and decreased in the other groups at this point, with *post hoc* LSD finding significant differences between SW and ED conditions (p=0.019), and SW and NT conditions (p=0.015).

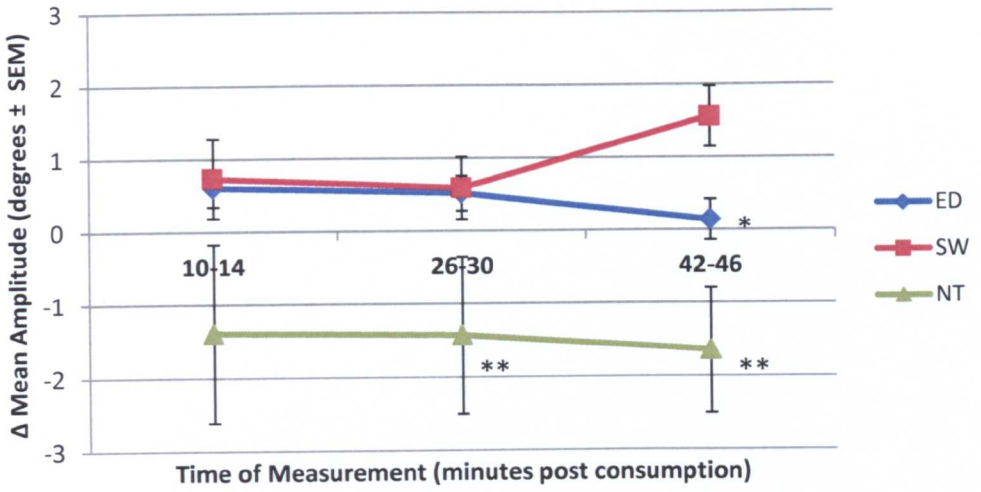

Figure 3.6: Δ Mean Saccadic Amplitude in the LAT task for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from SW condition ($p<0.05$, ANOVA and LSD). ** indicates significant difference from ED and SW conditions.

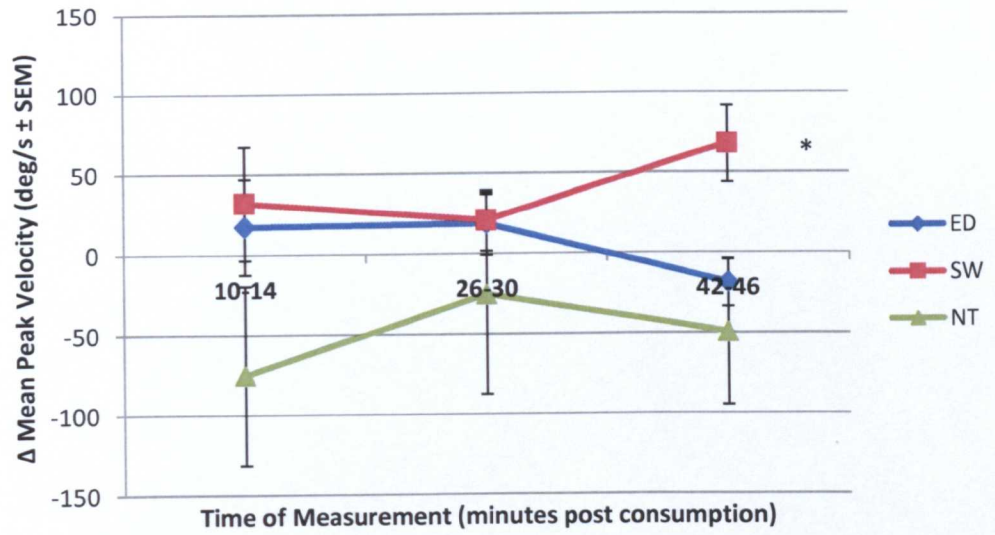

Figure 3.7: Δ Mean Saccadic Peak Velocity in the LAT task for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from all other treatments ($p<0.05$, ANOVA and LSD).

The results from the LAT task do not appear to show an influence of treatment on measures of alertness, as no impact was observed on latency. The impacts of the treatments on saccadic amplitude, duration and peak velocity are difficult to interpret, though they may be related. Saccade amplitude is a measure of focal

accuracy with regards to the peripheral target (the light that the participant is required to make a saccade towards), and as saccade amplitude significantly increased with SW treatment (saccades overshot the peripheral target), and tended to decrease with NT (participants saccades did not reach the peripheral target), duration and velocity of those saccades may be affected. It is possible though, that poor calibration prior to the start of group of trials could have led to type I error, as this would affect amplitude readings significantly.

Peripheral Conflict with Gap Task

Results for prosaccade trials and antisaccade trials are presented separately due to the different mechanisms involved in each trial type.

Prosaccade Trials

Table 3.3 outlines the results from the prosaccade trials of the PCG task, showing mean saccadic latency, accuracy, duration, amplitude and peak velocity at baseline and corresponding SEM values at baseline and each post treatment measurement. Table 3.3 shows a tendency for reduced latency in the ED and SW groups compared with NT, however ANOVA found no significant between treatment effects on latency. ANOVA did find significant effect of treatment on mean Δ latency at 30-34 minutes (p=0.017), with figure 3.8 showing mean Δ latency to be significantly lower with ED and SW treatments compared with NT (ED vs NT p=0.005; SW vs NT p=0.008, LSD).

As observed in Table 3.3, saccadic duration was longer at 14-18 minutes (p=0.011, ANOVA), 30-34 minutes (p=0.005) and 46-50 minutes (p=0.013) post treatment in the SW group than both other groups (14-18 minutes: SW vs ED, p=0.011, SW vs NT, p=0.008; 26-30 minutes: SW vs ED, p=0.005, SW vs NT, p=0.003; 46-50 minutes: SW vs ED, p=0.011, SW vs NT, p=0.006). Figure 3.9 displays mean Δ duration, with SW treatment significantly increasing duration at 30-34 minutes post consumption compared with NT (ANOVA p=0.020, LSD p=0.026) and at 46-50 minutes compared with both ED (ANOVA p=0.003, LSD p=0.017) and NT (p=0.002).

Table 3.3: Results from prosaccade trials of the PCG task. Table presents mean and standard error mean (SEM) values for latency, accuracy, duration, amplitude and peak velocity data for energy drink (ED), still water (SW) and no treatment (NT) conditions. * indicates significantly different from ED and NT conditions ($p<0.05$, ANOVA and LSD). ** indicates significantly different from SW condition ($p<0.05$, ANOVA and LSD).

Measure	Treatment	Baseline		14-18 min post cons.		30-34 min post cons.		46-50 min post cons.	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Latency (ms)	ED	298.56	23.63	274.88	15.05	265.94	14.37	269.63	14.45
	SW	349.50	29.40	317.81	30.66	325.88	27.84	337.75	27.28
	NT	319.56	7.93	316.63	7.36	328.19	17.54	325.44	21.87
Accuracy (% correct)	ED	85.67	4.83	89.55	4.00	94.42	3.50	91.38	3.27
	SW	96.62	1.67	92.55	3.50	91.72	2.79	94.66	2.66
	NT	94.92	2.08	96.72	1.43	96.13	1.72	94.19	2.54
Duration (ms)	ED	44.64	1.25	47.05	1.29	47.68	1.83	47.87	1.16
	SW	49.65	2.30	52.60*	2.48	54.51*	3.52	54.48	2.85
	NT	44.96	1.69	46.19	2.11	45.97	1.27	45.42	1.77
Amplitude (degrees)	ED	10.14	0.47	10.08	0.81	10.89	0.55	10.76	0.42
	SW	9.94	0.20	10.30	0.35	11.47	0.98	9.84	0.29
	NT	10.37**	0.24	9.80	0.37	10.00	0.29	10.44	0.34
Peak Velocity (deg/s)	ED	469.29	24.78	476.45**	35.66	488.93	34.84	474.94	12.38
	SW	425.85	22.75	423.86	26.77	457.09	36.55	404.85	25.98
	NT	483.26	25.70	445.41	33.70	499.68	27.96	509.19	28.19

Treatment had no significant effects on accuracy, amplitude or peak velocity of saccades in prosaccade trials of the PCG task.

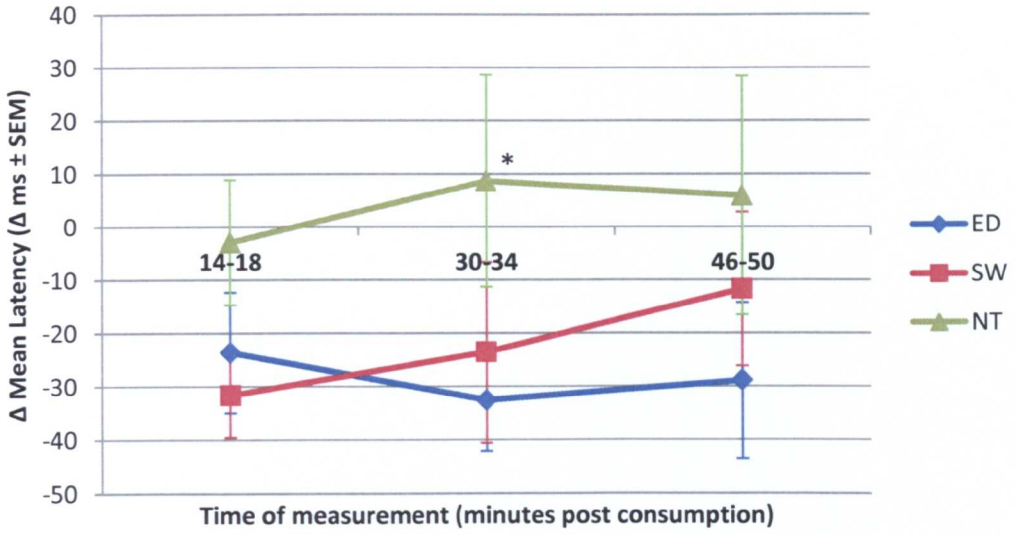

Figure 3.8: Δ Mean Saccadic Latency in PCG prosaccade trials for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from ED and SW conditions ($p<0.05$, *post hoc* LSD).

In reaction time tasks with a decision making element, latency and accuracy are often a trade off where when one improves, the other suffers (Hick 1952; Wickelgren 1977). In this task ED and SW consumption have improved latency with no negative effect on accuracy (in fact ED tended to improve accuracy, though this effect was not significant), suggesting a significant effect of the treatment on measures of attention. This could be caused by a direct influence of mechanisms discussed above (section 1.2.1.1.5) on attention, though the results from antisaccade trials must be considered before making any claims.

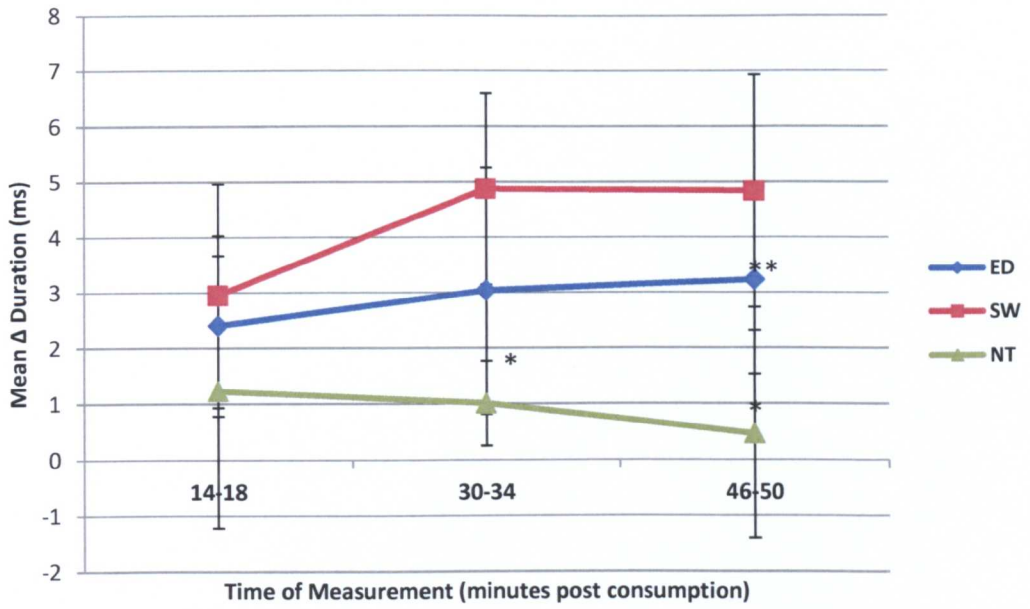

Figure 3.9: Δ Mean Saccadic Duration in PCG prosaccade trials for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from SW condition ($p<0.05$, *post hoc* LSD). ** indicates significant difference from SW and NT conditions ($p<0.05$, *post hoc* LSD).

Antisaccade Trials

Table 3.4 outlines the results from the antisaccade trials of the PCG task, showing mean saccadic latency, accuracy, duration, amplitude and peak velocity and corresponding SEM values at baseline and each post-treatment measurement. Table 3.4 appears to show a trend for decreased latency with ED consumption post-treatment compared with baseline, however these changes failed to reach significance. ANOVA found borderline significant effect of treatment on Δ mean latency at 46-50 minutes post consumption ($p=0.64$, and as is shown in figure 3.10 the SW group had borderline significantly higher Δ latency than the ED group ($p=0.052$, LSD).

Table 3.4: Results from antisaccade trials of the PCG task. Table presents mean and standard error mean (SEM) values for latency, accuracy, duration, amplitude and peak velocity data for energy drink (ED), still water (SW) and no treatment (NT) conditions. * indicates significantly different from SW and NT conditions ($p<0.05$, ANOVA and LSD).

Measure	Treatment	Baseline		14-18 min post cons.		30-34 min post cons.		46-50 min post cons.	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Latency (ms)	ED	320.13	21.73	294.06	12.13	289.50	33.03	289.38	26.18
	SW	358.43	23.38	359.00	20.71	382.07	21.85	369.07	17.67
	NT	352.56	25.63	349.00	27.22	350.75	19.66	337.38	29.85
Accuracy (% correct)	ED	72.76	5.92	63.47	9.47	55.40*	12.02	65.20*	11.53
	SW	80.97	6.85	82.09	6.60	82.59	9.74	81.28	11.31
	NT	88.20	2.29	82.19	4.71	80.25	3.98	80.77	6.34
Duration (ms)	ED	66.88	6.26	65.21	7.35	61.26	7.44	66.11	6.37
	SW	64.53	3.73	61.67	4.63	64.71	4.23	64.36	5.99
	NT	62.98	4.60	64.40	8.90	75.34	12.00	64.68	8.96
Amplitude (degrees)	ED	12.95	1.97	12.71	2.15	11.82	1.84	12.63	2.14
	SW	11.14	1.02	10.53	0.97	11.08	0.78	10.46	0.96
	NT	12.94	1.64	12.20	1.50	14.00	2.79	12.78	2.10
Peak Velocity (deg/s)	ED	414.75	38.42	421.35	45.55	398.50	52.02	403.40	39.28
	SW	387.03	34.30	384.41	33.87	372.05	25.88	360.57	33.42
	NT	441.50	32.31	421.48	38.27	454.70	46.07	432.44	37.24

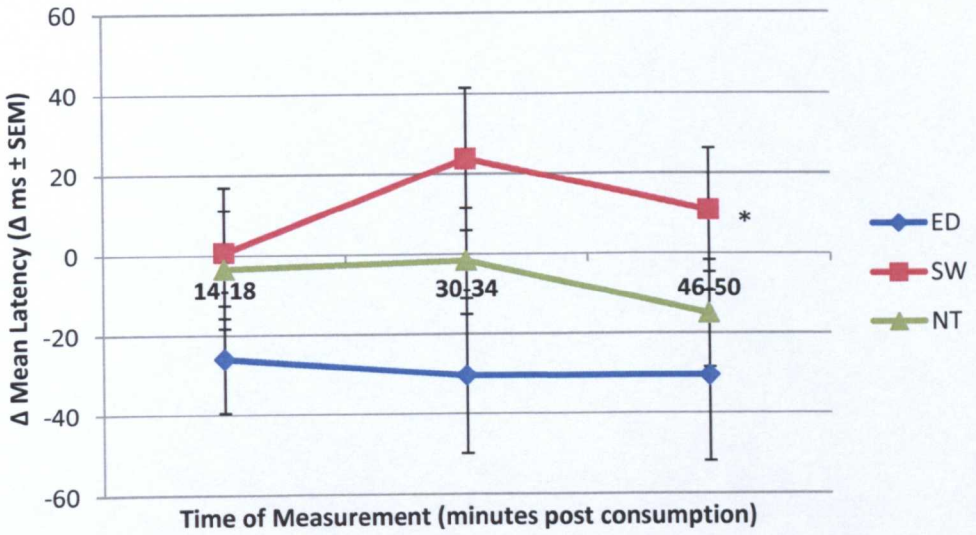

Figure 3.10: Δ Mean Saccadic Latency in PCG task antisaccade trials for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from all other treatments ($p<0.05$, ANOVA and LSD).

ANOVA found significant between treatment effects on accuracy at 30-34 minutes ($p=0.031$) and 46-50 minutes ($p<0.001$). As displayed in table 3.4, ED consumption significantly reduced mean accuracy compared with both the SW group ($p=0.021$, LSD) and the NT group ($p=0.024$, LSD) at 30-34 minutes. Though no significant effect of treatment was observed on Δ mean accuracy at 30-34 minutes, Figure 3.11 shows a tendency for ED treatment to reduce accuracy, particularly at 30-34 minutes.

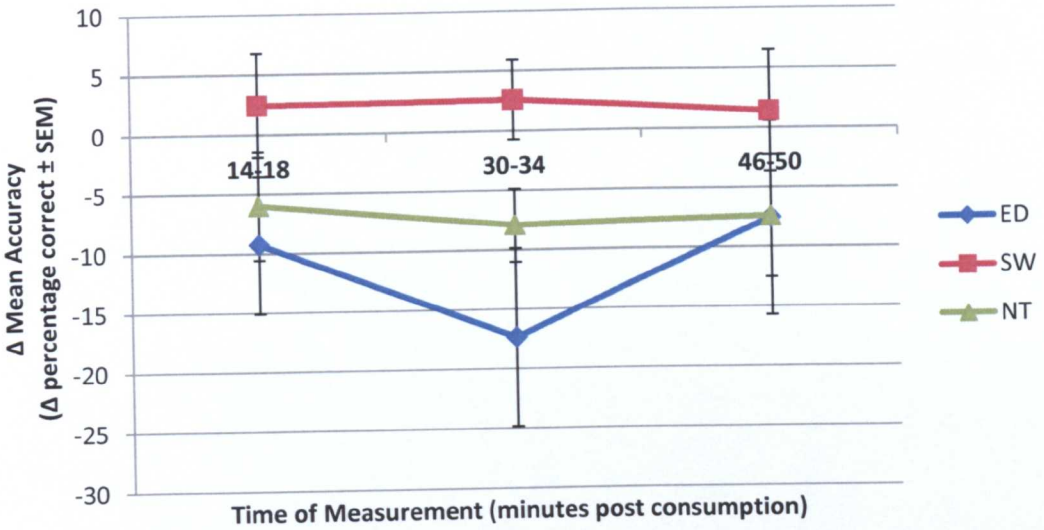

Figure 3.11: Δ Mean Accuracy in PCG task antisaccade trials for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions.

Comparing these results with those observed in the prosaccade trials, ED treatment has had tended to reduce reaction times, significantly so in prosaccade trials, however ED has not affected accuracy in prosaccade trials but decreased accuracy in antisaccade trials. This suggests that despite an apparent improvement in alertness as shown by the tendency for reduced reaction times with ED, accuracy is being negatively affected in antisaccade trials. This could be caused by an increased likelihood to generate a saccade in the direction of the peripheral target irrespective of the information provided by the cue (poorer response inhibition). This could indicate either an increase in arousal that leads to increased latency with the cost being poorer information processing as a result of less processing time or an inhibition of top down (goal-orientated) control of attention that would normally

inhibit saccade generation towards newly presented peripheral targets in antisaccade trials.

Peripheral Conflict with Overlap Task

Prosaccade Trials

Table 3.5 outlines the results from the prosaccade trials of the PCO task, showing mean saccadic latency, accuracy, duration, amplitude and peak velocity and corresponding SEM values at baseline and each post-treatment measurement. Treatment had very little effect on any of the outcome measures. A tendency for reduced latency following ED treatment was observed, however this failed to reach significance.

Table 3.5: Results from prosaccade trials of the PCO task. Table presents mean and standard error mean (SEM) values for latency, accuracy, duration, amplitude and peak velocity data for energy drink (ED), still water (SW) and no treatment (NT) conditions. * indicates significantly different from ED and NT conditions ($p<0.05$, ANOVA and LSD). ** indicates significantly different from all other treatment conditions ($p<0.05$, ANOVA and LSD).

Measure	Treatment	Baseline		18-22 min post consumption		34-38 min post consumption		50-54 min post consumption	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Latency (ms)	ED	345.94	29.27	305.50	26.14	312.88	18.93	299.63	20.85
	SW	387.56	43.39	365.75	32.37	377.63	22.22	369.38	25.15
	NT	351.75	23.69	367.88	22.22	371.06	22.12	357.63	36.17
Accuracy (% correct)	ED	89.91	4.61	92.02	3.47	89.62	3.76	90.96	3.98
	SW	94.64	1.69	92.13	2.64	92.74	1.91	96.96	1.68
	NT	92.47	3.31	87.40	3.65	91.24	3.19	92.98	2.32
Duration (ms)	ED	48.37	3.90	45.92	1.08	42.90	1.16	46.63	1.03
	SW	51.01	2.52	51.94*	3.60	54.00	3.58	51.22	2.95
	NT	46.25	2.01	44.59	2.07	44.96	1.71	44.22	1.09
Amplitude (degrees)	ED	10.43	0.37	10.96	0.43	9.87**	0.82	9.69	0.75
	SW	9.80	0.41	11.54	1.07	10.12**	0.38	10.15	0.36
	NT	11.05	0.70	10.59	0.65	10.30**	0.24	10.49	0.58
Peak Velocity (deg/s)	ED	470.43	38.16	508.71	19.35	490.72	39.59	437.67	33.71
	SW	422.25	35.35	484.83	43.19	431.12	47.39	424.26	27.07
	NT	518.86	35.22	502.50	26.73	522.83	31.58	544.49	43.96

Table 3.5 shows that at 18-22 minutes post consumption ANOVA found significant effect of treatment on saccade duration (p=0.028), with *post hoc* LSD finding that duration in the SW group significantly longer than the ED (p=0.033) and NT (p=0.020) groups. ANOVA also found a significant effect of treatment on saccadic amplitude at 34-38 minutes post consumption (p=0.001). LSD found all three treatment conditions to be significantly different (as shown in table 3.5: ED vs SW, p=0.001; ED vs NT, p<0.001; SW vs NT, p=0.002).

Antisaccade Trials

Table 3.6 outlines the results from the antisaccade trials of the PCO task, showing mean saccadic latency, accuracy, duration, amplitude and peak velocity and corresponding SEM values at baseline and each post-treatment measurement. ANOVA found no between treatment effects on any of the outcome measures.

Table 3.6: Results from antisaccade trials of the PCO task. Table presents mean and standard error mean (SEM) values for latency, accuracy, duration, amplitude and peak velocity data for energy drink (ED), still water (SW) and no treatment (NT) conditions.

Measure	Treatment	Baseline		18-22 min post consumption		34-38 min post consumption		50-54 min post consumption	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Latency (ms)	ED	367.13	30.48	354.63	16.93	328.00	23.16	347.13	19.18
	SW	456.14	35.11	431.14	23.28	432.00	23.26	444.57	22.27
	NT	413.07	25.14	418.86	30.74	394.79	35.05	381.00	31.42
Accuracy (% correct)	ED	65.52	8.17	64.21	8.82	65.68	12.28	61.99	11.14
	SW	75.03	8.78	76.29	8.81	72.74	12.03	77.10	12.93
	NT	73.63	8.41	81.91	5.78	73.71	9.45	79.26	11.74
Duration (ms)	ED	70.09	7.02	62.18	6.09	62.65	6.75	64.95	6.21
	SW	65.72	6.61	64.64	5.41	63.97	6.97	65.18	6.94
	NT	61.41	6.37	58.08	7.55	69.59	13.34	59.23	7.06
Amplitude (degrees)	ED	12.86	1.81	12.01	1.91	13.24	2.61	11.83	1.88
	SW	10.20	1.25	11.61	1.07	10.75	1.42	11.53	1.01
	NT	12.54	2.01	12.14	2.14	15.45	3.65	12.33	1.93
Peak Velocity (deg/s)	ED	423.83	50.76	409.90	42.21	424.83	51.95	379.30	39.48
	SW	349.60	43.05	388.47	25.15	361.53	47.40	401.93	36.29
	NT	471.42	35.93	449.42	27.12	481.78	40.27	459.72	28.67

It is clear from the PCO task results that the removal of the 200 ms gap (from the PCG task) has a significant impact on the attentional effects of ED consumption. These results suggest that perhaps ED consumption affects processes involved in the PCG task but not the PCO task where there is a greater inhibition of the re-engagement of attention to the new cue. Although, as the PCG and PCO tasks were performed separately, order effects could have influenced performance. Looking at the latency scores for each treatment at 42-54 minutes post consumption, it is clear learning did not improve performance as the experimental session continued; however glucose levels might have peaked during at 30-34 minutes post-consumption, only affecting performance in the PCG task at this time. The observed effects may in fact be caused by the slower response times observed in the PCO task allowing greater time to process the information conveyed by the stimulus and therefore reducing any negative impact of the drinks on antisaccade trials.

3.3.1.3 Effects of Time of Day

Peripheral Conflict with Gap Task

Prosaccade Trials

Time of day (TOD) significantly impacted on mean accuracy at baseline (p=0.015), participants completing the task in the morning had significantly greater response accuracy than those in the afternoon, as is displayed in figure 3.12.

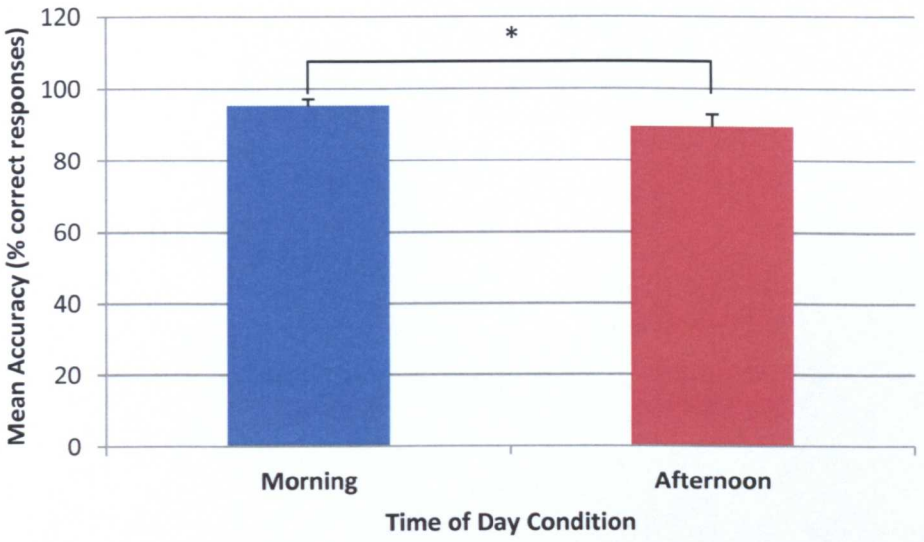

Figure 3.12: TOD effects on mean accuracy at baseline in prosaccade trials of the PCG task. * indicates significant difference between morning and afternoon groups at time of measurement (P<0.05 for ANOVA and *post hoc* LSD).

Antisaccade Trials

Figure 3.13 shows mean accuracy for the morning and afternoon groups at baseline and all three post-treatment assessments, illustrating that accuracy was significantly higher for those who participated in the morning than those who participated in the afternoon at 30-34 minutes (p=0.031) and at 46-50 minutes (p<0.001).

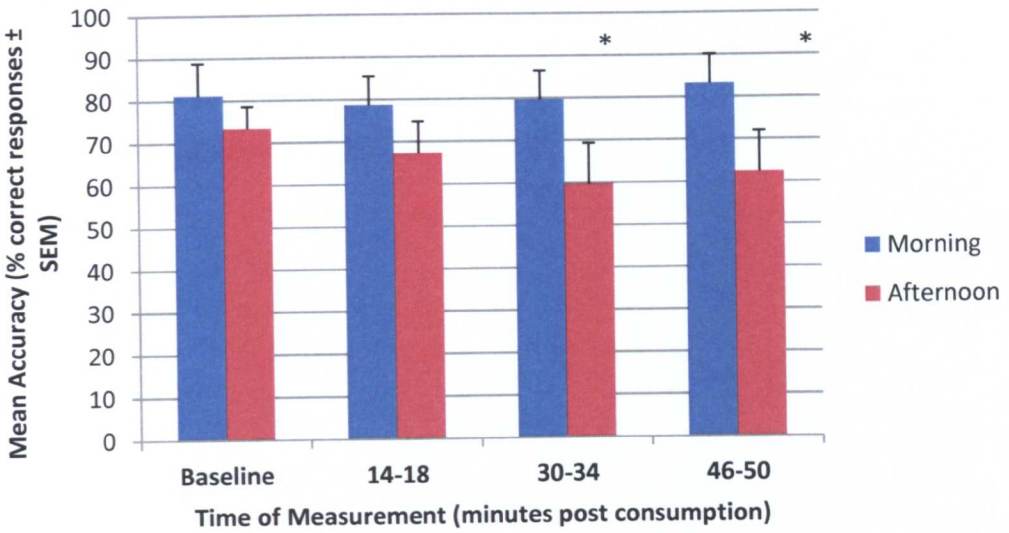

Figure 3.13: TOD effects on mean accuracy in antisaccade trials of the PCG task. * indicates significant difference between morning and afternoon groups at time of measurement ($P<0.05$ for ANOVA and *post hoc* LSD).

It appears there may have been interactive effects of treatment and TOD on Δ mean accuracy. Figure 3.14A shows the Δ mean accuracy from baseline at post-treatment assessments and figure 3.14B shows the Δ mean accuracy at 30-34 minutes post consumption for the morning and afternoon groups according to their treatment group. Figure 3.14A shows that Δ mean accuracy tended to be lower for the afternoon group at 30-34 minutes post consumption, though no significant difference was observed ($p=0.138$). Figure 3.14B highlights a tendency for decreased in accuracy following ED consumption in the afternoon group, but not for those who arrived in the morning.

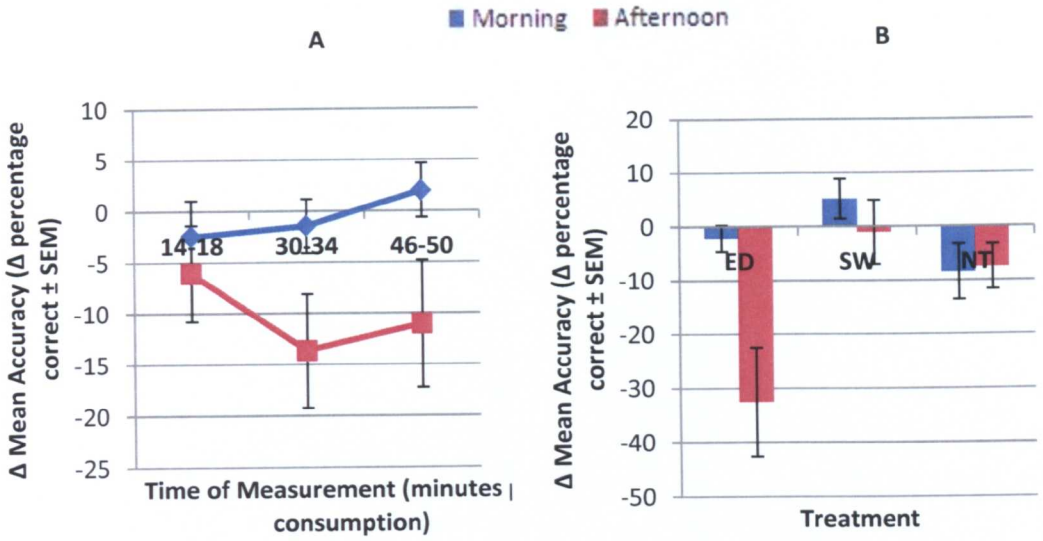

Figure 3.14: Δ Mean Accuracy in PCG task antisaccade trials for TOD. ; B. Change in mean latency at 30-34 minutes post consumption showing values for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions with values for morning (blue) and afternoon (red) displayed. * indicates borderline significant differences between groups (p=0.052, ANOVA).

Peripheral Conflict with Overlap Task

Antisaccade Trials

Figure 3.15 shows mean accuracy in the PCO task for the morning and afternoon groups at baseline and each post-treatment assessment. Mean accuracy tended to be higher in those who participated in the morning than those who participated in the afternoon, with this tendency reaching significance at 36-40 minutes (p=0.028) and at 50-54 minutes post consumption (p=0.040, ANOVA).

The results presented allude to time of day effects interacting with the alerting effects of ED consumption. These interactions affected the PCG task most significantly, where the main effects of ED consumption were observed. Specifically ED consumption appears to decrease accuracy in antisaccade trials in the afternoons but perhaps not in the morning.

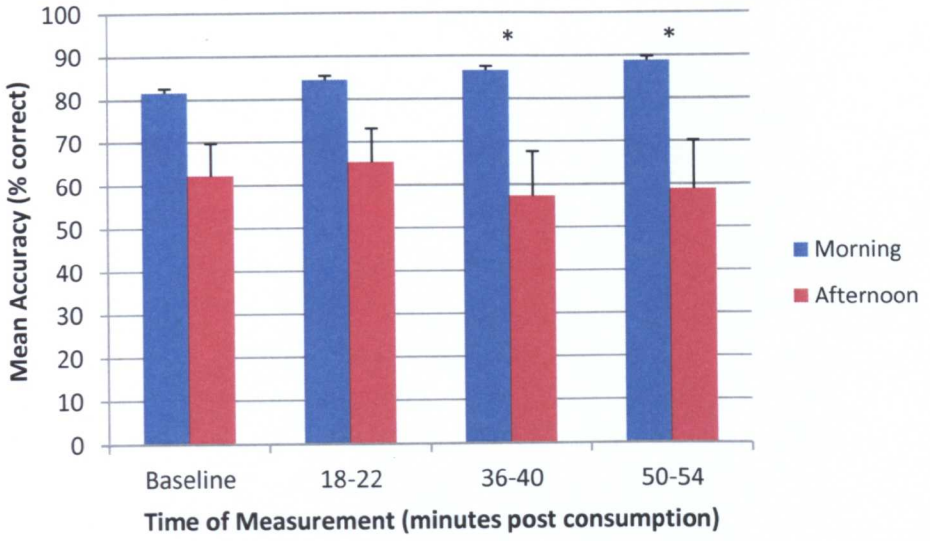

Figure 3.15: TOD effects on mean accuracy in antisaccade trials of the PCO task. * indicates significant difference between morning and afternoon groups at time of measurement ($P<0.05$ for ANOVA and *post hoc* LSD).

3.3.2 UWIST Mood Adjective Checklist

Table 3.7 shows mean mood scores for energetic arousal, tense arousal, hedonic tone and ager/frustration and SEM values for each treatment at baseline assessment and each post treatment assessment. Within treatment ANOVAs found no significant effects between baseline and any post treatment measurements.

3.3.2.1 Energetic Arousal

No significant effects of treatment, time of day or gender were found by ANOVA on measures of EA.

3.3.2.2 Tense Arousal

There were no significant effects observed on TA score for treatment, time of day, or gender.

3.3.2.3 Hedonic Tone

There were no significant effects observed on HT score for treatment, time of day, or gender.

Table 3.7: UWIST Mood Questionnaire results. Table presents mean and standard error mean (SEM) values for Energetic Arousal (EA), Tense Arousal (TA), Hedonic Tone (HT) and Anger/Frustration scales for energy drink (ED), still water (SW) and no treatment (NT) conditions.

Measure	Treatment	Baseline		0 min		22 min		38 min		54 min	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
EA	ED	25.25	1.16	22.75	1.39	22.50	1.59	1.63	1.07	25.25	1.16
	SW	19.38	1.85	19.38	2.30	21.25	1.57	0.63	1.02	19.38	1.85
	NT	18.88	1.88	18.00	2.21	19.00	1.94	0.38	0.53	18.88	1.88
TA	ED	14.75	1.37	14.13	1.20	14.75	1.49	0.00	1.12	14.75	1.37
	SW	14.50	1.24	13.75	0.82	12.88	0.55	-1.00	1.12	14.50	1.24
	NT	13.88	1.84	14.13	1.47	14.38	1.72	-0.75	0.53	13.88	1.84
HT	ED	28.25	1.03	27.13	0.85	27.63	1.00	0.75	0.45	28.25	1.03
	SW	24.13	1.56	25.25	1.29	27.13	0.95	2.00	0.85	24.13	1.56
	NT	26.13	1.53	25.50	1.82	26.50	1.27	-0.50	0.53	26.13	1.53
AF	ED	5.75	0.31	5.63	0.32	5.50	0.27	-0.13	0.13	5.75	0.31
	SW	8.00	1.34	7.25	0.75	6.13	0.61	-0.88	0.81	8.00	1.34
	NT	7.75	1.18	7.63	1.22	7.50	1.15	0.25	0.41	7.75	1.18

ANOVA found significant effect of treatment on mean ΔHT score from baseline at 0 minutes ($p=0.010$), following consumption (see figure 3.16). However *post hoc* LSD found no significant differences between treatment conditions.

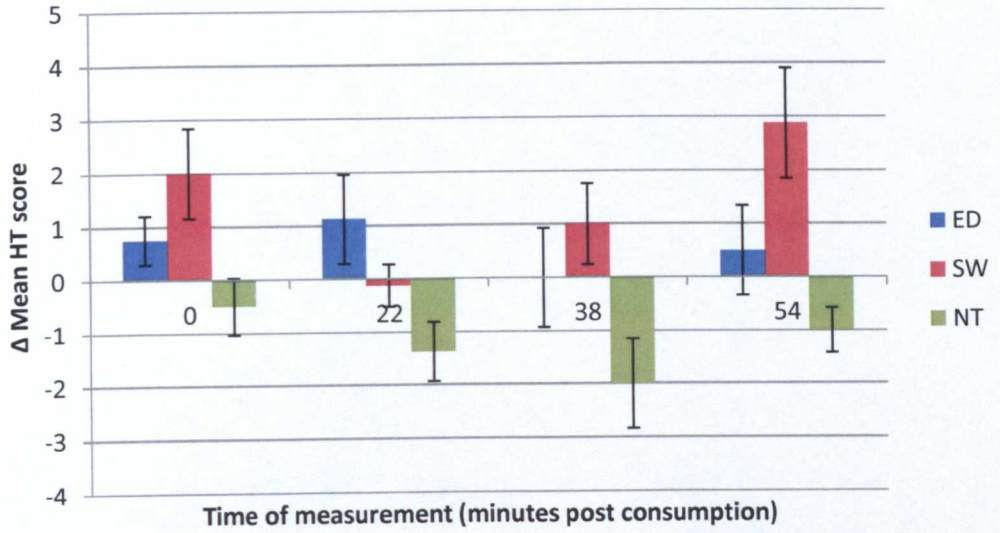

Figure 3.16: Δ Mean HT score for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions.

3.3.2.4 Anger/Frustration

ANOVA found significant effects of treatment on Δ mean AF score at 0 minutes following consumption (p=0.010). However *Post hoc* LSD found no significant differences between treatments. There appears to be a trend for increased AF scores at 22, 38 and 54 minutes post treatment, however ANOVA failed to find significance of treatment.

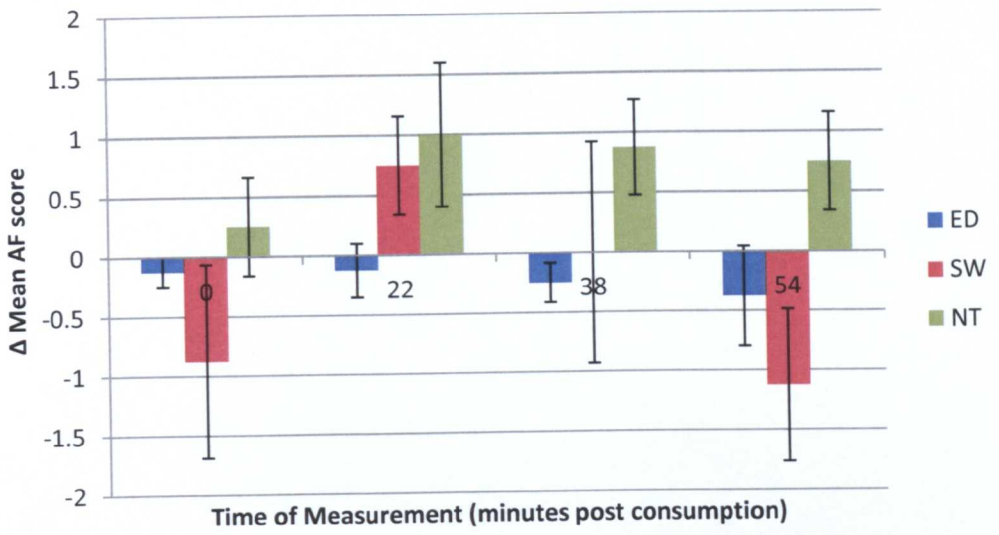

Figure 3.17: Δ Mean AF score for energy drink (ED), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference from all other treatments at that time point (P<0.05, ANOVA and *post hoc* LSD).

3.4 Discussion

The main aims of this study were as follows:

- Measure the postprandial impact of drinks on cognitive performance and mood, in order to confirm effects of energy drinks observed in the literature using our own methodology.
- Determine at what time following ED consumption potential cognitive effects are likely to be strongest.
- Investigate any potential interactive effects of time of day or gender on cognitive effects of energy drink consumption.

- Investigate sensitivity/ability of saccadometer and mood questionnaire to detect small variations in cognitive ability that may be inferred by postprandial (and possibly sensory) impact of EDs.

It was hypothesised that energy drinks could influence cognitive task performance and subjective mood through the modulation of neurotransmitter systems in the brain. Whilst the results of this study cannot establish the specific mechanism behind the action of ED treatment, the effects on task performance (in the PCG task) are relatively clear.

The most interesting result is the influence the energy drink had on the peripheral conflict task with the gap paradigm, but not in the overlap condition. This leads to a possible influence of the drink on the 'gap effect', a phenomenon whereby saccadic latencies are speeded by the removal of the fixation point for a period prior to stimulus presentation, and a decrease in accuracy is also observed (Saslow 1967). One theory for the mechanism of this effect is that removal of a fixation point allows attentional disengagement (Fischer and Breitmeyer 1987; Fischer and Weber 1993), that is to allow attention to shift from one point to another, attention must be disengaged from the first target before it can shift to a peripheral target. Another hypothesis is the competitive inhibition of two neural systems in the superior colliculus, one responsible for making fixations, and another for initiating saccades. When the eye is already fixating it is more difficult to initiate a saccade when there is no gap due to the inhibition of the neural system responsible for saccades by that responsible for fixation (Reuter-Lorenz, Hughes et al. 1991). With either hypothesis, the ED has only influenced task performance when the fixation point was unable to inhibit the onset of the reaction due to the presence of a gap, suggesting that either only neural systems responsible for initiating saccades are affected, or EDs can only improve visual perception when the attention has been disengaged. The influence of the drink on accuracy also supports that disinhibition of cells responsible for initiating eye-movements had an impact: the decrease in accuracy in anti-saccade trials may be caused by the decrease in RT (or by the

increase in 'readiness' to respond), preventing sufficient processing of the information contained within the cue, resulting in directing a saccade at the newly presented peripheral cue, irrelevant of central cue colour. In order to determine whether inhibition in the shift of attention or inhibition of neural pathways responsible for starting saccades is responsible for these effects, perhaps a manual (i.e. finger pressing) version of the task could be used. A significant beneficial effect on a manual choice reaction time task with ED consumption would indicate the former, and no effect would suggest the latter.

The strongest effects of ED consumption occurred 30-50 minutes post consumption, backing up findings in the literature of ED consumption on visual information processing (Kennedy and Scholey 2004), simple reaction time (Horne and Reyner 2001; Smit, Cotton et al. 2004) and driving performance (Horne and Reyner 2001).

The effects of treatment on saccadic amplitude in the LAT task are interesting. Change in amplitude increased in the SW group and decreased in the NT group at 42-46 minutes post consumption whilst remaining close to zero in the ED group. An increase in amplitude suggests that participants in the SW group were making saccades that resulted in fixations past the point of peripheral target, and conversely the NT group were making saccades that did not reach the peripheral target. This could signify that fatigue played a role as this was the simplest task at 42-46 minutes post treatment (the fifth time the task had been completed including familiarisation trials, over an hour after the experimental procedures had begun). It should be noted that at baseline amplitude for the SW group was as low as 9.26 deg (peripheral targets were presented at 10 deg) and as high as 11.20 deg in the NT group, so these effects may not necessarily be caused by fatigue. In fact, the effects observed post treatment could be a result of facilitation, as this was the first task carried out where experimental data was recorded (though this is unlikely as latencies don't appear to have been affected by a facilitation effect).

The possible interactive effect of treatment and time of day on accuracy was a very interesting. If accurate this agrees with research by Horne and Reyner, who suggest

that for the effectiveness of stimulants to be assessed measurements should take place when arousal is at its lowest (Horne and Reyner 1996; Reyner and Horne 2000). That is, at the circadian nadir and mid-afternoon drop (~0600 and 1600 hours respectively; Horne and Reyner 1995). These first two studies investigated the impact caffeine has on counteracting sleepiness in driving tasks, however other studies have been able to demonstrate beneficial effects of caffeine supplementation during the mid-morning (~0900-1000), when arousal is generally at its peak, on reaction time (Smit and Rogers 2000; Brice and Smith 2002), vigilance (Lieberman, Wurtman et al. 1987; Temple, Warm et al. 2000), attention (Warburton 1995), visual information processing and mood (Smit and Rogers 2000). The present study suggests that the beneficial cognitive effects of EDs are limited to afternoons, however these time of day effects may be task specific and further research is necessary.

There appeared to be a small but insignificant increase in energetic arousal in the first two measurements following ED consumption. The lack of significant effect observed on EA following ED treatment was unexpected, as findings by Smit and Rogers (2002) and Smit, Cotton *et al.* (Smit, Cotton et al. 2004) found significant impact of ED treatments, increasing energetic arousal from around 30 minutes following consumption up 100 minutes. These studies however, used different mood questionnaires: a 12-item unipolar visual analogue scales questionnaire based on the Profile of Mood States (PoMS) and an unnamed questionnaire that used a 9-point scale with adjectives presented in a random order respectively. The second of these studies found that hedonic tone reduced over time following placebo energy drink and water consumption, an effect that was attenuated with functional ED treatment. Perhaps if power was increased by increasing the number of participants, the present study may have observed significant effects on energetic arousal. The results presented in this study show that the anger/frustration scores tended to increase following treatment in the no treatment condition. Though not significant, this trend could be attributed to the fact that participants were not blind to the treatment they were receiving. Having

volunteered for a study into 'the effects of energy drinks' and subsequently been told they were receiving no energy drink, these participants' AF score may have been affected. This type of effect has been observed before, Mikalsen, Bertelsen *et al.* (2001) explained that participants told they were receiving a caffeinated drink, irrespective of what drink was actually administered a decrease in subjective 'calmness' was observed.

Of the three saccadometer tasks used, it appears that only one was sensitive to the postprandial rise in glucose and caffeine levels associated with ED consumption. With regards to the LAT task, it is perhaps too simple and ceiling effects preventing improvement in performance may have contributed to the lack of effect. Another explanation could be that, with more difficult tasks there is sometimes a decrease in performance over time associated with control/placebo treatments which can be attenuated (temporarily) with ED consumption (Kennedy and Scholey 2004). The differing effects observed in the PCG and PCO tasks have been explained above, however a direct *post hoc* comparison of performance in the two tasks may not be appropriate as tasks were always completed in the same order, with changes that may be related to the postprandial effects of treatment possibly occurring. In order to directly compare performance in gap and overlap trials during the postprandial period, a task that runs both trial types either in series or at random would allow calculation of the 'gap effect', the difference in reaction time for gap and overlap trials.

With the choice of placebos used in this task, it was impossible for participants to be blind to the treatment they received and as alluded to above, with the mood questionnaire being subjective there was always the chance that expectancy effects would impact on results. With no rise in energetic arousal following ED treatment, it is unlikely that an expectancy effect has had any influence. In order to prevent expectancy from affecting any results of future studies, it would be wise to use a 'sensory-matched' placebo energy drink in addition to still mineral water and no treatment. In addition to this, presenting the treatments in a balanced crossover

design, rather than the between subjects design used in this study, would add to the power of the experiment.

CHAPTER 4: THE INFLUENCE OF SENSORY AND POSTPRANDIAL PROPERTIES OF ENERGY DRINKS ON ATTENTION IN HUMANS

4.1 Introduction

This study aimed to investigate the influence that the sensory qualities of EDs can impart on cognitive performance, and compare these effects with those observed postprandially. It is difficult to predict what task types chemosensory perception of an ED might influence, however it would be logical to hypothesise that chemosensory perception might be similar to that observed postprandially, particularly if reward value and energy content were to drive such an effect. The study outlined in chapter 3 discovered that ED treatment could reduce saccadic latencies in the postprandial period when a gap was present prior to the stimulus appearing; however it was unclear whether this was caused by greater ability to re-engage attention to the stimulus or an improvement in ability of neural pathways to begin saccades. If the former is true, reaction times in a manual choice reaction time task (CRTT) with gap/overlap paradigm will be affected by the ED treatment.

As it appears that ED treatment may affect ability to re-engage attention following the removal of a cue, it may be that the attentional blink phenomenon is affected too. The attentional blink (described in Raymond, Shapiro et al. 1992) describes the lowered ability to detect a second target stimulus presented soon after a first target stimulus. The mechanism of this phenomenon is explained by Sperling and Weichselgartner (1990), stating that attention acts as a gate that opens once the first target is presented. This allows information regarding the stimuli to enter working memory, however the rapid presentation of stimuli overflows working memory, resulting in the closing of the 'attentional gate' and inability to detect stimuli in a given period following presentation of the first target. If as predicted by the previous study, ED treatment could theoretically allow participants to reengage attention faster, shortening the period of the attentional blink, a change in the ability to detect the second target should be observed.

4.2 Materials and Methods

4.2.1 Participants

Fifteen (nine female, six male; mean age 23.5 years, SD 3.6) volunteers gave informed consent to participate in this study. Those who were pregnant, diabetic, smokers or suffered from any condition that could affect their ability to take part were excluded from the study. Participants were asked to attend five 1.5 hour sessions (one familiarisation session and four experimental sessions), arriving for each experimental session at approximately 10am – having been asked to eat a small breakfast before 8am, not to eat or drink anything (except water) for the two hours before arriving for the test session, and to abstain from caffeine consumption on the day of each session. Participants were required to estimate their weekly habitual intake of caffeinated products and average daily caffeine intakes were calculated using figures given by the Food Standards Agency (2008). A blank example of the 'case report form' containing information for participants, consent forms, screening questions and space for experimenter notes is included in appendix 4 (required by GlaxoSmithKline). Those who completed all five sessions were given a monetary reward for their participation. Ethical approval was granted by the University of Nottingham School of Psychology Ethics Committee.

4.2.2 Samples

In order to investigate both sensory and postprandial impact of the ED and placebo, four experimental treatments were used (see table 4.1), two non-carbonated ED (one active, one placebo) treatments[4] and two control treatments (Evian still mineral water with orange colouring (Dr Oetker Ltd.) and no treatment). An artificially sweetened placebo treatment designed to taste 'similar' to the active ED treatment was used as a means of delivering a tastant that would be perceived as sweet without activating the proposed oral nutrient receptors; still mineral water was used as a control that activated somatosensory neural pathways and no treatment was used as a somatosensory control.

[4] Full composition of the energy drink treatments cannot be included in the thesis due to a confidentiality agreement between the lead experimenter and GlaxoSmithKline.

Table 4.1: Summary of treatments used and ingredients present.

	Glucose (g/100ml)	Caffeine (mg/100ml)	Aspartame (mg/100ml)	Acesulfame K (mg/100ml)	Orange Colour (Y/N)
Functional ED (ED)	17.9	12.1	0	0	Y
Placebo ED (P)	0	0	16.3	4.5	Y
Still mineral water (SW)	0	0	0	0	Y
No treatment (NT)	n/a	n/a	n/a	n/a	n/a

In each of the four experimental sessions, one treatment was administered, with the four sessions being delivered in a randomised and balanced design (MacFie, Bratchell et al. 1989). ED and P treatments were created by the lead experimenter using ingredients supplied by GlaxoSmithKline and administered in a double-blind method in unbranded packaging.

4.2.2.1 Sensory Analysis of Energy Drink Samples

The two energy drink treatments were intended to be 'taste-matched' and not be perceived as different by participants. To determine whether test drinks were truly 'taste-matched', sensory analysis was carried out on the samples in the form of similarity testing using a triangle test, adhering to the guidelines set out in the British Standard for the sensory analysis technique, the triangle test (British Standards Institution 2007) in the Sensory Science Centre at the University of Nottingham. The Fizz Acquisition programme was used to design and run the tests using a randomised double-blind design.

To obtain protection from falsely concluding that the samples are similar, β=0.05 was accepted, with the maximum allowable proportion of discriminators identified as p_d =20%. However with α=0.2, 86 assessors would be required (using table A.3 in the British Standard), so a compromise of α=0.2, β=0.01 and p_d=30% was accepted, requiring 64 assessors (using table A.3 in the British Standard). 66 assessors (staff and students at the University of Nottingham) were recruited for the study.

Assessors each completed one triangle test, where three 20ml samples (two of one sample, one of the other) were presented in 30 ml plastic pots each identified by a three digit code. Assessors were asked to taste each sample in the order presented and to identify the odd sample. The presentation order of the samples in each test

was randomised using a Latin-square design, with six possible presentation orders. Still water and crackers were provided for panellists to cleanse their palates between each sample.

4.2.3 Tasks

Results from chapter 3 suggested that a commercially available ED would improve reaction time in a choice reaction time task when a 200 ms gap was present between a pre-stimulus cue and presentation of the stimulus, but not when the gap was absent. For this reason a choice reaction time task (CRTT) including a gap condition with a high difficulty level that would prevent a ceiling effect and potentially be sensitive to effects of glucose postprandially (glucose appears to influence cognitive performance when task difficulty is high (Donohoe and Benton 1999; Messier 2004)) was chosen. A no-gap (overlap) condition was also included as a comparison. Reaction time and accuracy in RT tasks does not give the full picture as to how improvements in performance have occurred; a task that includes signal detection allows us to determine whether improved reaction time performance is caused by greater responsivity (i.e. without necessarily processing the information fully) or though greater/faster ability to discriminate and process information. For this reason a visual information processing task where a phenomenon known as the attentional blink is observed was also used (Raymond, Shapiro et al. 1992).

Participants were asked to complete two computer based tasks (a choice reaction time task and an attentional blink task, both written using the E-prime programme) lasting approximately 20 minutes in total, three times each session.

4.2.3.1 Choice Reaction Time task

Each trial began with the presentation of a black fixation cross on a gray background for a duration of 800 ms prior to the presentation of the stimulus. In one half of trials, the stimulus appeared as soon as the fixation point disappeared (overlap condition, figure 4.1), and in the other half a 200ms gap was present (gap condition, figure 4.2). There were four possible stimuli that could be presented: a blue square,

a blue circle, a yellow square or a yellow circle, presented for a duration of 150 ms. The participant was to respond as quickly as possible by pressing the left button on the response pad (displayed in figure 4.3) using their left index finger when either a blue square or yellow circle was presented, and by pressing the right button with their right index finger when either a yellow square or blue circle were presented. The interval between trials was 1050 ms following gap trials and 1250 ms following overlap trials). The fixation-cross and stimuli were all presented in front of a grey background. The task was made up of 128 trials lasting approximately 7 minutes in total, with the opportunity for a break after 64 trials.

Reaction time (in milliseconds) and response (left or right button press) were recorded for each trial. At the completion of the 128 trials there were 6 parameters recorded: median reaction time (RT) and mean accuracy (ACC; [n correct responses / n total trials * 100]) gap trials, RT and ACC for overlap trials, and scores for the impact of gap presence were calculated by taking the RT and ACC scores in gap trials from the RT and ACC scores obtained in overlap trials.

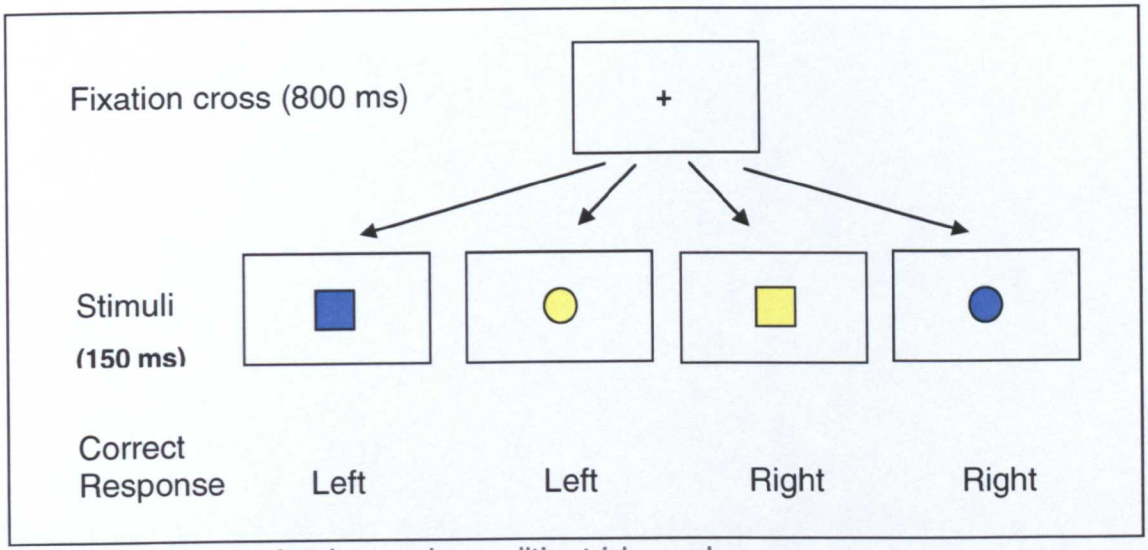

Figure 4.1: Choice reaction time overlap condition trial procedure.

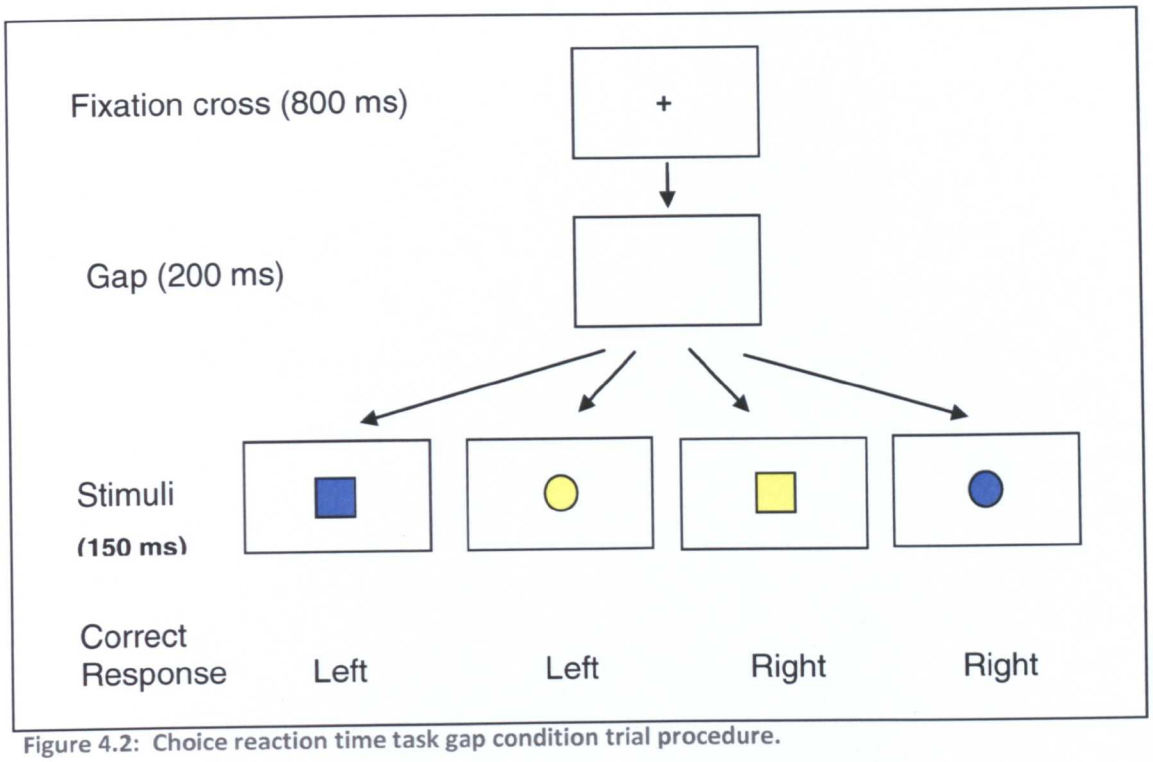

Figure 4.2: Choice reaction time task gap condition trial procedure.

4.2.3.2 Attentional Blink Task (ABT)

The attentional blink task is a type of rapid serial visual presentation task, where two different salient target stimuli are presented within close proximity to each other within a series of non-target stimuli, first described by (Raymond *et al.* (1992). In the variation used in this study (figure 4.4), each trial began with the participant being asked to press the space bar to continue (allowing a break whenever necessary), and consisted of rapid presentation of a 16 letter sequence, with one white letter appearing between positions 3-8. All of the other letters were black, and a grey background was used for the entire task. In 50% of trials, the letter X was presented within the eight letters following the white letter. At the end of each trial, the participant was asked two questions, 'What was the white letter?' and 'Was the letter X presented?'. The task consisted of 96 trials (12 trials for each possible position of the white letter, with eight of each having the letter X present) and lasted roughly 13 minutes.

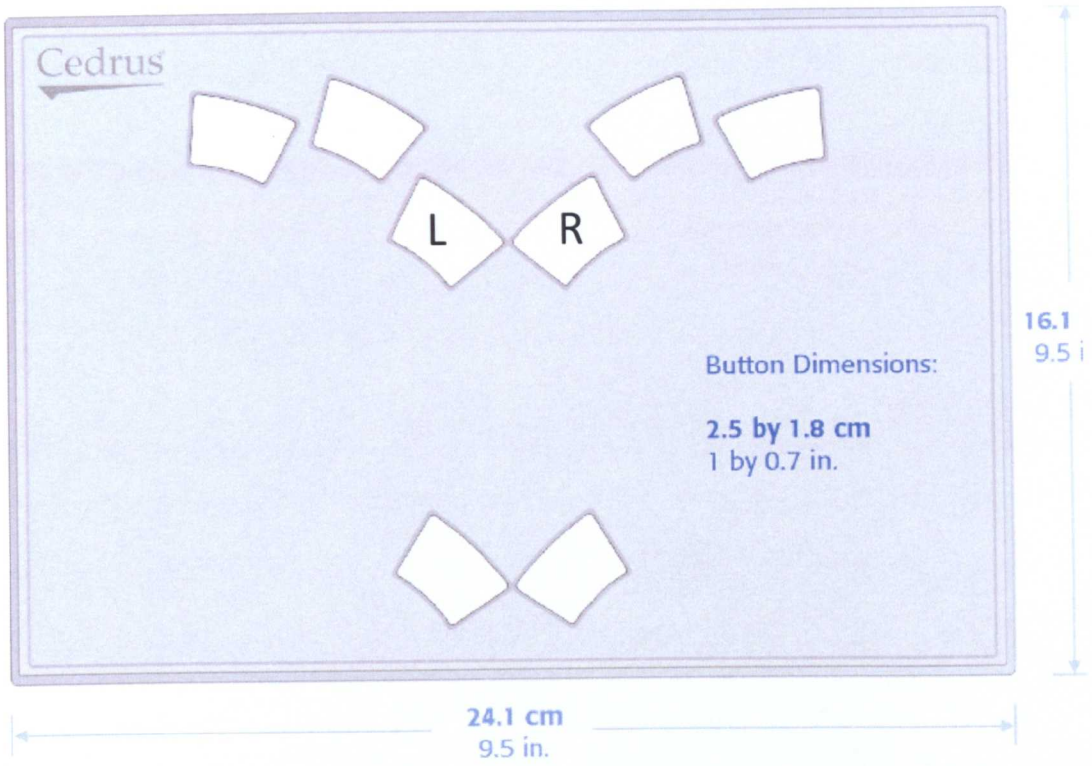

Figure 4.3: Cedrus RB-830 Response Pad - used to collect responses in Choice RT task (Cedrus 2009).

Accuracy in response to each question (number of correct responses / total number of trials * 100) was calculated. Response to question 2 (Was the letter X presented?) was also assessed using a signal detection analysis. The number of hits, misses, correct rejections and false alarms were calculated (explained in table 4.1). Based on the proportions of these responses, an estimate of sensitivity to the stimulus, d' ($Z_{(hit\ rate)} - Z_{(false\ alarm\ rate)}$) was calculated for each participant.

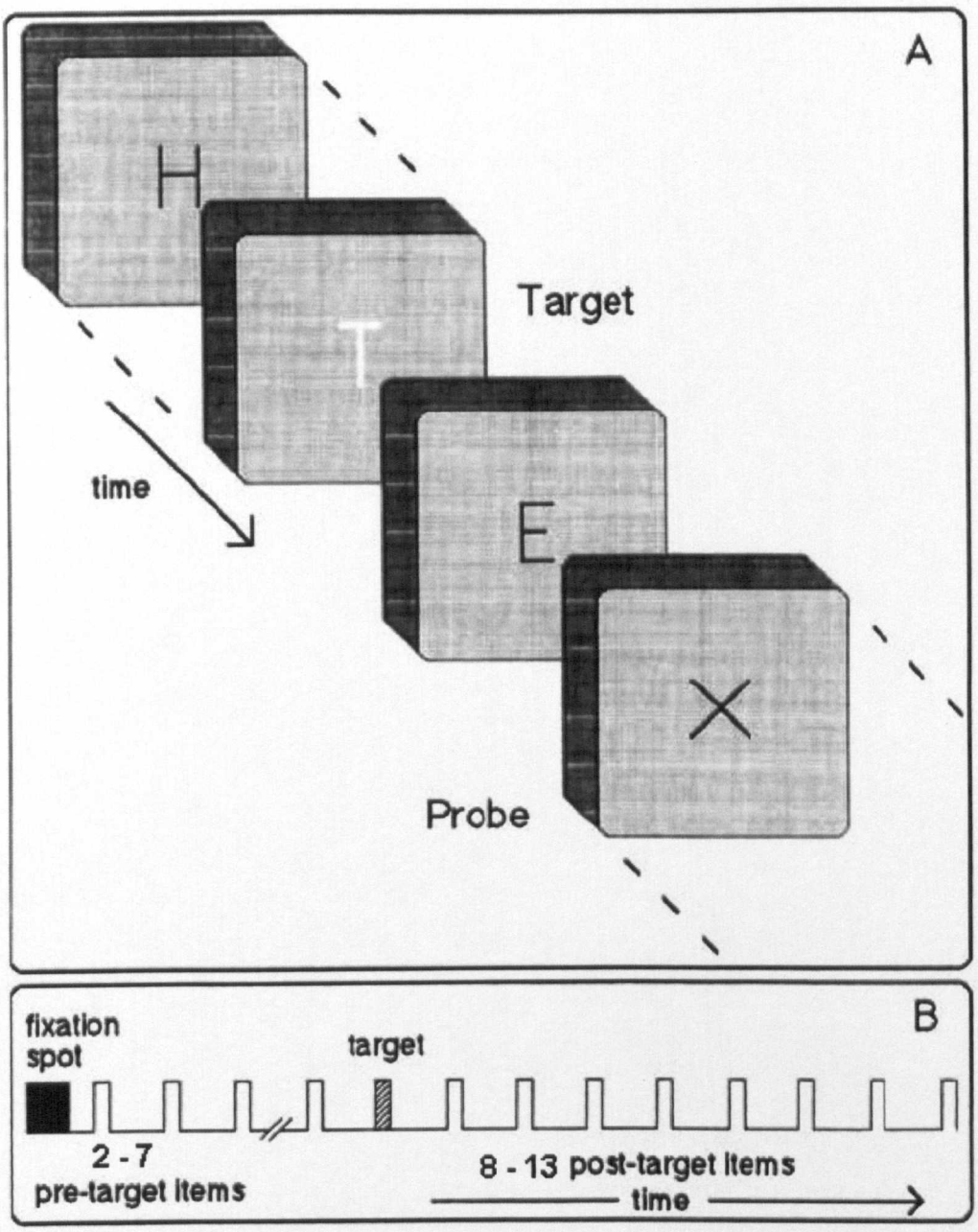

Figure 4.4: Panel A illustrates the presentation of stimuli during the attentional blink task. Embedded in the 16-letter series is a white letter, occurring between positions 3 and 8 in the series. The letter X was presented within 8 letters following the white letter in the series in 50% of trials. Panel B illustrates the stimulus presentation over time. Figure adapted from Raymond *et al.* (1992).

Table 4.2: Explanation of the categorisation of responses in signal detection tasks.

		Response	
		'Present'	'Absent'
Stimulus	Present	Hit	Miss
	Absent	False Alarm	Correct Rejection

4.2.4 Experimental Design

Table 4.3 outlines the schedule of the experimental procedure. After arrival for their familiarisation session, participants were given an information sheet to read and consent form to complete. Participants were sat at a computer where they received information about how to complete the tasks and completed 20 trials of the CRTT and 15 trials of the ABT to re-familiarise themselves with the tasks (approximately 10 minutes in total) before measurement of baseline performance (20 minutes).

Table 4.3: Schedule of experimental procedure.

Time	Trial Type	Task	Duration (min)
~10.00		Participant arrives, reads information sheet and signs consent form.	1
10.00	Familiarisation	Choice RT task	5
10.05		Attentional Blink task.	5
10.10	Baseline	Choice RT task	7
10.17		Attentional Blink task	13
10.30	Sensory	Choice RT task	7
10.37		Attentional Blink task	13
10.50	Break	Participant receives drink	
10.50		Break	20
11.10	Postprandial	Choice RT task	7
11.17		Attentional Blink Task	13
11.30		End	91 (total)

For all trials, even those where no test samples were delivered to the mouthpiece, participants were required to hold the mouthpiece from the pump in their mouths, with the pump active (although not connected to the mouthpiece, to ensure that sounds that were made by the pump during the measurement of sensory impact were present for all trials).

In the sensory trials (figure 4.5 shows an illustration of the participant sat at the computer during the sensory trials), 80ml of the test drink was be delivered to participants' mouths in regular 0.5ml doses (in the CRTT 64 x 0.5ml doses were delivered every other trial starting with the first trial, and in the ABT 96 x 0.5ml doses delivered each trial during stimulus presentation) for the duration of the 20 minute test. The test drink was kept within sight of the participant while the tasks were being performed, and a cardboard screen was placed between the participant and the pump to prevent the participant from being distracted by the pump. The test drinks were delivered using an Autoclude Programmable Easy Tube Load Peristaltic Pump (Verder Ltd.) with Verderprene tubing (1.6mm bore x 1.6mm wall; food grade quality; Verder Ltd.) and two 800mm Narrow Bore Extension Sets (Portex) which were placed on either end of the Verderprene tubing with one used as a sterile mouthpiece and the other placed in the test drink bottle. In the familiarisation session, still mineral water was delivered through the pump during the sensory trials to allow the participant to become accustomed to performing the tasks whilst consuming a test drink.

300ml of the test drink was given to the participant after completion of the sensory trials (therefore a total of 380ml was administered, the volume of a standard bottle of Lucozade Energy Original), and given a 20 minute break before repeating the two tasks a final time, as measurement of the postprandial impact of the drink.

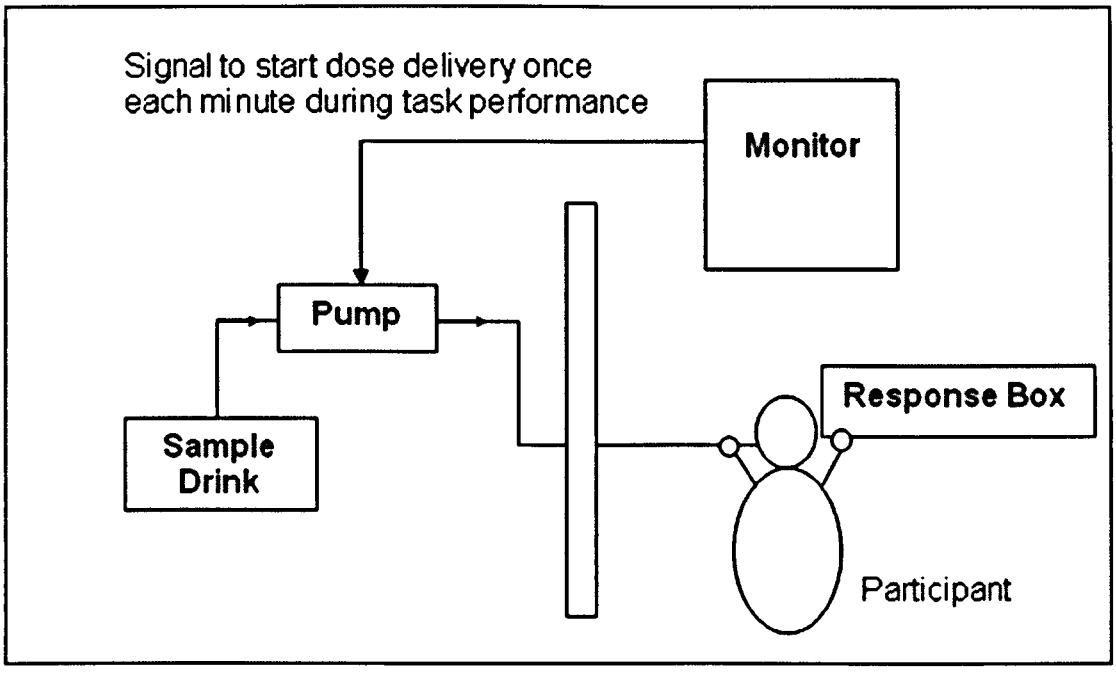

Figure 4.5: Illustration of experimental booth and equipment.

4.2.5 Data Analysis

4.2.5.1 CRTT

Trials where participants responded before 100 ms or after 1000 ms following stimulus presentation, or responded incorrectly were excluded from the analysis. Each time the task was performed, median reaction time (RT) was calculated. Mean change in median RT scores (Δ RT) between baseline and each post-treatment measurement were also calculated.

Each time the task was performed, the percentage of correct responses was calculated (accuracy or ACC). Mean change in percentage correct responses between baseline and each post treatment measurement were also calculated (Δaccuracy or ΔACC).

Between treatment effects on group mean of individual median RT, mean accuracy, mean ΔRT and mean Δaccuracy were analysed using ANOVA and *post hoc* LSD test. Within treatment effects were monitored using a separate ANOVA where measurement (e.g. baseline or time of post-treatment measurement) was the only

fixed factor and *post hoc* Dunnett Test was used to compare mean median baseline scores with each post treatment score.

4.2.5.2 ABT

For each time the task was performed, number of correct detections of T1 and T2 for each lag position (i.e. Lag 1-8) and percentage correct responses for each question (i.e. accuracy at all lag positions) were recorded. Ability to detect T2 relative to T1 was calculated by subtracting the number of correct detections of T1 from correct detections of T2 at each lag position (titled L1, L2, L3...L8 to avoid confusion with Lag1, etc.). A signal detection analysis was carried out on ability to detect T2, meaning hit, miss, false alarm and correct rejection rates were calculated in order to determine d'. Between treatment effects on accuracy in response to each question, miss rate, false alarm rate, d' and L1-L8 an mean change in each of these measures were analysed using ANOVA and *post hoc* LSD test. Within treatment effects were monitored using a separate ANOVA where measurement (e.g. baseline or time of post-treatment measurement) was the only fixed factor and *post hoc* Dunnett Test compared mean median baseline scores with each post treatment score.

4.2.5.3 Effects of Treatment, Habitual Caffeine Intake, Age and Gender

In the ANOVA, treatment was inserted as a fixed factor in order to determine the effects of treatment on the measures outlined above. Habitual caffeine intake was calculated for each participant as in section 3.2.4.5, and median scores for HCI and age were used to split participants into high/low caffeine consumers and 'older'/'younger' participants. Age, HCI and Gender were all included as fixed factors in the ANOVA analysis.

4.2.5.4 Post Hoc ABT Task Analysis

In order to determine whether the attentional blink phenomenon was in fact elicited by the ABT, a post hoc analysis compared T2 detection at lag positions L2, L3 and L4 (the period during which target detection is expected to be inhibited) with detection at positions L6, L7 and L8. The percentage of correct detections of T2 at

positions L2-4 and L6-8 were calculated, and paired t-test was used to compare these scores at baseline, sensory and postprandial measurements.

4.3 Results

4.3.1 Sensory analysis of energy drink samples

A total of 46 out of 66 assessors correctly identified the odd sample in the test. Referring to table A.2 in the British Standard (British Standard British Standards Institution 2007), in the row corresponding to n=66 and column α=0.2, the maximum number of correct responses required for significance is 26, therefore with 46 correct responses it can be concluded that the samples were not perceived to be similar.

4.3.2 Cognitive Assessment

Due to a computer error, data recorded from one participant in the CRTT, and from three participants in the ABT was excluded from the study. Therefore, for data pertaining to the CRTT, n=14 (median age = 23.5; 6 male, 8 female) and the data concerning the ABT, n=12 (median age 23; 5 male, 7 female). Results from the CRTT are shown in table 4.1, with results from the ABT shown in Tables 2 (overall data) and 3 (target detection at each lag position).

4.3.2.1 Postprandial Effects of Treatment

CRTT

Results from the CRTT are summarised in table 4.4, where RT and ACC scores for gap and overlap trials are presented for each treatment. No within treatment effects were observed with any of the treatments on any of the outcome measures in the CRTT. Postprandially, ED consumption has very significantly reduced RT in overlap trials compared with all three other treatments as is displayed in figure 4.6 (p=0.021, ANOVA; ED vs P, p=0.035; ED vs SW, p=0.033; ED vs NT, p=0.001).

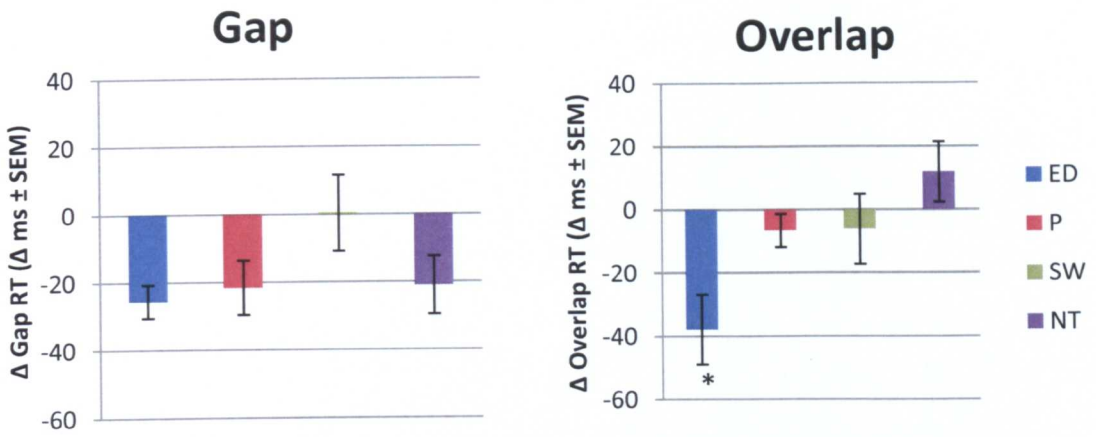

Figure 4.6: Charts displaying change in mean RT (ΔRT) scores between baseline and postprandial measurement for energy drink (ED), placebo (P) still mineral water (SW) and no treatment (NT) conditions. Chart A shows mean RT(±SEM) in gap trials; Chart B shows mean RT (±SEM) in overlap trials. * indicates significantly different from all other treatments ($p<0.05$, ANOVA and *post hoc* LSD).

Treatment was also found to influence the 'gap effect' on RT postprandially. ANOVA found a significant effect of treatment ($p=0.037$) with *post hoc* LSD finding differences between ED and NT ($p=0.016$) and SW and NT ($p=0.003$) conditions (see figure 4.7). Treatment also significantly impacted on Δ 'Gap Effect' RT ($p=0.029$, ANOVA), with scores for NT significantly higher than ED and SW treatment (NT vs. ED $p=0.002$; NT vs. SW $p=0.006$) and the score for P treatment significantly higher than for ED treatment ($p=0.048$), displayed in figure 4.7.

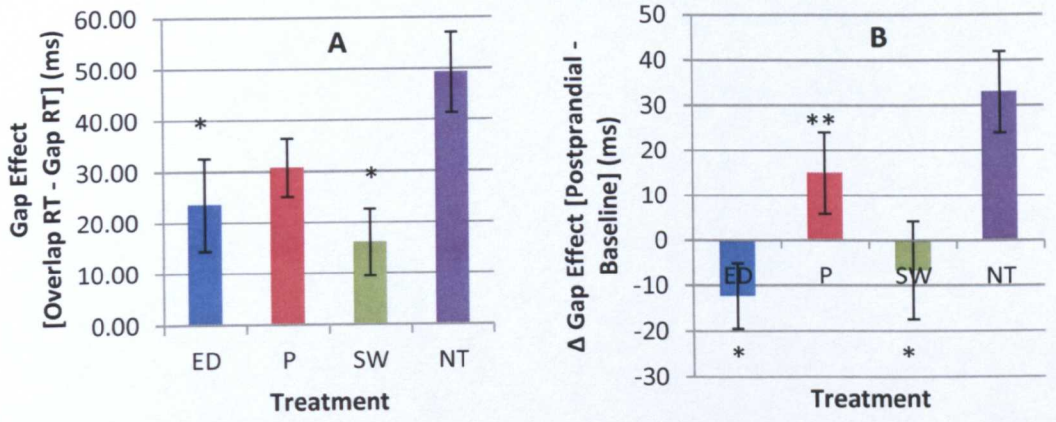

Figure 4.7: Chart A shows mean gap effect for energy drink (ED), placebo (P) still mineral water (SW) and no treatment (NT) conditions postprandially. Chart B shows Δ gap effect for each treatment postprandially. * indicates significant difference from NT ($p<0.05$ ANOVA and LSD). ** indicates significant difference from ED ($p<0.05$ ANOVA and LSD).

Table 4.4: Results from Choice Reaction Time Task. Table presents mean and standard error mean (SEM) values for each measurement (baseline, sensory and postprandial) for all four treatments: functional energy drink (ED), placebo energy drink (P), still mineral water (SW) and no treatment (NT).

Measure	Treatment	Baseline		Sensory		Postprandial	
		Mean	SEM	Mean	SEM	Mean	SEM
Gap RT	ED	521.29	22.15	501.04	20.31	495.68	22.86
	P	529.89	24.16	503.50	23.10	508.29	21.78
	SW	510.68	20.63	511.00	21.11	511.07	23.49
	NT	524.25	28.18	523.00	28.72	503.21	24.83
Overlap RT	ED	557.00	27.06	537.93	25.92	519.14	22.81
	P	545.61	23.13	541.36	24.72	539.04	22.82
	SW	533.32	21.35	541.68	21.88	527.14	20.53
	NT	540.57	27.75	550.46	27.06	552.50	25.57
Gap Acc	ED	85.08	3.25	87.52	2.47	88.45	2.47
	P	88.74	2.36	86.26	3.59	88.40	3.29
	SW	87.48	2.18	83.92	2.48	86.75	2.61
	NT	88.31	2.45	81.74	3.71	87.55	2.73
Overlap Acc	ED	86.39	3.04	86.55	2.81	87.98	2.80
	P	87.70	3.01	86.29	3.49	85.37	3.88
	SW	87.78	2.34	80.99	3.46	87.62	2.00
	NT	87.80	2.94	84.37	2.93	85.74	2.81
Gap Effect on RT	ED	35.71	9.57	36.89	8.86	23.46	5.75
	P	15.71	6.38	37.86	7.56	30.75	9.07
	SW	22.64	8.15	30.68	10.60	16.07	6.55
	NT	16.32	4.43	27.46	6.92	49.29	7.82
Gap Effect on Acc	ED	1.31	1.29	-0.97	2.06	-0.47	1.15
	P	-1.04	1.12	0.03	1.40	-3.03	1.10
	SW	0.29	2.12	-2.94	2.25	0.87	1.45
	NT	-0.51	1.82	2.63	2.13	-1.80	1.64

No significant effects of treatment were observed on accuracy postprandially, however as seen in figure 4.8, accuracy tended to increase from baseline scores with ED treatment, with no change or even a tendency to fall with other treatments.

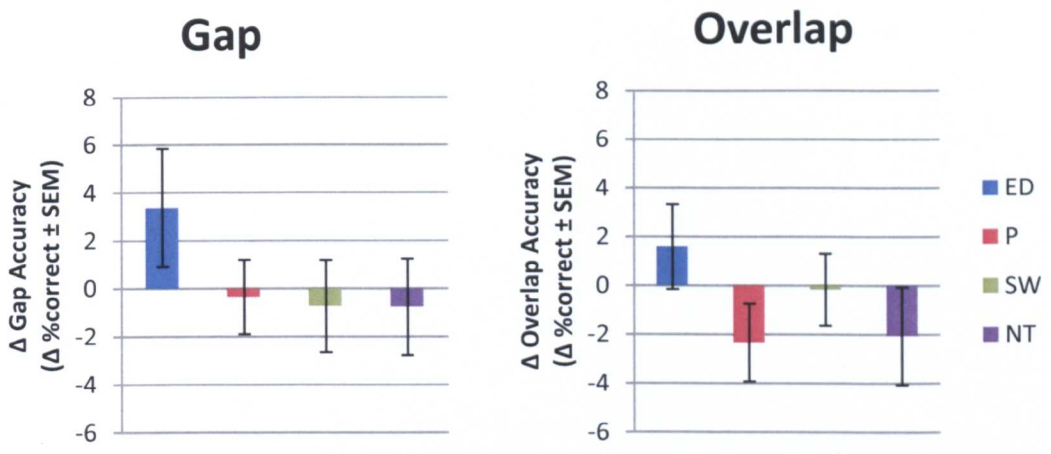

Figure 4.8: Charts displaying change in mean accuracy (Δ percentage correct) scores between baseline and postprandial measurement for energy drink (ED), placebo (P) still mineral water (SW) and no treatment (NT) conditions. Chart A shows mean ΔAcc (±SEM) in gap trials; Chart B shows mean ΔAcc (±SEM) in overlap trials. * indicates significantly different from all other treatments ($p<0.05$, ANOVA and *post hoc* LSD).

ABT

Table 4.5 displays the results from the ABT, showing accuracy for each of the questions asked, miss rate, false alarm rate and sensitivity (d') for each treatment. No significant effects of any of treatment on any of these measures were observed.

Table 4.5: Overall Results from the Attentional Blink Task. Table presents mean and standard deviation (SD) values for each measurement (baseline, sensory and postprandial) for all four treatments: functional energy drink (ED), placebo energy drink (P), still mineral water (SW) and no treatment (NT).

Measure	Treatment	Baseline		Sensory		Postprandial	
		Mean	SD	Mean	SD	Mean	SD
T1 Acc (White Letter)	ED	78.56	14.203	75.17	19.088	78.65	20.712
	P	74.48	18.737	77.34	18.803	77.17	18.794
	SW	78.30	13.039	73.52	17.490	76.91	17.011
	NT	77.26	18.014	76.48	18.657	76.82	19.229
T2 Acc (Letter X)	ED	73.61	9.235	72.05	9.880	72.83	9.583
	P	71.53	7.316	72.92	8.154	73.78	10.107
	SW	72.83	8.720	72.57	8.719	72.14	10.944
	NT	75.52	9.807	76.48	8.147	74.74	9.350
Misses	ED	19.77	6.809	21.25	8.237	20.00	7.977
	P	21.08	5.534	21.00	6.994	20.00	6.876
	SW	18.92	7.255	21.17	7.614	21.33	7.726
	NT	18.00	7.261	16.85	6.958	19.58	7.633
False Alarms	ED	5.00	3.717	5.33	3.774	6.17	4.064
	P	6.25	4.025	4.92	3.655	5.08	4.502
	SW	5.33	4.735	5.25	4.712	5.42	3.895
	NT	5.42	4.274	5.67	3.939	4.92	3.679
D'	ED	2.23	2.030	1.83	1.470	1.80	1.684
	P	1.70	1.434	2.18	1.921	1.96	1.619
	SW	2.21	1.997	2.20	2.058	2.44	2.476
	NT	2.34	2.093	1.72	0.623	2.31	1.986

Table 4.6: Results from the Attentional Blink Task, presenting 'T1 accuracy' minus 'T2 accuracy' at each lag position. Table presents mean and standard deviation (SD) values for each measurement (baseline, sensory and postprandial) for all four treatments: functional energy drink (ED), placebo energy drink (P), still mineral water (SW) and no treatment (NT).

Measure	Treatment	Baseline		Sensory		Postprandial	
		Mean	SD	Mean	SD	Mean	SD
L1	ED	-0.50	2.747	-0.67	1.670	-0.83	2.125
	P	-1.08	2.109	-0.33	2.348	0.00	1.954
	SW	-1.50	2.153	-0.83	1.992	-0.42	2.610
	NT	-0.33	2.462	0.44	2.262	-0.83	2.209
L2	ED	-2.50	2.236	-2.75	2.927	-2.67	2.387
	P	-2.83	2.125	-3.00	1.758	-2.25	2.598
	SW	-2.25	1.960	-2.25	2.527	-3.42	2.353
	NT	-2.17	2.368	-2.50	2.236	-2.17	2.855
L3	ED	-2.33	1.303	-2.50	2.316	-2.42	3.059
	P	-2.67	1.371	-2.25	2.261	-2.58	1.975
	SW	-2.58	1.929	-2.33	2.839	-3.17	1.992
	NT	-2.42	2.644	-2.63	1.886	-2.42	1.676
L4	ED	-2.07	2.136	-2.67	1.435	-2.00	2.000
	P	-2.00	1.477	-2.33	2.103	-1.92	2.021
	SW	-1.17	1.586	-1.08	1.505	-1.17	1.899
	NT	-0.50	1.314	-0.58	1.832	-1.25	2.179
L5	ED	-1.00	1.477	-0.83	1.337	-1.08	1.782
	P	-0.17	1.749	-1.33	2.060	-0.75	1.865
	SW	-1.00	1.954	-1.08	1.240	-1.33	1.231
	NT	-0.83	2.209	-0.73	1.507	-0.92	1.240
L6	ED	0.00	1.477	0.08	1.676	-0.17	1.697
	P	0.42	1.505	-0.42	2.065	0.50	1.446
	SW	0.17	1.115	-0.25	1.815	0.17	1.697
	NT	0.17	1.642	0.40	1.359	0.17	1.467
L7	ED	-0.33	0.888	0.00	0.739	0.00	1.706
	P	0.00	1.044	0.00	0.853	-0.08	0.900
	SW	0.08	1.165	0.17	1.193	-0.08	1.084
	NT	-0.08	0.996	0.08	0.900	0.33	1.435
L8	ED	-0.33	0.888	0.25	1.765	0.58	1.621
	P	0.75	1.357	0.33	0.985	0.50	1.087
	SW	0.00	1.044	0.42	1.311	-0.17	1.337
	NT	-0.33	1.155	0.87	1.080	0.08	1.084

4.3.2.2 Sensory Effects of Treatment

CRTT

No within treatment effects on any outcome measures were observed by ANOVA. ANOVA found significant effects between treatment conditions on gap RT (p=0.041). Placebo treatment significantly reduced Δ gap RT from baseline scores in compared with SW (p=0.022, LSD) and NT (p=0.030 conditions, when perceived in the mouth (displayed in figure 4.9). Differences in Δ gap RT for ED treatment compared with SW and NT approached significance (ED vs. SW p=0.072; ED vs. NT p=0.096).

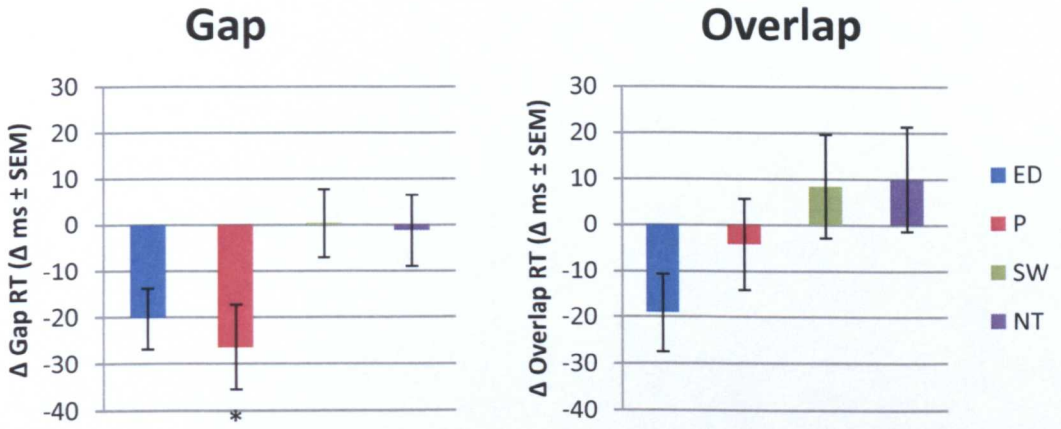

Figure 4.9: Charts displaying change in mean RT (ΔRT) scores between baseline and sensory measurement for energy drink (ED), placebo (P) still mineral water (SW) and no treatment (NT) conditions. Chart A shows mean RT(±SEM) in gap trials; Chart B shows mean RT (±SEM) in overlap trials. * indicates significantly different from SW and NT treatments (p<0.05, ANOVA and *post hoc* LSD).

ANOVA found borderline significant effect of treatment on accuracy in gap trials (p=0.051). As can be seen in Figure 4.10, Δ accuracy in gap trials was found to be significantly higher with ED treatment than with SW (p=0.045) and NT (p=0.004) treatments. For overlap trials SW treatment appeared to reduce Δ accuracy, however ANOVA found no effect of treatment (p=0.203).

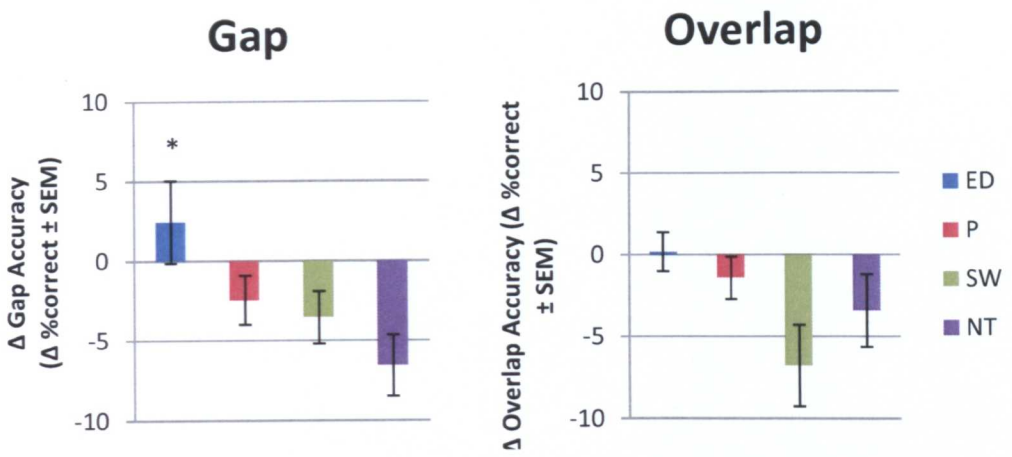

Figure 4.10: Charts displaying change in mean accuracy (Δ percentage correct) scores between baseline and sensory measurement for energy drink (ED), placebo (P) still mineral water (SW) and no treatment (NT) conditions. Chart A shows mean ΔAcc (±SEM) in gap trials; Chart B shows mean ΔAcc (±SEM) in overlap trials. * indicates significantly different from SW and NT treatments ($p<0.05$, ANOVA and *post hoc* LSD).

Impact of gap presence on reaction times

Table 4.7 displays the size of the 'gap-effect' (overlap RT minus gap RT). Gap presence was observed to significantly reduce RTs, as differences between gap and overlap RT scores did not cross zero at baseline, sensory or postprandial measurements, as shown in table 4.7. This finding confirms that the gap presence had the effect it was designed to have, and that responses to overlap trials are either hindered by an inhibitory effect of attentional engagement or greater cognitive demand is required to decide how to respond.

Table 4.7: 'ANOVA Estimated Marginal Means' results showing impact of gap presence on reaction time. Mean estimates for scores for Overlap RT minus Gap RT are shown, alongside the standard error of mean and 95% confidence intervals. * indicates significant difference between gap and overlap RT as 95% CI does not cross zero.

Measurement	Mean difference (ms)	Std Error	Lower 95% CI	Upper 95% CI
Baseline	22.598*	3.768	15.026	30.170
Sensory	33.223*	4.378	24.425	42.022
Postprandial	29.893*	3.667	22.523	37.262

ABT

No significant effects of treatment were observed on any measures recorded in the ABT.

4.2.3.3 Effects of other fixed factors on performance

Age

Figure 4.11 displays mean gap and overlap accuracy in the CRTT for participants younger than the median age (23.5 years old) and older than median age, showing that accuracy in the CRTT was significantly affected by age, with participants aged below the median age being less accurate than older participants.

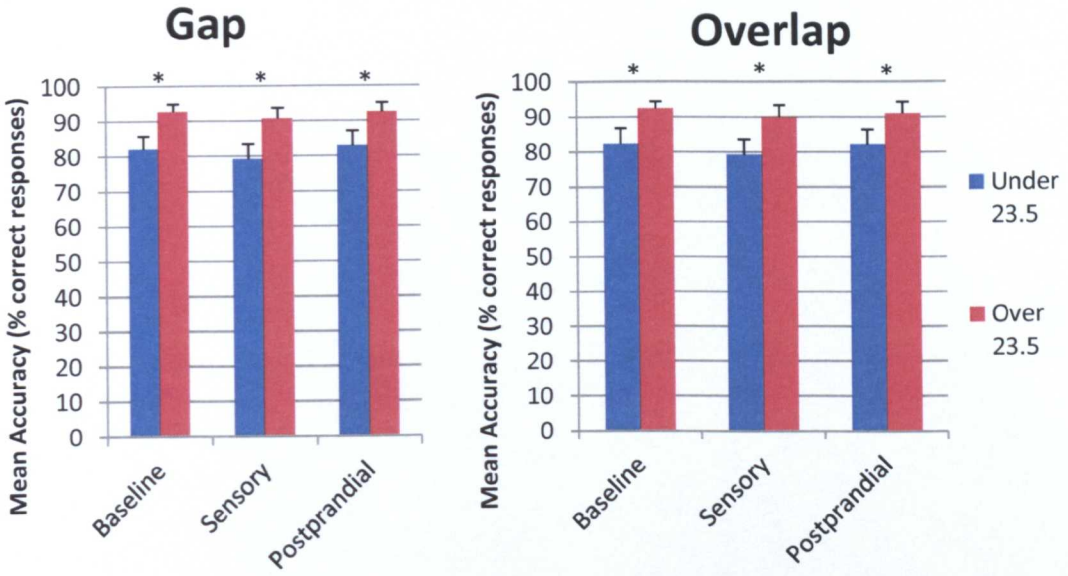

Figure 4.11: Impact of Age on Gap and Overlap Accuracy in the CRTT. * indicates significant difference between participants aged under 23.5 and participants aged over 23.5 ($p<0.050$ ANOVA).

4.2.5.4 Post Hoc ABT Task Analysis

Table 4.6 shows a trend for lower 'T1 minus T2' scores at lag positions L2, L3 and L4 than at other positions. Figure 4.12 displays this attentional blink effect using measurements recorded at baseline for all four treatments. Figure 4.13 displays scores for L2-4 and L6-8 for each measurement (baseline, sensory and postprandial). Paired t-test found significant differences between L2-4 and L6-8 at baseline ($p<0.001$), sensory ($p<0.001$) and postprandial ($p<0.001$) measurement.

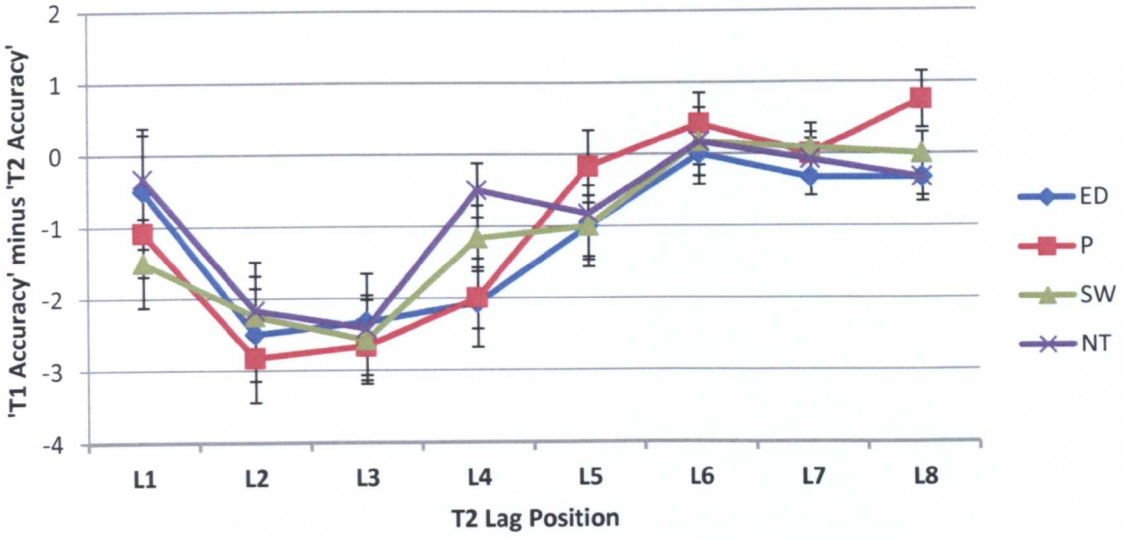

Figure 4.12: Chart displays mean 'T1 minus T2' (±SEM) scores at baseline measurement for energy drink (ED), placebo (P), still mineral water (SW) and no treatment (NT) conditions.

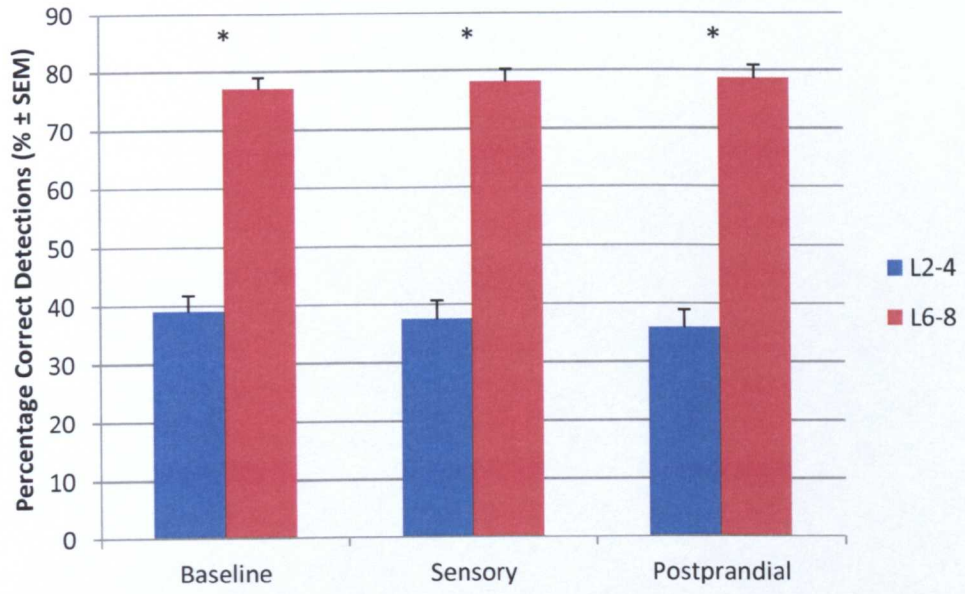

Figure 4.13: Chart displays mean percentage correct detections of T2 (±SEM) scores at baseline measurement for energy drink (ED), placebo (P), still mineral water (SW) and no treatment (NT) conditions. * indicates significant difference between L2-4 and L6-8 ($p<0.001$, paired t-test).

4.4 Discussion

This study aimed to investigate the postprandial and sensory effects of ED consumption on two cognitive tasks that assess attentional ability. The main hypotheses of this study were as follows:

- ED treatment would reduce reaction time in gap trials of the CRTT in the postprandial period, with the decrease possibly influencing response accuracy.
- ED treatment would shorten the attentional blink in the ABT in the postprandial period.
- ED treatment would confer behavioural effects that would not be observed with P treatment when perceived orally, due to the presence of calorific carbohydrates (ONR hypothesis).
- That the 'gap effect' would occur in the CRTT, a shortening of RTs caused by the presence of a 200ms gap between cue and stimulus presentation.
- That the attentional blink phenomenon will reduce perception of T2 when presented in close proximity to T1 in the ABT.

4.4.1 Postprandial effects of treatment

Energy drink treatment appeared to exert beneficial effects on RT in overlap trials but not gap trials in the postprandial period. Although this was the opposite of what was hypothesised, this resulted in significant differences between these treatments on the gap effect, with lower difference between gap RT and overlap RT observed with ED treatment compared with P and NT treatments. It appears that ED treatments affect reaction time tasks with gap paradigms differently when conducted manually than through saccadometry. The ABT could have examined however no significant effects of treatment were observed in this task. Any change in ability to detect T2 with shorter lag periods could have related to overlap RT trials, and longer lag periods with gap trials. Looking again at the CRTT results, ED treatment had a clear effect by reducing reaction time in overlap trials compared

with every other treatment, including placebo. These effects are most likely explained by the well documented effects of glucose and caffeine on cognitive performance in the postprandial period, outlined in sections 1.2.1.1 and 1.2.1.2. The difference between gap and overlap trials is that the presence of the gap causes more speeded responses by affecting ability to re-engage attention with the stimulus. In overlap trials the cue appears to produce inhibitory effects on either re-engaging attention or on information processing/decision making processes. Here ED reduced reaction times in overlap trials only, where there is a greater inhibition of either attentional re-engagement or processing the information conveyed by the stimulus slowing the decision making process. Without further study it is difficult to conclude what mechanism has caused this effect.

The effects of the functional ED are clear, and somewhat agree with previous study into the effects of EDs on cognition. As outlined in table 2.1, EDs have been observed to beneficially affect a range of cognitive processes, most significantly decision making tasks or other reaction time tasks (Smit and Rogers 2002; Kennedy and Scholey 2004; Rao, Hu et al. 2005; Adan and Serra-Grabulosa 2010). In this study it is difficult to make any conclusions regarding the cognitive processes that have been modified by ED consumption, however as the main effects were on overlap trials perhaps the effect was caused by an increase in ability to disengage attention from the pre-stimulus cue. Alternatively, the effect could be caused by an increase in readiness to respond, an effect that may be cancelled out in gap trials by removal of the cue, increasing readiness to respond regardless of treatment.

4.4.2 Sensory effects of treatment

Based on the findings of Frank *et al.* (2008), Chambers *et al.* (2009) and the nutrient-reward hypothesis proposed by Kringelbach *et al.* (2004) it was hypothesised that sensory perception of the ED would impart a beneficial effect on cognitive performance with the involvement of an oral nutrient receptor, an effect that would not be observed with an artificially sweetened placebo. The sensory effects exerted by ED and P treatments on CRTT in this study performance are very interesting. Both ED and P treatments appeared to improve gap RT compared with

SW and NT (though ED failed to reach significance), but had little effect on overlap trials. In gap trials, ED alone appears to have attenuated the decrease in accuracy observed with SW and NT groups. The apparent sensory effects of ED treatment replicate those produced in the previous chapter. However, these effects do not match those observed postprandially in this study, where the most significant effects were on overlap trials. This suggests that the sensory effects of energy drinks on the CRTT, that may be elicited by reward value of the drink, may only occur when attention has been allowed time to disengage from the pre-stimulus cue. Perhaps these effects are inhibited when the gap is absent and participants are less primed to respond.

The effects that P treatment exerted on gap reaction time are also interesting. It may be that reaction time in gap trials is more sensitive to subjective effects, as indicated by the observed effects of ED and P treatment, or that there is actually a rewarding effect of both treatments. This would suggest that the oral nutrient receptor hypothesis is not the only mechanism causing effects on cognition here. The attenuation of decline in accuracy observed with the ED could point to stronger alerting effects of this treatment. These stronger effects with ED are most likely accounted for by an oral nutrient receptor, which means there might be more than one mechanism. Of course the results could also be explained by a combination of the oral nutrient receptor hypothesis and a placebo effect. Imaging studies have shown that calorific oral carbohydrate stimuli activate taste pathways in humans differently from artificial sweeteners, with glucose and sucrose eliciting stronger activations in the striatum and anterior cingulate cortex which are involved in modification of behaviour and the representation of reward value (de Araujo, Rolls et al. 2003; Chambers, Bridge et al. 2009). This may be another explanation for the stronger effect observed with ED treatment compared with placebo.

Another explanation for the observed effects could involve the reward value of sweet/pleasant flavours acting as positive reinforcers that raise mood and arousal levels, which could then produce behavioural effects. These effects have been

observed using physical outcome measures that relate to psychological functions. Villemure *et al.* (2003) observed that odours can increase pain tolerance in a cold-pressor task (CPT) in adult humans through their impact on mood, an effect that appears to be limited to "sweet-smelling" odours (caramel) and not observed with odours that were non-sweet but pleasant (aftershave) and neither (civet) (Prescott and Wilkie 2007). This effect has also been observed with sweet tasting solutions, sweet taste improved length of pain tolerance in the CPT by 18.1% compared with spring water (Lewkowski, Ditto et al. 2003). Sweet taste was also associated with lowering pain perception in newborn babies with both sucrose and aspartame reducing the period of time newborn babies cried for following the onset of spontaneous crying (Barr, Pantel et al. 1999). Perhaps in the current study the reward value of odourants have led to changes in mood/arousal that have influenced participants ability to attend/react to stimuli, and perhaps the representation of flavour has differing effects when odour is combined with different qualities of sweet tastes (calorific or artificial). In order to determine how these effects are mediated, future work in the area should look at the influence of sweet tastants and odourants on their own and in combination on task performance: this study would however be difficult to perform since the full perception of an odourant involves both orthonasal (breathing in before food is placed in the mouth) and retronasal (breathing out after swallowing of food) stimulation (Pierce and Halpern 1996), and odourants when placed in the mouth in solution without flavour enhancers such as sugar or salt will 'taste' very unpleasant.

The effects observed may in part be explained again by a placebo/expectancy effect, which would account for the improvement in gap RT observed with P treatment; however this would not account for the stronger effect ED treatment imparted on gap RT and the effect on overlap RT.

4.4.2.1 Somatosensory input

In this study, two control treatments were used, still mineral water and no treatment. No treatment was used as a somatosensory control, as there was the possibility that perception of any tastant in the mouth may influence cognitive

performance. The results rule out the possibility that the experimental treatments imparted an effect through somatosensory perception, as very few effects were observed with SW and NT. In fact, the SW and NT treatments both significantly reduced accuracy in the CRTT, impacts that were significantly different to that of ED. However, as said above, a confounding factor though is the negative reinforcement caused by the disappointment of receiving SW or NT rather than a pleasant tasting ED. These negative effects of the control treatments could also be attributed to fatigue, which if true would suggest that ED and P treatment attenuate fatigue. This however is unlikely due to there being no decline in performance postprandially with these treatments.

4.4.3 Suitability of Cognitive Assessments used in the detection of sensory and postprandial effects of treatments

4.4.3.1 CRTT

As the CRTT observed significant effects of treatments on measures of RT, response accuracy and the 'gap effect' to be monitored, it can be concluded that the CRTT is a suitable task for measuring the discrete alterations in cognitive functioning elicited by energy drink treatments postprandially and sensory perception of tastants. As discussed above, the task has allowed further investigation of the impact that ED treatment can have on speed of responses, finding that ability to reengage attention with and without the presence of a gap was improved, an effect not observed with other treatments.

4.4.3.2 ABT

It had been hypothesised that as ED treatment may influence the ability to reengage attention following the removal of a pre-stimulus cue, it may also influence the ability to detect two targets placed within close proximity to each other; however the results presented show that no effect was observed. As attention actually had to be focussed on the first target, ability to attend to additional stimuli will be affected differently than if a pre-stimulus cue was presented first. The analogy comparing attention to an opening and closing gate

(Sperling and Weichselgartner 1990) might be particularly fitting here, as in the ABT the working memory is flooded with information, inhibiting attentional focus differently from the mechanism involved in the 'gap effect' which would not involve working memory. It appears then that this task type is not sensitive to the effects of ED treatment or sensory perception of foods, possibly because there is no impact of these treatments on the inhibitory effect of overloading working memory on attention. This concurs with the findings by Scholey and Kennedy (2004) that although there was an influence of ED consumption on some measures of memory and attention (including quality of memory, secondary memory, speed of memory and speed of attention), no influence was observed on working memory using the Cognitive Drug Research computerised assessment battery.

Post hoc Attentional Blink Task Analysis

The small number of trials where T2 was presented at each lag position (T2 was presented in 48 of the 96 trials, and was presented just six times in each lag position) may have contributed to the lack of effect observed. With only six trials at each position it is possible that the small amount of data could weaken the sensitivity of the task to treatment effects. In spite of this, the experiment has successfully demonstrated the attentional blink phenomenon, as displayed in table 4.7 and figures 4.12 and 4.13.

CHAPTER 5: INFLUENCE OF SENSORY PROPERTIES OF ENERGY DRINKS ON HUMAN PERFORMANCE IN A VIGILANCE TASK.

5.1 Introduction

Malinauskas, Aeby *et al.* (2007) observed that 67% (n=253) of US college students questioned on their use of energy drinks consumed them to counteract insufficient sleep with 65% using them in order to 'increase energy'. These subjective motivations for energy drink consumption go some way to justify the criticism of studies into the cognitive effects of energy drinks and their ingredients, that attention should be paid to their influence on sleepiness and vigilance rather than measures of information processing (Horne and Reyner 1996; Reyner and Horne 2000; Horne and Reyner 2001; Reyner and Horne 2002). Until now this project has concerned itself with tasks that assess ability to attend to a rapid stream of stimuli with participants required to remain alert for the entire duration of each task. Energy drinks are often used as a means of preventing declines in vigilance (for example by drivers (van den Berg and Landstrom 2006) and air traffic controllers (Gander, Barnes et al. 1998)) and as a result several studies have investigated their effects on driving performance (Reyner and Horne 2000; Horne and Reyner 2001; Reyner and Horne 2002; van den Berg and Landstrom 2006) and vigilance tasks (Anderson and Horne 2006; Jay, Petrilli et al. 2006).

Until now, no research has been carried out into the effects of sensory perception of tastants on sleepiness or vigilance, and as with previous chapters it is reasonable to hypothesise that any sensory influence will be similar to that of the postprandial properties. The influence of energy drinks on these measures however is not entirely clear. Although positive effects on reducing the number of 'driving incidents', reaction times and reducing subjective sleepiness were recorded by Horne and Reyner (2001), studies by Anderson and Horne (2006) and Jay *et al.* (2006) found no improvement in Psychomotor Vigilance Task (PVT) performance and in fact a greater number of lapses were observed with ED treatment 70-80 minutes post consumption. These improvements and declines in performance will

obviously be related to the peak and dip in blood glucose levels that occur in the postprandial period, particularly following glucose consumption, however any potential sensory effect is likely to be similar to the effects observed with the rise in blood glucose, not the fall later in the postprandial period.

The present study aims to determine whether the sensory properties of energy drinks can impart any benefit on vigilance performance using a sustained attention task. Tasks measuring sustained attention and vigilance generally involve the presentation of few target stimuli amongst several non-target stimuli; therefore participants are required to maintain attention to the stream of information meaning that when the rare targets are presented an inhibitory effect may be placed on the ability to respond.

It was hypothesised that sensory perception of ED treatment may influence performance by stimulating reward centres in the brain, either via stimulation of the oral nutrient receptor with glucose, or through the rewarding sensation of sweet/pleasant tastes. If the latter, a placebo energy drink treatment may elicit similar effects. It was also theorised that measures to diminish performance might allow greater beneficial impact of treatments, therefore it was decided that the hydration status of participants and the impact of this on treatment effects would be monitored. Dehydration has been shown to negatively affect attention in adults and in children (Suhr, Hall et al. 2004; Edmonds and Jeffes 2009), therefore scope for an improvement in performance with treatments is increased with dehydrated participants.

5.2 Materials and Methods

5.2.1 Participants

Twenty students from the University of Nottingham (median age 24.5 years, 10 male 10 female) volunteered to attend five sessions lasting approximately 33 minutes each. The first session was carried out to familiarise the participants with the experimental procedures and no data was recorded. In each of the 4 experimental sessions - the impact of one experimental treatment was assessed, outlined below. Participants arrived in the morning following an overnight fast,

having not consumed any food or drink (including water) since midnight. Those who were pregnant, diabetic, suffered from any disorder affecting attention or smokers were excluded from the study. Participants were required to estimate their weekly habitual intake of caffeinated products and average daily caffeine intakes were calculated using figures given by the Food Standards Agency (2008). A blank example of the 'case report form' containing information for participants, consent forms, screening questions and space for experimenter notes is included in appendix 4 (required by GlaxoSmithKline). Those who completed all five sessions were given a monetary reward for their participation. Ethical approval was granted by the University of Nottingham School of Psychology Ethics Committee.

5.2.2 Treatments

Four experimental treatments were used (see table 5.1), two non-carbonated ED (one active, one placebo) treatments and two control treatments (Evian still mineral water with orange colouring and no treatment). In each of the four experimental sessions, one treatment was administered, with the four sessions being delivered in a randomised and balanced design (MacFie, Bratchell et al. 1989).

Table 5.1: Summary of treatments used and ingredients present.

	Glucose (g/100ml)	Caffeine (mg/100ml)	Aspartame (mg/100ml)	Acesulfame K (mg/100ml)	Orange Colour (Y/N)
Functional ED (ED)	17.9	12.1	0	0	Y
Placebo ED (P)	0	0	16.3	4.5	Y
Still mineral water (SW)	0	0	0	0	Y
No treatment (NT)	n/a	n/a	n/a	n/a	n/a

In order to study the effects of levels of thirst on performance effects of EDs, one half of participants (water group) were asked to drink a 500 ml bottle of Evian still mineral water prior to the recording of experimental data in each session. The other half of participants (no water group) were not given a drink before the experiment began.

5.2.3 Task

A vigilance task was used to assess participants' responses to infrequently occurring target stimuli. In the task, the numbers 1-9 were presented in a random order and participants were asked to respond by pressing the R button on the Cedrus

Response Box (Cedrus 2009; displayed in figure 4.3) only when the numbers 3 or 5 were presented; the probability of a target stimulus appearing was 0.125. Target and non-target stimuli were presented for 150 ms in black Courier New font size 60, 100 and 140 (to reduce the possibility of participants responding to a perceptual feature of the stimuli rather than the numbers (Helton 2009)) on a grey background. During the inter-stimulus interval, which lasted 1,100 ms, a black rectangle appeared to remove any after image. The task lasted 10 minutes, with 48 stimuli (approximately 6 target stimuli) appearing each minute. In each trial reaction time and response accuracy were recorded.

5.2.4 Thirst Ratings

Subjective thirst was assessed using a 10 cm visual analogue scale (VAS) following measurement of cognitive performance both at baseline and in response to the experimental treatment. A number of studies show that although the use of VAS scales for assessing thirst is subjective, ratings show good correlation with hydration status (Rolls, Wood et al. 1980; McKenna and Thompson 1998), show sensitivity to experimental manipulations and show good reproducibility (Stubbs, Hughes et al. 2000).

5.2.5 Experimental Procedure

A simplistic diagram of the experimental booth is shown in figure 4.5, with a list of the events taking place during each session shown in table 5.1.

When completing the vigilance task, participants were required to hold the mouthpiece used to deliver sample drinks to the participants' mouths between their lips, even when no drink was being delivered (i.e. during familiarisation and baseline testing and in between sample deliveries during performance whilst receiving treatment). The pump also remained active each time the task was completed, as it produced sounds with each sample delivery, however participants were asked to wear headphones playing white noise. Although the screen used in previous studies helped prevent noise distractions, there was no guarantee that it completely prevented the noise generated by the pump or that other noises (for

example from the corridor) would not influence performance. It was felt that this precaution would eliminate any potential problems in these regards.

Participants arrived for experimental sessions between 9 and 11 am and after giving informed consent were asked to mark on the VAS how thirsty they felt. Participants were then required to complete familiarisation trials of the vigilance task to prevent any learning effects over the session. Participants in the water group were at this point given 500 ml to drink – if participants could not finish the 500 ml they were asked to drink until they were full and the volume remaining was recorded. Those participants who received water were asked to complete another thirst questionnaire. All participants then completed the vigilance task once again to obtain a baseline measurement of performance. Cognitive performance was then assessed in response to the experimental treatment – a 1 ml dose of the treatment was delivered every 10 seconds (8 trials) with a flow rate of 12 ml/min (therefore 4 stimuli were presented during dose delivery – meaning a dose was being delivered during one half of trials, and no dose was delivered during one half of trials). Once the task was complete, subjective thirst was measured once more, and the session was complete.

Table 5.2: Experimental protocol - order of events during experimental session. Events only completed by participants in the water group are shown with a grey background.

Event	Duration
Arrival	
Thirst Questionnaire	1 minute
Vigilance Task – Familiarisation	10 minutes
Water group received water	<5 minutes
Thirst Questionnaire (water group only)	1 minute
Vigilance Task – Baseline	10 minutes
Vigilance Task – with treatment	10 minutes
Thirst Questionnaire	1 minute
End of Session	**32-38 minutes**

5.2.6 Data Analysis

5.2.6.1 Thirst

Subjective thirst was calculated as the distance in mm that participants marked from the left hand side of the scale (anchored "not thirsty"). Mean subjective thirst

was calculated for the water group and no water group. Paired t-tests were used to compare initial thirst ratings between water and no water groups, initial rating in the no water group with the post-water rating in the water group, and initial and post-water within the water group.

5.2.6.2 Vigilance Task

Latency Data

Latency data was analysed using the same methods as described in section 4.2.5.1.

Accuracy Data

Each time the task was performed, the rate of omission errors (OE; percentage of target stimuli presented where no response was made) and commission errors (CE; percentage of non-target stimuli where a response was made) were calculated. Mean change in OE and mean change in CE between baseline and post treatment measurement were also calculated (ΔOE, ΔCE). Between treatment effects on OE rate, CE rate, ΔOE and ΔCE were analysed using ANOVA and *post hoc* LSD test. Within treatment effects were monitored using a separate ANOVA where measurement (e.g. baseline or time of post-treatment measurement) was the only fixed factor and *post hoc* Dunnett Test comparing mean median baseline scores with each post treatment score.

Effects of Treatment, Thirst Condition, Habitual Caffeine Intake, Age and Gender

In the ANOVA, treatment was inserted as a fixed factor in order to determine the effects of treatment on the measures outlined above. Habitual caffeine intake was calculated for each participant as in section 3.2.4.5, and median scores for HCI and age were used to split participants into high/low caffeine consumers and 'older'/'younger' participants. Age, HCI and Gender were all included as fixed factors in the ANOVA analysis.

5.3 Results

5.3.1 Impact of overnight food and drink restriction on measures of subjective thirst

Mean pre-treatment thirst ratings are displayed in figure 5.1. Initial ratings of subjective thirst between water and no water groups were not significantly different ($p=0.068$). Ratings made by the not-thirsty group post water consumption were significantly lower than their initial ratings ($p<0.001$), and the ratings made by the other group ($p<0.001$).

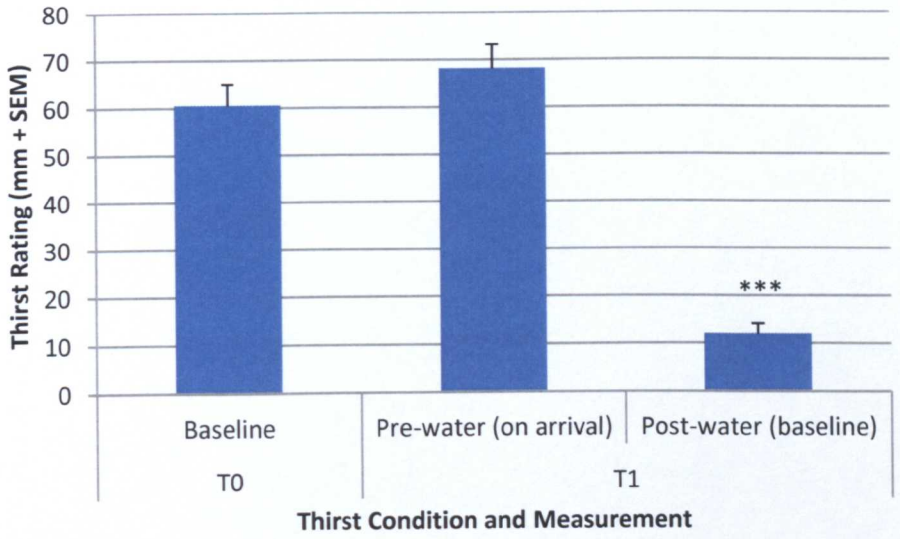

Figure 5.1: Effect of overnight fast and thirst condition on ratings of subjective thirst. *** indicates significant difference from all other thirst measurements ($p<0.001$)

5.3.2 Impact of samples on task performance:

5.3.2.1 Reaction Time

Figure 5.2 displays the ΔRT scores for each treatment in all trials (overall), while drink was delivered, or would have been delivered and pump was active with NT (Deliv) and between drink deliveries (Ndeliv). As can be observed in figure 5.2, a tendency for increased ΔRT was observed with the SW and NT groups, however ANOVA found no significant differences between these ΔRTs and other treatments

(Overall, p=0.393; Deliv, p=0.475, NDeliv, p=0.505). In all trials, the estimated 95% CI for ΔRT in the NT condition did not cross zero (mean: +13.02 ms, lower: +1.48 ms, upper: +24.56 ms), indicating a significant increase in scores from baseline with this treatment. In Deliv trials the estimated 95% CI for ΔRT in the SW condition did not cross zero (mean: 16.54 ms, lower: +0.10 ms, upper: +32.98 ms), indicating that SW increased RTs significantly.

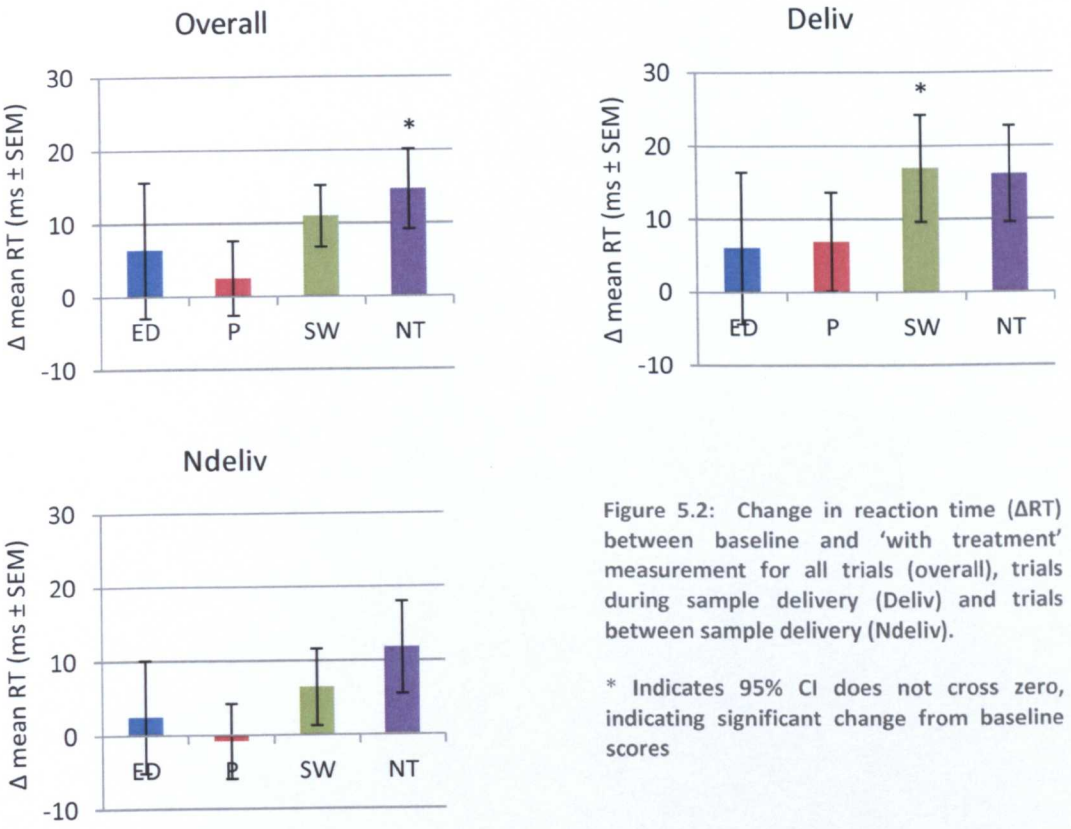

Figure 5.2: Change in reaction time (ΔRT) between baseline and 'with treatment' measurement for all trials (overall), trials during sample delivery (Deliv) and trials between sample delivery (Ndeliv).

* Indicates 95% CI does not cross zero, indicating significant change from baseline scores

5.3.2.2 Errors of Omission

Figure 5.3 shows the change in rate of omission errors (ΔOE). No effects of treatment on rate of OEs or ΔOE were observed when all trials or only Deliv trials were considered. For NDeliv trials, ANOVA found significant effect of treatment on ΔOE (p=0.041) with a *post hoc* LSD finding significant differences between SW and NT (p=0.005, see figure 5.3).

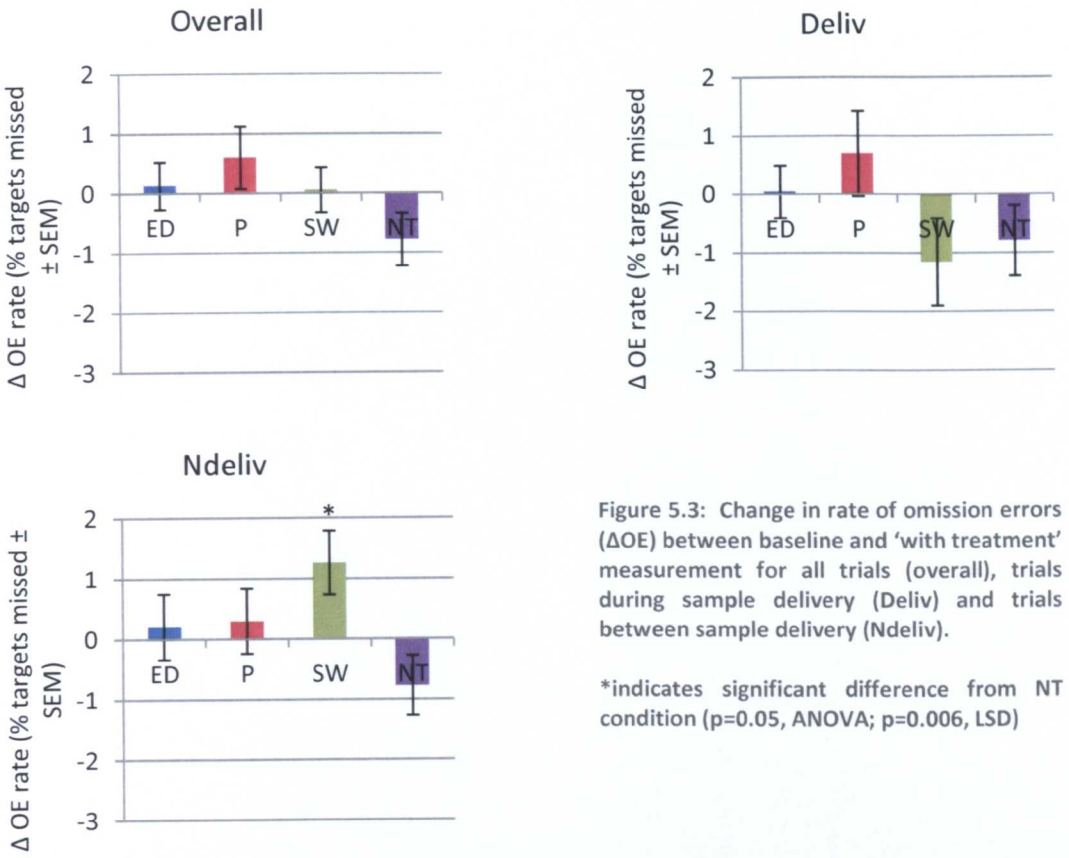

Figure 5.3: Change in rate of omission errors (ΔOE) between baseline and 'with treatment' measurement for all trials (overall), trials during sample delivery (Deliv) and trials between sample delivery (Ndeliv).

*indicates significant difference from NT condition (p=0.05, ANOVA; p=0.006, LSD)

5.3.2.3 Errors of Commission

The change in rate of commission errors (ΔCE) results are displayed in figure 5.4. ANOVA found no significant effects of treatment on rate of CEs or ΔCE in any trial type. In the NDeliv trials, CE rate was increased with the SW treatment as the estimated 95% CI for ΔCE did not cross zero (mean: +0.19%, lower: +0.003%, upper: +0.382%).

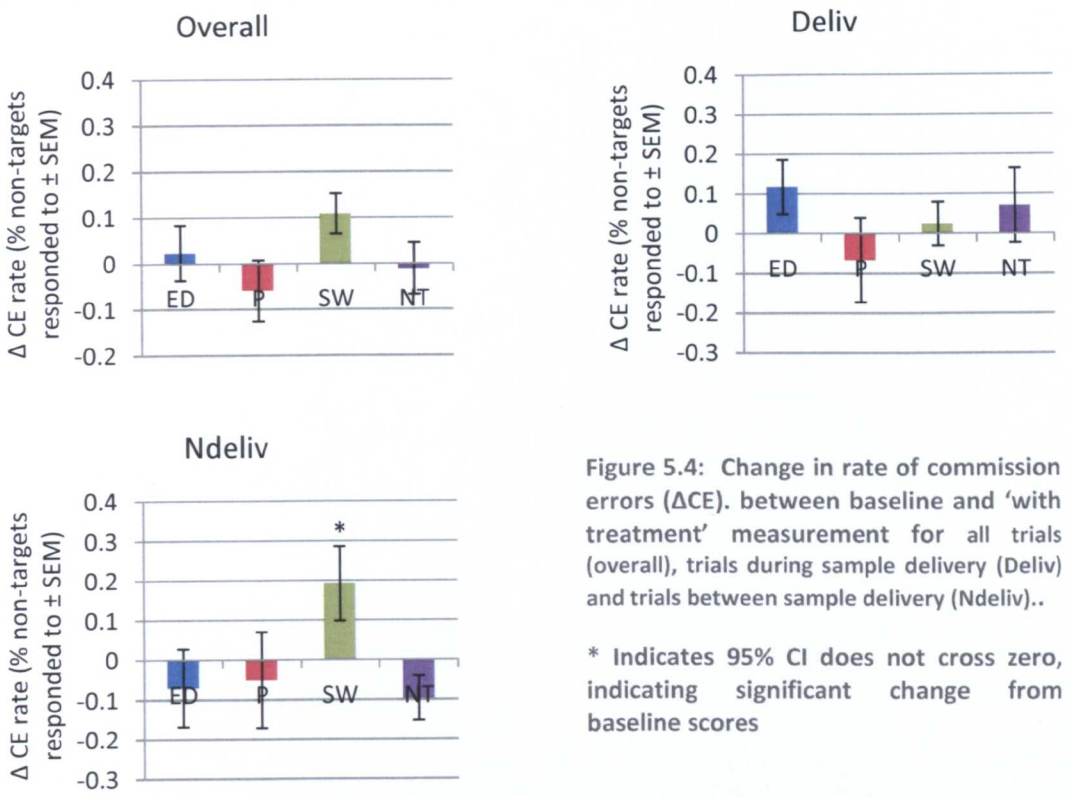

Figure 5.4: Change in rate of commission errors (ΔCE). between baseline and 'with treatment' measurement for all trials (overall), trials during sample delivery (Deliv) and trials between sample delivery (Ndeliv)..

* Indicates 95% CI does not cross zero, indicating significant change from baseline scores

5.3.3 Impact of thirst condition/subjective thirst on performance:

Reaction time scores for each thirst condition at baseline and 'with-treatment' are displayed in figure 5.5. Participants in the no water group (T0) had borderline significantly faster reactions times compared with those in the water group (T1), in all trials (Overall, p=0.067), during drink delivery (Deliv, p=0.058) and between drink deliveries (p=0.091) as can be observed in figure 5.5. No interactive effects were observed between thirst condition and treatment.

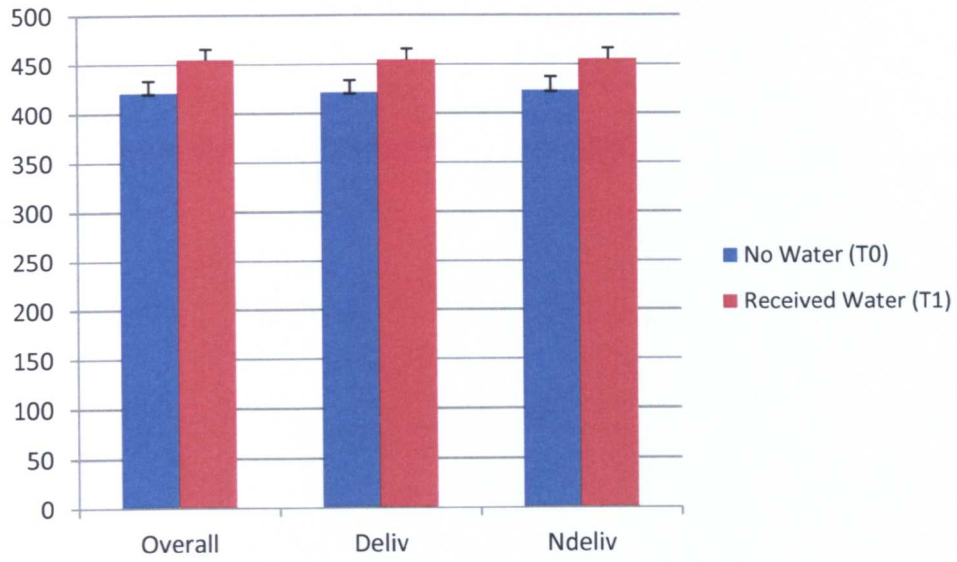

Figure 5.5: Overall, Deliv and NDeliv reaction time measurements for both thirst conditions at RT1 (baseline) and RT2 (sensory) measurements.

5.3.4 Impact of other fixed factors on cognitive performance

5.3.4.1 Habitual Caffeine Intake

Figure 5.6A shows the OE rates for participants with below median HCI and above median HCI. Median HCI was 124.3 mg/day caffeine. Mean change in reaction time was greater for participants with below median HCIs (mean 49.6 mg/day caffeine) than those with higher HCIs (mean HCI 233.4 mg/day caffeine; p=0.048, ANOVA). ANOVA found significant effect of HCI on ΔOE rate (p=0.009), as displayed in the right hand side of figure 5.6.

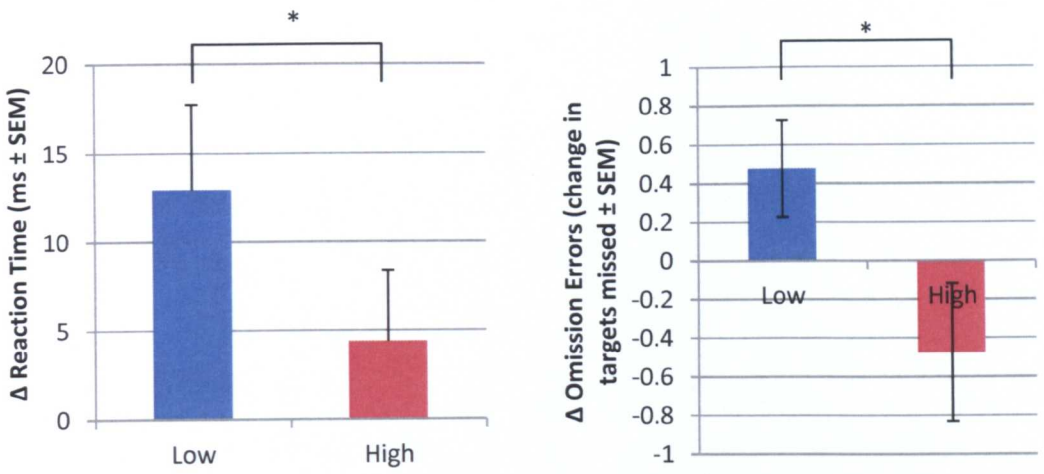

Figure 5.6: A. Overall Change in Mean Reaction Time and; B Overall Mean Change in Omission Error for Low (below median) and High (above median) Habitual Caffeine Consumers. * indicates significant difference in error rate between HCI conditions ($p<0.05$, ANOVA).

5.2.4.2 Age

Figure 5.7 displays mean Omission Error rates for participants below and above median age (24.5 years old). Significant effect of age was observed, with younger participants (mean age 22.7) missing target stimuli more frequently than older participants (mean age 29.4, $p=0.029$).

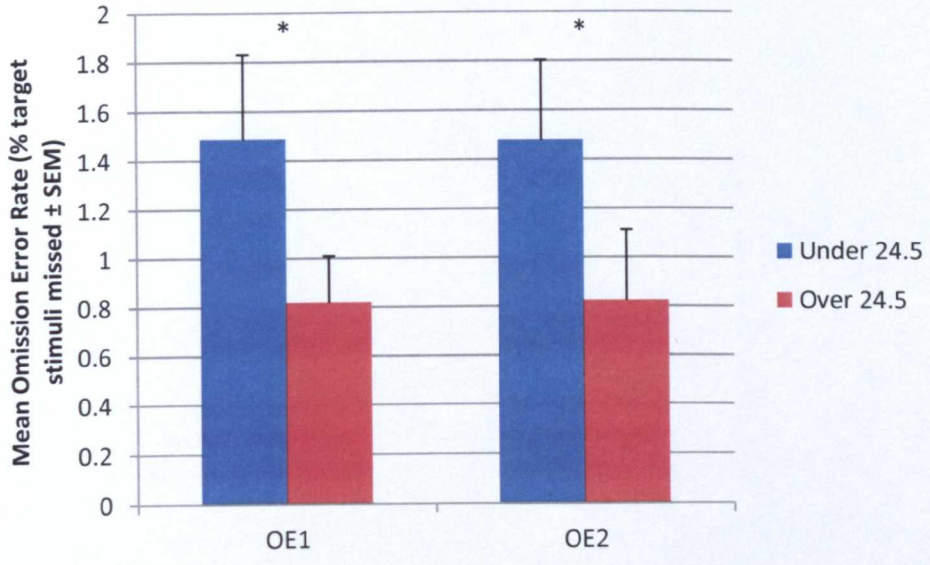

Figure 5.7: Overall Mean Omission Error Rates for participants below median age and above median age. * indicates significant difference in error rate between Age conditions ($p<0.05$, ANOVA).

5.4 Discussion

5.4.1 Influence of treatments on cognitive performance

The main aim of this study was to investigate the influence of the sensory properties of functional and placebo energy drinks on performance in a vigilance task, and how any potential impact interacted with hydration/thirst status. It was hypothesised that through its glucose content, perception of a functional ED might influence vigilance via the stimulation of an oral nutrient receptor that recognises the energy value of carbohydrates. No significant effects of ED or P treatment were observed suggesting that the measures used in this study were not sensitive to the sensory effects of ED treatment observed in previous chapters. There could be several explanations for this, one being that vigilance tasks are not sensitive to the cognitive effects of sensory perception of EDs. In the task used, error rates were very low (often lower than 1%), meaning that any improvement in performance was likely to be observed on reaction time. In this task, the decision to respond was much simpler than in tasks used in previous chapters as there was less information to be processed and the choices of response were either to respond or to withhold a response. The high frequency of non-target stimuli had an inhibitory effect on response times to target stimuli, as the participants were not as used to responding as they were in the CRTT. It seems though that although ED treatment could affect the inhibitory effect of a pre-stimulus cue in chapters 3 and 4, the inhibitory effect of expectation remains unaffected in this study.

There were tendencies for increased response time and error rates with the SW and NT which did not occur with ED and P treatment. Therefore it is possible that ED and P attenuated these effects, however no difference between treatments was observed. In order to increase the sensitivity of this task to the sensory effects of treatments used, it would be worth repeating this experiment in the afternoon to monitor if any effect is observed on sustained attention via sensory perception when arousal is at its midday dip, as in studies carried out by Anderson and Horne (2006) who actually observed a decrease in performance (postprandially) with energy drink supplementation. Another option would be to include both a vigilance task with greater difficulty (i.e. one where target-stimuli are difficult to differentiate

from non-target stimuli) alongside go/no-go task that measures response inhibition. If target stimuli were more difficult to detect, error rates would most likely increase and may be more susceptible to small changes in performance (Temple, Warm et al. 2000).

5.4.2 Impact of Thirst condition on performance

5.4.2.1 Effectiveness of methods to control hydration levels

The methods used to induce low hydration status appear to have been successful, as were the rehydration methods in the water group. At baseline thirst for both groups was elevated, however the borderline increase in thirst for the water group is intriguing. It is possible that due to the crossover nature of the study, and participants becoming familiar with the fact that they are to receive water, their perception of their thirst is amplified (Toates 1979). After the water group received their drink, subjective assessment of thirst was almost zero, indicating an effective increase in hydration status. One issue with this inference though, is that the second thirst rating made by the water group was made immediately after drink consumption, and as a result the rating may be decreased by subjective measures and the feeling of fullness. Initial thirst ratings are also subject to the effect of knowledge of the experimental conditions, as participants have been asked to abstain from food and drink consumption overnight. To prevent these unwanted effects participants could be asked to complete a mood questionnaire incorporating a thirst scale, or blood samples could be taken and plasma osmolality measured (Armstrong 2007).

The increase in reaction time observed with the water group was an unexpected but explainable finding; Aarts, Dijksterhuis *et al.* (2001) observed a decrease in reaction times to emotional stimuli related to thirst or drinking. In a study investigating voluntary dehydration in college athletes, dehydration resulted in improved memory performance, decreased vigilance performance and negative mood ratings (D'Anci, Vibhakar et al. 2009). Whilst there is a great deal of evidence supporting the cognitive benefits of good hydration status (Suhr, Hall et al. 2004;

Lieberman, Bathalon et al. 2005; Benton and Burgess 2009; Edmonds and Jeffes 2009), it is clear that there is still more work to be carried out in this field.

CHAPTER 6: INFLUENCE OF SENSORY PERCEPTION OF SWEET TASTANTS ON COGNITIVE PERFORMANCE

6.1 Introduction

The effects of energy drinks once the ingredients have been digested and absorbed into the bloodstream is already well-documented, however this sensory impact is until now yet to be investigated; the question was then posed, how is this effect mediated? Previous chapters have investigated the impact of the sensory and postprandial properties of a non-carbonated Lucozade Energy drink on cognition, and observed that both functional (glucose and caffeine containing) and placebo (contains artificial sweeteners) drinks could reduce reaction time when perceived in the mouth, and postprandially – perhaps via some influence on arousal and decision making processes. Possible theories are that the pleasant taste (or in fact presence of a taste) increased arousal allowing faster response times to on screen stimuli, or that perception of sweet tastants (or in fact any of the tastants present in the drinks) on the tongue influence behaviour, improving awareness/attention and allowing better performance. There is evidence to suggest that sweet flavours, particularly those that provide energy when consumed, can influence response to particular stimuli and performance via an oral nutrient receptor. The next logical step would then be to investigate the influence of sweet tasting solutions on performance when perceived in the mouth.

Although calorific and artificial sweeteners are both perceived in the mouth by T1R3 receptors in combination with the T1R2 receptors on the tongue (Damak, Rong et al. 2003; Zhao, Zhang et al. 2003; Chandrashekar, Hoon et al. 2006), it appears that they activate taste pathways in the brain differently (Frank, Oberndorfer et al. 2008). Primary taste regions (anterior insula and frontal operculum) were activated by both sucrose and sucralose; however sucrose also activated secondary taste pathways in the striatum, caudate nucleus and the thalamus, where the reward value of taste/flavour perception is formed. This suggests that sucrose and calorific sweeteners bestow a greater reward value, probably as a result of their greater

nutritional value. Behavioural studies based on these results have been designed to test the hypothesis that foods containing greater nutritional value offer greater reward and will alter behavioural performance. Li, Glaser *et al.* (2009) investigated the preference for sweet stimuli in a range of animal species, finding that a number of carnivorous species displayed preference for calorific sweeteners ahead of artificial sweeteners explained by gene expression of the T1R2 receptor. This suggests that an evolutionary role is involved in preference for energy containing nutrients. The greater reward elicited by perception of calorific sweeteners may also influence behaviours other than feeding. Sensory information converges in the brain in the secondary taste cortex, with projections then passing information on to brain regions such as the anterior cingulate cortex, responsible for attention, error detection and motivation (de Araujo, Rolls et al. 2003; Rolls, Critchley et al. 2010).

This study aims to investigate the potential influence that sweet taste perception may exert on performance in a CRTT already observed to have been sensitive to the sensory perception of a functional energy drink. It is hypothesised that this cognitive effect is mediated by glucose perception via an oral nutrient receptor, therefore this study compares the effects of glucose with a 'taste-matched' artificially sweetened solution. There is also the possibility that the effect is mediated by perception of sweet tastants irrespective of calorific content – which suggests both treatments would exert a positive effect; or even by flavours associated with sweet taste present in EDs, which would predict no effect would be observed in the present study.

6.2 Materials and Methods

6.2.1 Participants

24 healthy participants (median age = 21 years; 10 male, 14 female) gave informed consent to take part in this study; those who were pregnant, colour-blind, smokers or diabetic were excluded. Participants attended one session, lasting approximately 70 minutes, arriving in the morning (between 9 and 11 am) having fasted for two hours and abstained from caffeine consumption on the morning of the trial. Participants were awarded an inconvenience allowance for attending the session.

Blank examples of consent forms, information sheets and case report forms are included in appendix 6.

6.2.2 Samples

The impact of two test samples were assessed, along with water and no treatment controls. The test samples were a glucose solution and a solution containing the artificial sweeteners aspartame and acesulfame K. The two solutions were matched for sweetness with Lucozade Energy Original, using paired comparison tests following the methods outlined below.

The impacts of all four treatments were assessed in one session, and were administered in a randomised and balanced order, outlined by MacFie, Bratchell *et al.* (1989).

6.2.2.1 Sensory Testing

In order to determine the concentrations of glucose syrup and artificial sweeteners to be used in the study, a series of paired comparison (similarity) tests were carried out following the instructions found in British Standard for paired comparison tests (British Standards Institution 2007) to determine what concentrations of these solutions were perceived to be equi-sweet to non-carbonated Lucozade Energy samples used in a previous study.

The sensory evaluation was conducted in the Sensory Science Centre at the University of Nottingham. The Fizz Acquisition programme was used to design and run the tests using a randomised, balanced double-blind design. The testing booths adhered to sensory analysis guidelines, with northern hemisphere lighting (British Standards Institution 2007). To obtain protection from falsely concluding that the samples are similar, β=0.05 was accepted, with the maximum allowable proportion of discriminators identified as p_d=30%. With an alpha risk of α=0.2 96 assessors were required for a two-sided test (using table A.3 in the British Standard).

Ninety-six assessors were recruited for this part of the study and each assessor completed three paired comparison tests, where two ~20ml samples – one glucose solution, one artificially sweetened solution – were presented in 30 ml plastic pots

identified by three digit codes, and asked to identify the sample they perceived to be the sweetest. The solutions used in the three tests were as follows:

- Paired Comparison Test 1
    - 106 mg Aspartame, 29.3 mg Acesulfame K / L
    - 115 g glucose syrup / L
- Paired Comparison Test 2
    - 106 mg Aspartame, 29.3 mg Acesulfame K / L
    - 120 g glucose syrup / L
- Paired Comparison Test 3
    - 106 mg Aspartame, 29.3 mg Acesulfame K / L
    - 125 g glucose syrup / L

The presentation order of the samples in each test was randomised, with two possible presentation orders. Still water and crackers were provided for panellists to cleanse their palates between each sample. The glucose solutions were created by weighing the desired weight of glucose syrup and diluting this down in Evian still mineral water (Danone) to 1 L. The artificial sweetener solutions were created by making a base solution containing 1.63 g aspartame and 0.45 g acesulfame K and diluting to 1 L; the test solutions contained 65 ml and 68 ml of the base solution per litre respectively.

6.2.3 Cognitive Assessment

6.2.3.1 Choice Reaction Time Task

Each trial began with the presentation of a black fixation cross on a gray background for a duration of 800 ms prior to the presentation of the stimulus. In one half of trials, the stimulus appeared as soon as the fixation point disappeared (overlap condition, figure 4.1), and in the other half a 200ms gap was present (gap condition, figure 4.2). There were four possible stimuli that could be presented: a blue square, a blue circle, a yellow square or a yellow circle, presented for a duration of 150 ms. The participant was to respond as quickly as possible by pressing the left button on the response pad (displayed in figure 4.3) using their left index finger when either a blue square or yellow circle was presented, and by pressing the right button with

their right index finger when either a yellow square or blue circle were presented. The interval between trials was 1050 ms following gap trials and 1250 ms following overlap trials). The fixation-cross and stimuli were all presented in front of a grey background. The task was made up of 128 trials lasting approximately 7 minutes in total, with the opportunity for a break after 64 trials. Median reaction time and percentage accuracy (n correct trials / n total trials * 100) for gap trials and for overlap trials were calculated for each participant.

6.2.4 Procedure

On arrival, participants were given an information sheet and asked to give written informed consent, stating their awareness that they were free to withdraw from the study at any point without the need to give a reason. Participants completed 10 minutes of familiarisation trials of the CRTT before repeating the task four more times, alongside each of the four experimental treatments in a randomised counterbalanced order of presentation (MacFie, Bratchell et al. 1989) after becoming familiarised with the task, and were given £5 inconvenience allowance. Participants were given a 5 minute break between completing the task and starting it again for the subsequent treatment.

Participants received 30 ml of each test drink during a session. Whilst performing the cognitive task (outlined below), a 3 ml dose was delivered to the participant's mouth once every minute (for 10 minutes) during a short (~10 second) break from the task. Participants were asked to hold the solution in their mouth for approximately 5 seconds before swallowing and continuing with the task. This was repeated for each treatment. An experimental schedule is shown in table 6.1. A diagram of the equipment set-up is shown in figure 4.5.

Table 6.1: Experimental Schedule

Time	Task	Duration (min)
~10.00	Participant arrives, reads information sheet and signs consent form.	1
10.00	Familiarisation Trials – No Treatment	10
10.10	Break	5
10.15	Choice Reaction Time Task – Treatment 1	10
10.25	Break	5
10.30	Choice Reaction Time Task – Treatment 2	10
10.40	Break	5
10.45	Choice Reaction Time Task – Treatment 3	10
10.55	Break	5
11.00	Choice Reaction Time Task – Treatment 4	10
11.10	End of session	71 (total)

6.2.5 Data Analysis

6.2.5.1 CRTT

Between treatments data from the CRTT was analysed using the methods described in section 4.2.5.1. No analysis of within treatment effects was carried out as no baseline measurement of performance was recorded. Effects of HCI, Age and Gender were also assessed as in section 3.2.4.5, with these fixed factors included in the ANOVA.

6.2.5.1 *Post hoc* Analysis

Due to comments made by several participants taking part in the study, indicating that they subjectively felt faster/more accurate for some stimuli than others, a *post hoc* analysis comparing mean gap, overlap and global (gap and overlap scores combined) RT and Acc for each stimulus type (blue square, yellow square, yellow circle and blue circle), stimulus colour, stimulus shape and appropriate response (left vs right) was carried out. Mean RT and Acc for stimulus type were compared using the GLM Multivariate ANOVA function and *post hoc* LSD in SPSS, with Paired samples T-tests used to compare RT and Acc for stimulus colour, stimulus shape and appropriate response.

6.3 Results

6.3.1 Sensory analysis of samples

Using table A3 from the British Standard for paired comparison tests (British Standards Institution 2007), the maximum number of assessors choosing one sample over another that will allow the samples to be concluded as similar, for 96 panellists (with β=0.05 and p_d=30%) was 54. Table 6.2 shows the results from the paired comparison tests, highlighting that the solutions used in the first and second tests were similar tasting, as fewer than 54 participants chose one particular solution as sweeter than the other. As the 115g glucose solution used in the first test was closest to the 106 mg aspartame plus 29.3 mg acesulfame K, artificially sweetened solution. The combination of these two sweeteners was used as these sweeteners were present at this ratio in the placebo drink, designed by the manufacturers as giving a similar quality of sweetness to glucose.

Table 6.2: Frequency of responses for each paired comparison (PC) test.

	PC test 1	PC test 2	PC test 3
Art. Sw. Solution	49	46	39
Glucose Solution	47	50	57

6.3.2 Effects of treatment

Results from the CRTT are displayed in table 6.3. No significant effects of treatment were found by ANOVA on any RT or Accuracy measures.

6.3.3 Effects of other fixed factors

6.3.3.1 Age

Significant effects of age were found by ANOVA on RT measures ($p<0.001$ for gap trials and overlap trials). Participants under the age of 21 had significantly faster reactions than those aged 21 or over, see table 6.4.

6.3.3.2 Habitual Caffeine Intake

No significant effects of habitual caffeine intake were observed on any measures of RT or accuracy.

6.3.3.3 Gender

No significant effects of gender were observed on any measures of RT or accuracy.

Table 6.3: Results from Choice Reaction Time Task. Table presents mean, standard error of mean (SEM) and confidence interval values for all four treatments.

Measurement	Treatment	Mean	SEM	95% Confidence Interval	
				Lower	Upper
Gap RT (ms)	Glucose	477.02	20.483	434.648	519.394
	Art. Sw.	474.35	18.531	436.019	512.689
	Still Water	477.13	17.868	440.162	514.088
	No Treatment	494.02	17.452	457.918	530.123
Overlap RT (ms)	Glucose	496.10	18.493	457.849	534.360
	Art. Sw.	500.54	18.979	461.280	539.803
	Still Water	502.56	18.010	465.306	539.819
	No Treatment	512.27	19.485	471.963	552.579
Gap Acc (% correct responses)	Glucose	19.08	5.392	7.928	30.238
	Art. Sw.	26.19	4.769	16.322	36.053
	Still Water	25.44	5.930	13.170	37.705
	No Treatment	18.25	4.815	8.290	28.210
Overlap Acc (% correct responses)	Glucose	82.22	2.335	77.392	87.052
	Art. Sw.	84.65	1.869	80.787	88.519
	Still Water	84.72	1.833	80.931	88.513
	No Treatment	85.07	1.987	80.958	89.181
Gap Effect RT (ms)	Glucose	82.57	2.426	77.550	87.589
	Art. Sw.	84.31	1.973	80.223	88.388
	Still Water	83.61	2.016	79.442	87.781
	No Treatment	84.72	2.420	79.717	89.727
Gap Effect Acc (% correct responses)	Glucose	0.35	1.187	-2.108	2.803
	Art. Sw.	-0.35	1.501	-3.453	2.759
	Still Water	-1.11	1.012	-3.204	0.981
	No Treatment	-0.35	1.528	-3.508	2.814

Table 6.4: Estimated Mean Gap RT and Overlap RT with Standard Error and 95% Confidence Intervals for Age condition in CRTT.

		Mean	Std. Error	95% Confidence Interval	
				Lower	Upper
Gap RT	Under 21 years old	430.36	15.75	398.90	461.82
	21 years or over	525.47	13.70	498.11	552.83
Overlap RT	Under 21 years old	449.62	15.56	418.53	480.72
	21 years or over	547.73	13.54	520.69	574.77

6.3.4 *Post Hoc* Analysis

Effect of Stimulus Type

ANOVA did not find significant effect of stimulus type on RT (p=0.133) or Acc (p=0.114) measures, however there was a clear tendency for reduced RT (p=0.031, *post hoc* LSD) and increased Acc (p=0.018) for trials where a blue square (mean RT: 457.7, SEM: 17.2; mean Acc: 88.6%, SEM: 2.1%) was presented compared with a blue circle (mean RT: 511.2 ms, SEM: 19.6 ms; mean Acc: 80.7%, SEM: 2.8%), as shown in figure 6.1.

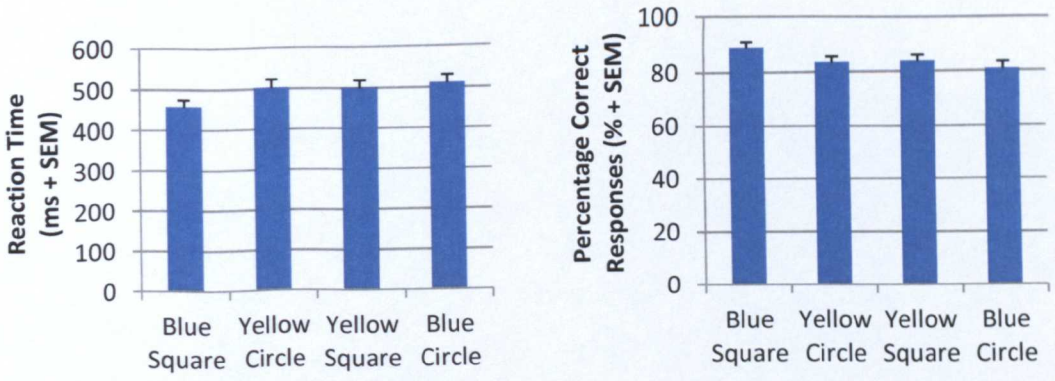

Figure 6.1: Impact of stimulus type on global reaction time and accuracy.

Effect of Stimulus Shape

Paired t-test found that participants responded faster to square stimuli (mean 476.7 ms, SEM 16.8 ms) than to circle stimuli (mean 507.5 ms, SEM 20.0 ms), as is outlined in figure 6.2, (p=0.017). A borderline significant impact of stimulus shape was found on accuracy, with a tendency for greater accuracy with squares (mean: 86.1 ms, SEM: 1.9 ms) than for circles (mean: 81.9, SEM: 2.2 ms).

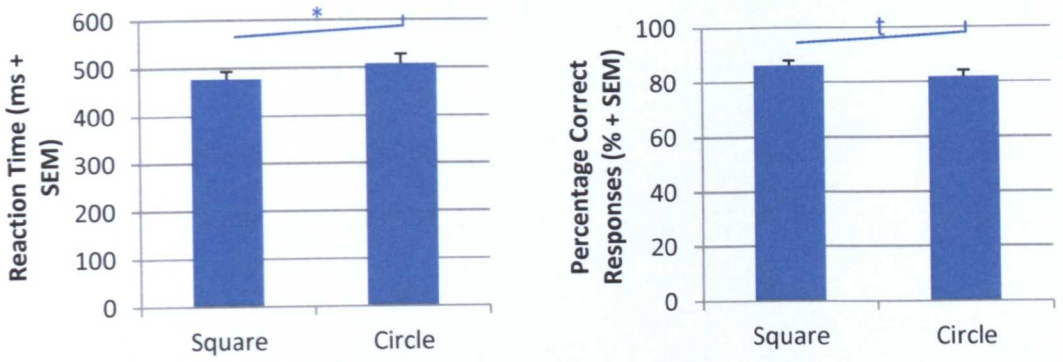

Figure 6.2: Impact of stimulus shape on global reaction time and accuracy. * indicates p=0.017, paired t-test. t indicates p=0.052, paired t-test.

Effect of Stimulus Colour

Paired t-test found that participants responded faster to blue stimuli (mean 477.8 ms, SEM 17.6 ms) than to yellow stimuli (mean 500.7 ms, SEM 17.8 ms), as is outlined in figure 6.3, (p=0.017). Although no significant impact of stimulus colour was found on accuracy, a trend for greater accuracy when responding to blue stimuli (mean: 85.9 ms, SEM: 1.9 ms) than to yellow stimuli (mean: 82.1, SEM: 2.2 ms) was observed.

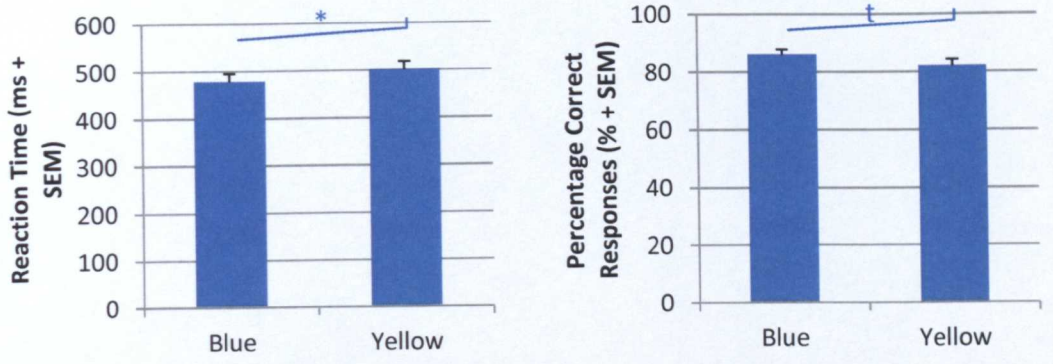

Figure 6.3: Impact of stimulus colour on global reaction time and accuracy. * indicates p=0.001, paired t-test. t indicates p=0.096, paired t-test.

Effect of hand used for response

Paired t-test found that participants responded faster when required to respond with the left hand (mean 478.9 ms, SEM 16.3 ms) than with the right (mean 500.1 ms, SEM 19.8 ms), as is outlined in figure 6.4, (p=0.017). No significant impact of

stimulus colour was found on accuracy, though there was a tendency for higher accuracy with the left hand (mean: 85.9 ms, SEM: 1.9 ms) than the right (mean: 82.1, SEM: 2.2 ms).

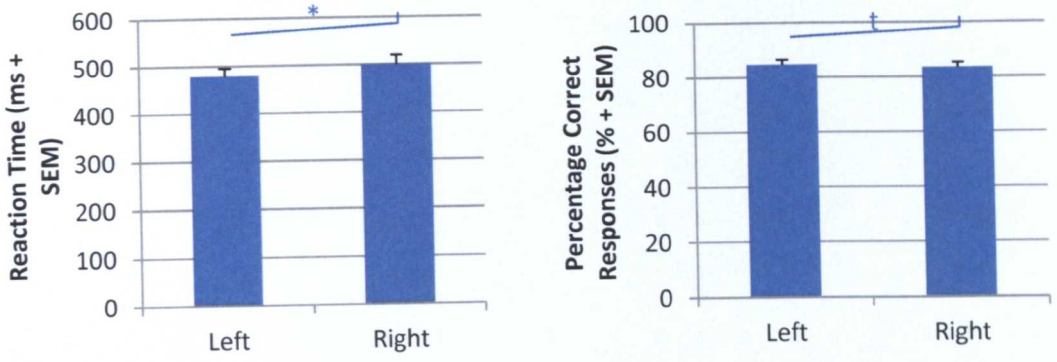

Figure 6.4: Impact of hand used for response on global reaction time and accuracy. * indicates p=0.006, paired t-test. t indicates p=0.157, paired t-test.

6.4 Discussion

6.4.1 Cognitive effects

Influence of sweetness on performance

In this study no significant differences were observed between either of the sweet tasting solutions and the still water and no treatment conditions, contradictory to the hypothesis that glucose perception would influence reaction time and accuracy through the stimulation of an oral nutrient receptor or reward value of sweet tastants, based on the results from previous chapters. With regards to this hypothesis, it is impossible to come to any conclusions following the results observed. Tendencies for increased reaction time with the no treatment condition and a decrease in accuracy with the glucose treatment were observed; however the results presented have been affected by a number of limitations, most notably the flawed methods. It is possible that, had a baseline measurement been taken and effects of each treatment been measured on different test days, significant differences may have been observed. Conversely, there is the possibility that sweet taste alone does not impact on attention/arousal in the same way as has been observed with energy drinks (see chapter 4). The literature suggests that significant

behavioural effects can be elicited with sweet taste perception, as it can influence pain perception in infants (Barr, Quek et al. 1994; Blass and Shah 1995; Barr, Young et al. 1999; Barr, Pantel et al. 1999), children (Lewkowski, Barr et al. 2003) and adults (Lewkowski, Ditto et al. 2003; Lewkowski, Young et al. 2008), alter the onset of fatigue in cycling (Chambers, Bridge et al. 2009) and running time trials (Rollo, Williams et al. 2008) and influence motor output (Gant, Stinear et al. 2010). This evidence does not suggest that sweet taste perception should have influenced performance in the tasks used in this study, however it does suggest that sweetness is the driving force behind the cognitive effects observed with energy drink treatment in chapter 4. Alternatively, these effects may not be mediated solely by taste perception, and odour/flavour perception may play a larger role than initially thought. Prescott and Wilkie (2007)observed that 'sweet-smelling' odours influenced pain perception in a cold-pressor task, replicating the effects of sucrose observed by Lewkowski *et al.* (2003). Prescott and Wilkie (2007) explain that, although odours themselves cannot be sweet as sweetness is perceived as a taste by receptors on the tongue, odours related to sweet tastes (such as caramel) are often described as such, and perception of these odourants may influence reward centres in the brain in the same way that sweet solutions do. Eggleston, White *et al.* (2010) investigated the influence of adding cocoa flavour to a sweet tasting solution, hypothesising that due to cocoa's association with sweet taste it may have an additive effect on this analgesic effect. No analgesic effect was observed with the cocoa and sucrose solution despite being perceived by participants to have a similar level of sweetness to the sucrose solution that did influence performance in the CPT. There is evidence to suggest the lack of effect observed with the cocoa and sucrose solution was caused by the bitterness of the cocoa (Lewkowski, Ditto et al. 2003).

Influence of task stimuli on cognitive outcome measures

Clear effects of the stimuli on performance have been observed, with both shape and colour of the stimuli influencing response time and accuracy. It is tempting to look for a perceptual mechanism behind these effects, however a more likely mechanism lies in the paradigm used by participants to decide what response to

make at speed. The information given to participants pre-trial might explain this effect see Figure 6.5. Participants responded to the blue square quickest and with the greatest accuracy, which happened to be the first stimulus mentioned on the information page, and to the blue circle the slowest with poorest accuracy, this stimulus being the last to be mentioned. If this task is to be used to measure cognitive effects in the future, bench testing with a new information page should be carried out, aiming to remove this effect of information.

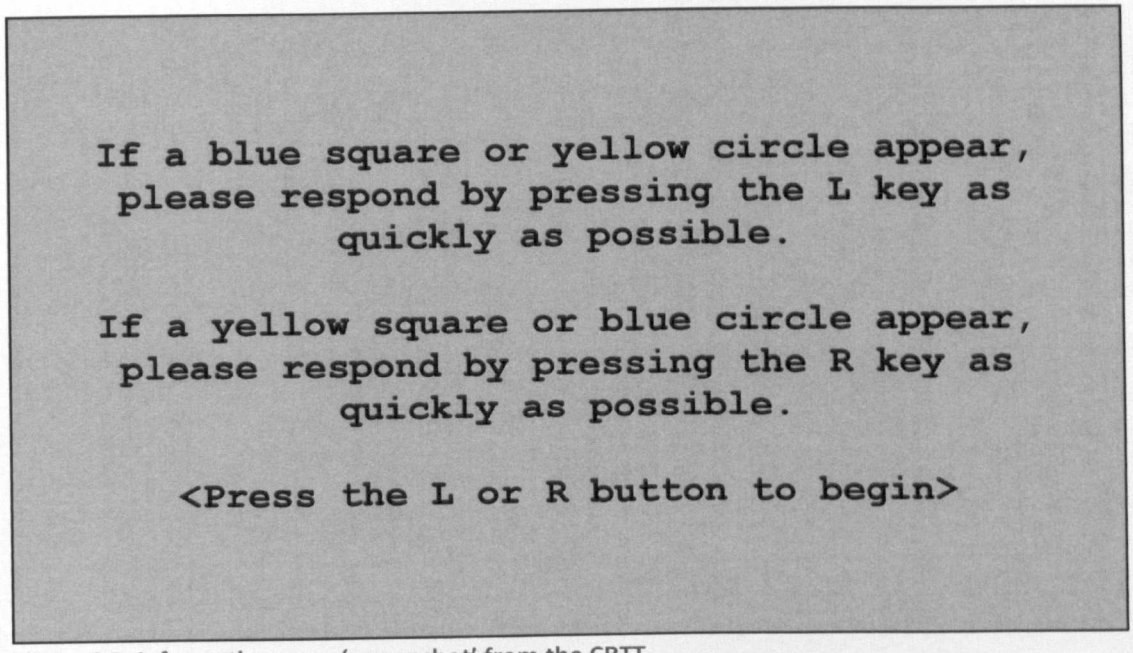

Figure 6.5: Information page 'screenshot' from the CRTT.

6.3.2 Limitations of the current study

Flaws in the methodology of this study prevented any beneficial impact of performance to be observed. Although this study used a randomised and balanced crossover design, as did the studies outlined in chapters 4 and 5, no baseline measurement was recorded in the present study, meaning no change in performance could be recorded. Previous studies found the most significant effects of treatment on measures of ΔRT and ΔACC, an effect that could not have been observed here.

Learning effects may also have influenced the results observed, as the effects of all treatments were measured in one session, as opposed to four experimental sessions plus one familiarisation session. Although familiarisation trials were

carried out at the start of each session, no testing was carried out to check that participants were performing at consistent levels and that learning effects had stopped. The interactive effect of learning on the outcome measures will have been reduced by the randomised presentation order of the sample solutions; however this will not have completely prevented learning from reducing the sensitivity of the task to measure discrete changes in performance.

CHAPTER 7: GENERAL DISCUSSION

7.1 Postprandial influence of Energy Drinks on cognitive functioning

Although the effects of energy drinks on cognition have been well documented, it was an important aim of this study to confirm these effects using methodologies that allowed comparison of the postprandial and sensory influences of energy drinks.

Results presented in chapter 3 observed that ED treatment reduced RT perhaps influencing accuracy, with no change accuracy in prosaccade trials and decreased accuracy in antisaccade trials. It appears that arousal/attention was increased significantly with the ED treatment, causing the improvement in reaction time, however according to the Yerkes-Dodson law increased arousal will improve performance to a point, and levels of arousal higher than this optimum will result in poorer performance (Yerkes and Dodson 1908). This may explain the effects observed on attention, as faster reaction times (a sign of increased arousal) limited the amount of time available to process the visual information contained by the stimulus resulting in participants responding with a prosaccade to the peripheral target, irrespective of whether a pro- or anti-saccade was appropriate. Applying this theory to the results presented in chapter 4, a significant decrease in reaction time was observed with ED treatment in overlap trials, however trends for increased accuracy were observed. Task difficulty in the antisaccade trials of the PCG task appears to be slightly higher than that observed in the CRTT, with mean accuracy in the former in the range of 65.2-88.2% and in the latter 85.8-88.3%, suggesting that the effects of over-arousal might not be so strong on CRTT performance. In addition to this, accuracy in antisaccade trials requires ability to inhibit a prosaccade towards the peripheral target presented, whereas the CRTT requires responding with either the left or right hand, where such conflict is not as great.

The impact of ED treatments on attention in the literature are not entirely consistent with the findings of this project, with the most significant effects of ED treatment concerning sustained attention/vigilance and simple RT tasks rather than choice reaction time. To date, only one study has investigated the combined influences of glucose and caffeine on attention using fMRI techniques; Serra-Grabulosa and Adan (2010) investigated the influence of glucose, caffeine and glucose + caffeine on cerebral haemodynamic activity during performance of a sustained attention task. Test samples were delivered double-blind and no impact of these samples was observed on task performance, however decreased left prefrontal cortex and bilateral parietal activation was observed in the glucose + caffeine group despite no change in performance. This was explained by the authors as increased efficiency in attention and working memory processes as a result of the combined effects of glucose and caffeine, as the ingredients on their own did not produce this effect. It is likely that stronger between treatment changes in cerebral haemodynamic activity would be observed using a task that was sensitive to the combined effects of the treatments used. This finding impacts greatly on any study of the behavioural effects of foods, as no effect of treatment was observed using behavioural data. However imaging data demonstrated a facilitation effect that would not otherwise have been recognised. The implications on this study are that lack of effects observed on cognition, such as performance in the LAT and PCO tasks might not be completely unaffected by EDs as first thought.

It is curious as to why no effects of ED treatment were observed on mood in the study outlined in chapter 3. There is the possibility that an increase in power could increase the significance of the trend in improved energetic arousal observed. Three studies by Smit *et al.*(Smit and Rogers 2002; Smit, Cotton et al. 2004; Smit, Grady et al. 2006), sponsored by GlaxoSmithKline consistently show greater levels of 'energetic arousal' following ED consumption compared with water and similar tasting placebo drinks using questionnaires derived from the Profile of Mood States (PoMS). Two of these studies involved intense testing batteries over the postprandial period, and ED treatment appeared to attenuate a decrease in energetic arousal observed with placebo and control treatments (Smit and Rogers

2002; Smit, Cotton et al. 2004). Scholey and Kennedy (2004), also funded by GlaxoSmithKline, found no significant influence of ED treatment on the PoMS or Bond-Lader mood questionnaires. The lack of effect observed in this thesis could be caused by the use of a questionnaire less sensitive to alterations in mood caused by ED consumption; however the lack of effect observed by Scholey and Kennedy despite using similar measures to Smit *et al.* suggests that mood effects of ED treatments are highly variable.

7.2 Sensory influence of Energy Drinks on cognitive functioning

Until now studies investigating the cognitive impact of energy drinks have only concerned the postprandial period, however there is growing evidence that chemosensory perception can influence cognition. This thesis outlines the first investigations into these potential effects sensory perception of energy drinks combining glucose, caffeine and flavourings on behaviour. It was hypothesised that sensory perception of energy drinks could influence cognitive performance via a proposed oral nutrient receptor (Frank, Oberndorfer et al. 2008; Chambers, Bridge et al. 2009) or rewarding effects of sweet tastes/flavours (Barr, Quek et al. 1994; Villemure, Slotnick et al. 2003; Prescott and Wilkie 2007). The results presented in chapter 4 confirm the experimental hypothesis, that sensory perception can influence behaviour. The functional energy drink appeared to reduce reaction time in gap trials (an effect that approachd significance) of the CRTT whilst appearing to attenuate the decrease in accuracy observed with still mineral water and no treatment. Energy drink treatment also appeared to attenuate the decrease in accuracy observed with still water treatment in overlap trials without any significant change in reaction time scores (in fact ED tended to (insignificantly) reduce RT in overlap trials too). Placebo treatment was also observed to reduce reaction times in gap trials, but did not affect accuracy scores, therefore it is possible that a subjective increase in arousal influenced speed of response, but did not influence decision making processes associated with choosing the appropriate response. As the sensory effects of ED treatment resembled those observed postprandially, a further study was designed to investigate the influence of sensory perception of the same samples on a sustained attention task, as EDs have been observed to

influence vigilance and sustained attention tasks significantly (see above). No significant effects of ED or P treatments were observed on reaction time, errors of omission or errors of commission, however it is possible that both treatments attenuated alterations in all outcome measures that occurred with SW treatment.

It is believed that the primary taste cortex and other sensory inputs project to areas involved in the mediation of behaviour, allowing sensory information, including taste, to influence behaviour through the reward system (de Araujo, Rolls et al. 2003; Rolls 2007). The changes observed on CRTT performance are most likely a result of the differing haemodynamic response to perception of calorific carbohydrates and artificial sweeteners. Greater activation is observed with calorific sugars in areas such as the striatum and anterior cingulate cortex than with saccharin (Frank, Oberndorfer et al. 2008; Chambers, Bridge et al. 2009; Smeets, Weijzen et al. 2011). The results presented by Chambers *et al.* (2009) also show behavioural effects of the calorific treatments, with an influence on perceived levels of fatigue during exercise that is not observed with placebo treatment. The most plausible explanation based on the results of this study and those reviewed in chapter 1 is that the behavioural effect is caused by the greater reward value of the energy containing nutrients, which must be perceivable by the human tongue as test drinks were not swallowed (Jeukendrup and Chambers 2010).

Another explanation however is the reward value of sweet taste/flavour and the influence of this on mood and/or arousal which would lead to similar effects to those observed postprandially (Prescott and Wilkie 2007). Although some effects were observed with placebo treatment in chapter 4, this mechanism would not explain why stronger effects were observed with the functional drink, however a combination of the two mechanisms could be involved.

In order to investigate the influence of a potential oral nutrient receptor, an experiment was designed to investigate the influence of sweet tasting solutions, containing either glucose or a combination of aspartame and acesulfame K on the CRTT, reported in chapter 6. The methods of this study were however flawed, and as a result it is impossible to make any conclusions regarding the mechanism of

these effects. There is the possibility of course that behavioural effects of chemosensory perception are not elicited by calorific sugars or even sweet tastants, but the reward value of pleasant flavours; however results from studies where behavioural effects of pleasant aromas and flavours have been assessed have not found any significance that can't be attributed to the presence of sugars

Whilst there is a great deal of evidence in support of the oral nutrient hypothesis theory, in order to determine if this is the mechanism leading to the sensory influence of energy drinks on cognitive performance a more in depth study incorporating functional brain imaging techniques together with the methods described in this thesis that successfully observed a sensory effect of energy drink treatments (particularly chapter 4) is necessary.

7.2.1 Wider Impact of Findings

Jenkins, Wolever *et al.* (1981) first proposed the idea of glycaemic index, the physiological basis of carbohydrate exchange in the postprandial period, and that by reducing the glycaemic load of a meal postprandial plasma glucose levels are lowered, which leads to implications on glucose tolerance and therefore diabetes. Although increasing glycaemic load lowers the peak in plasma glucose, levels remain elevated for longer as nutrients are absorbed over a longer period of time as the meal takes longer to be digested, which could lead to improved cognitive function in the postprandial period (Papanikolaou, Palmer et al. 2006). A study comparing the effects of sipping a glucose drink (50 g glucose in 700 ml water) over a 3.5 hour period with drinking it in five minutes found that despite similar plasma glucose responses, sipping the drink reduced insulin and C-peptide (involved in the process of synthesising insulin) levels. Combining the results of these studies with the results presented in this thesis outlining both sensory and postprandial influences of the drinks on cognition, as well as a possible health benefit of sipping energy drinks, there may also be a cognitive benefit due to the repeated sensory perception of the drinks and perhaps a prolonged postprandial benefit too.

7.3 Limitations

7.3.1 Time of Day Effects

One of the major findings of the study described in chapter 3 was the time of day interaction on the effects of energy drinks on arousal, however due to an error in the analysis this finding was not discovered until after the other three studies had been carried out. As it had been believed that time of day had not significantly interacted with the effect of the energy drinks in the study, it was decided that future studies would be carried out in the mornings as a means of consistency. By carrying out experimental sessions in the mornings, it was easier for participants to adhere to requests to abstain from food or caffeinated products for 2 hours/on the morning of sessions. High caffeine consumers, who may perceive themselves to be very reliant on caffeinated products throughout their working day might have been put off participation had they been asked to arrive caffeine deprived in afternoons rather than mornings. It is possible that had the study outlined in chapter 4 been carried out in afternoons, stronger effects of treatment may have been observed. This may also have prevented significant effects of treatments on cognitive measures used in chapters 5 and 6.

7.3.2 Expectancy Effects

The potential influence of expectancy and placebo effects may have resulted in beneficial effects on performance with ED treatments in all the results presented. It is unlikely that participants were completely unaware of the nature of these drinks, and will naturally have developed beliefs that these drinks do/don't produce alerting effects based on marketing and previous exposure (Faro 2010). In order to reduce expectancy effects, a placebo energy drink containing no calorific carbohydrate or caffeine was developed, which was administered in a double-blind crossover design in the experiments described in chapters 4 and 6 alongside the functional energy drink. Whilst there was some influence of the placebo treatment sensorially (on reaction times), stronger effects were observed with the functional drink (on accuracy and borderline significant effects on reaction time) sensorially and postprandially, which can only be explained by the sensory and postprandial influences of glucose and caffeine. The placebo drink was not a complete sensory

match, meaning that there were perceptible differences between the functional and placebo treatments, however as treatments were delivered on different test days and participants were blind to the nature of the drinks (i.e. they may have contained different levels of active ingredients, rather than one being a placebo) it is unlikely that participants could differentiate between the two or identify one or the other as being functional or placebo.

7.3.3 Effects of Reversal of Caffeine Withdrawal

As discussed in chapter 2, the postprandial influence of caffeine on performance has been cited by many to be caused by the reversal of the effects of caffeine withdrawal (Juliano and Griffiths 2004; Heatherley, Hayward et al. 2005; James and Rogers 2005; Rogers, Heatherley et al. 2005; James and Keane 2007; Ozsungur, Brenner et al. 2009). It appears from the results presented in chapter 3 that with stronger effects in the afternoon than in the morning, these effects could have been elicited by a longer period of caffeine abstention. Morning and afternoon groups will have been caffeine deprived for at least 10 hours and 14 hours respectively (though probably more, unless caffeinated beverages were consumed around midnight the night before experimental sessions), whilst reversal of withdrawal effects has been observed following a period of just 8 hours abstinence (Heatherley, Hayward et al. 2005). Positive effects of caffeine have, however been observed without prior abstinence (Warburton, Bersellini et al. 2001) and in non-habitual caffeine consumers (Haskell, Kennedy et al. 2005; Childs and de Wit 2006; Smith, Christopher et al. 2006). These studies conclude that whilst alerting effects of caffeine are not restricted to reversal of withdrawal in habitual consumers, it is likely that effects are more pronounced in heavier users. No effect of habitual caffeine intake was observed on performance in any of the studies described in this thesis. This suggests that caffeine withdrawal has not influenced cognitive performance, as performance in non-habitual consumers did not differ from habitual consumers. Taking this into account, it can also be concluded that time of day effects were not caused by length of caffeine withdrawal, but by typical alterations in arousal associated with circadian rhythm described at the start of section 7.3.

7.3.4 Possible Interaction of Rapid Absorption of Caffeine on Sensory Effects of Treatment

The methods used in chapters 4, 5 and 6 assume that any sensory effect of the treatments used result from the sensory perception of the treatment, however there is some argument that rapid absorption of caffeine could influence performance. Hindmarch, Quinlan *et al.* (1998) observed that only 10 minutes following consumption of caffeinated tea or coffee (each with 100 mg caffeine), performance in a discrimination task was improved compared with those who consumed non-caffeinated beverages, however no effect was observed on choice RT. Durlach, Edmunds *et al.* (2002) found significant improvement in CRTT performance immediately following administration of caffeine in the form of tea or a hot water drink (each containing 60 mg caffeine) compared with decaffeinated tea or plain hot water. It is highly unlikely however that this is the mechanism behind the effects described in chapter 4, as cognitive assessment was carried out alongside consumption of the test samples rather than immediately following it. During the CRTT (where significant effects were observed) which lasted only 7 minutes, only 32 ml of the ED was consumed in 64 x 0.5 ml doses, meaning only 3.87 mg caffeine and 5.73 g glucose had been consumed. The lowest reported dose of caffeine to elicit behavioural effects was 12.5 mg (Smit and Rogers 2000). For this reason, it appears safe to assume that the observed sensorial effects are not elicited by absorbed caffeine.

7.3.5 Sensitivity of Tasks Used

As has been discussed briefly above, lack of effect observed in some of the experimental work carried out does not signify a lack of cognitive impacts of treatments on that particular task type. Vigilance tasks like that used in chapter 5 can take many forms, and in this instance the target and non-target stimuli were very easily discriminated, making the task less demanding, and perhaps resulting in a ceiling effect. Additionally, results by Serra-Grabulosa *et al.* (2010) show that no behavioural effect needs to be elicited for a cognitive effect to be observed. Behavioural tasks use response time, accuracy and sometimes additional measures (as in the attentional blink task or driving simulator tasks) as a gauge allowing

assessment of cognitive function, however this does not tell the whole picture and cognitive events can go unnoticed.

The tasks used in the experimental chapters have been short in duration compared with some other behavioural studies (as described in section 2.3.1). This compromise in task duration (and therefore number of trials performed in each assessment) allowed investigation of change in treatment effects over the postprandial period and of the effects elicited by sensory perception of the treatments. Loh, Lamond et al. (2004) demonstrated that a 5 minute psychomotor vigilance task correlates well with and is a viable alternative a 10 minute psychomotor vigilance task. The durations of tasks used in the studies described in this thesis are similar to those used in the literature concerning energy drink treatments (Smit and Rogers 2002; Kennedy and Scholey 2004), in fact Alford, Cox et al. (2001) used a choice reaction time task with just 20 trials (though there is no evidence this is a valid measure of attention/alertness). Additionally, in studies where change in cognitive performance is measured over time, long experimental sessions (greater than 90 minutes) using short task durations (10 minutes or less) are useful to monitor any effects of fatigue and how treatment may affect fatigue.

7.3.6 Participants and Lack of Power

In the results discussed in chapters 3 and 4 there are several instances of effects of treatment approaching significance (ED effects on mean change in Gap Reaction Time in chapter 4 for example), that may have reached significance if a greater number of participants had been recruited. Although the number of participants appears low (24 in chapter 3 – eight in each treatment condition; 12-14 participants in chapter 4; 24 participants in chapter 5; and 20 participants in chapter 6), the studies described here use sample sizes typical of that in the literature. For example Smit *et al.* (2002) used 23 participants in a study investigating the effects of energy drinks in the postprandial period on a cognitive task battery; Horne and Reyner (2001) used 12 participants to assess the effects of an energy drink on driving performance; and Mucignat-Caretta (1998) used 12 participants investigating the effects of energy drinks on simple and go/no-go reaction time tasks. Each of these studies found significant effects of treatment conditions on

cognition. Studies into the cognitive effects of odour perception also used similar sample sizes, with Tubaldi *et al.* using 26 participants in two studies (2008; 2008) and 20 in another (2008). Although these studies justify the sample sizes used in this thesis, this does not mean that sample size did not affect power. It was recognised after the results of chapter 3 were analysed that power may be an issue, and further experiments utilised crossover designs. With hindsight, a power analysis should have been carried out following the first experiment described in this thesis; if further research is carried out into the sensory effects of energy drinks and carbohydrates on cognition, it will be crucial that a power analysis is conducted based on data gathered here (Cohen 1992).

7.4 Main Conclusions

To summarise, the results presented in this thesis have demonstrated clear functional effects of energy drink treatments both in the postprandial period and through sensory perception. There is already a wealth of evidence behind the mechanisms behind the drinks' postprandial influences, a combination of the alerting effects of elevated plasma glucose and caffeine concentrations following consumption, however there is still a lack of imaging data concerning the consumption of glucose and caffeine together on tasks sensitive to their alerting effects. The observation of a sensory influence of energy drinks on cognitive performance is a previously undiscovered finding, although there is proof that sensory inputs can influence behaviour in exercise, motor tasks and pain perception. These effects are most likely mediated by an oral nutrient receptor that detects the energy content of carbohydrates; however more work is required in order to accept this theory, and determine the extent of the cognitive benefits of sensory perception.

It is also clear that the time of day that cognitive effects are measured affects the effects of treatment, and future work should be carried out to determine if this effect is limited to the postprandial influence of the drinks or whether this also interacts with the sensory effects of energy drinks.

7.5 Future Work

Further investigations are necessary to determine the extent of the sensory influences of EDs on cognitive performance and the mechanism(s) by which the effects are elicited.

7.5.1 Extent of the influence of sensory properties of energy drinks on cognitive function

The sensory qualities of EDs have been observed to influence performance in CRTT, with similar effects observed postprandially; however it is possible that these cognitive effects influence other task types. The most significant effects of the postprandial influences of EDs observed in the literature are on vigilance and response inhibition in the form of continuous performance tasks. If sensory influences of energy drinks resemble the postprandial effects, it is likely that they will also affect these task types, therefore further studies into the extent of the sensory effects observed here should include these task types. The results presented in chapter 3 do also suggest that response inhibition may be influenced by energy drink consumption. Vigilance tasks typically include presentation of rare target stimuli amongst more frequent non-target stimuli, requiring sustained attention. Go/no-go tasks involve the presentation of frequent target stimuli amongst rare non-target stimuli, participants become accustomed to responding to the target stimuli and find it difficult to inhibit responses when the non-target stimuli are presented. Vigilance tasks typically see slower reaction times than go/no-go tasks as participants are less primed to respond, this means that errors of commission (responding to non-target stimuli) are less frequent, however errors of omission (not responding to target stimuli) may be higher. Such a study could replicate the methods used in chapter 4, using a randomised, balanced crossover design, participants could complete both tasks at baseline, whilst perceiving the treatments sensorially and during the postprandial period.

Due to the variation in mood effects observed postprandially in the literature, it may also be wise to incorporate subjective mood questionnaires into such a study. As no significance was observed using the UWIST mood questionnaire, it may perhaps be more useful to include a questionnaire that has been used in studies

that have measured significant effects of treatment on mood, such as the PoMS or variants of PoMS.

7.5.2 Mechanisms behind sensory influence of energy drinks on cognitive function

7.5.2.1 Behavioural studies

One method of investigating the mechanism involved in the sensory influence of EDs would be to repeat the behavioural tasks that have been observed to be sensitive to these sensory inputs using different test samples. It would be possible to investigate the oral nutrient hypothesis further by using glucose and artificially sweetened solutions in a methodology similar to that described above, using the CRTT and/or the vigilance and go/no-go tasks if they were proved to be sensitive to sensory influences of energy drinks. It would then be useful to investigate the influences of other fractions of the energy drink flavour (e.g. acidity, lemon/lime flavour or caffeine/bitterness) in isolation and in combination (e.g. glucose plus caffeine or glucose plus flavour). Investigating the impact of ED flavourings would be difficult, as flavour is made up of a combination of taste and smell, and model solutions containing flavourings would need to also include sweeteners, which may mediate cognitive effects. For this reason, the full drink containing all ingredients is the only sample that could deliver accurate flavour perception. Delivery of odours alone has been observed to influence motor performance (Tubaldi, Ansuini et al. 2008; Tubaldi, Ansuini et al. 2008) and pain perception (Villemure, Slotnick et al. 2003; Prescott and Wilkie 2007), however these methodologies would not allow full perception of the flavour, as odours are not delivered retronasally and sweet taste is an important part of flavour perception (Pierce and Halpern 1996).

7.5.2.2 Imaging studies

Due to the nature of the methods used to investigate the sensory effects of energy drinks and sweet stimuli (i.e. using cognitive tasks to make inferences about alterations in cognitive ability), it would be useful to modify the protocols for use in an imaging study that can identify more directly the mechanisms involved in the sensory and postprandial influences of energy drinks. As described above, one

study has investigated the combined influence of glucose and caffeine on attention in the postprandial period using fMRI methodologies; however this failed to use a task sensitive to the effects of the treatments (Serra-Grabulosa, Adan et al. 2010). Recording imaging data whilst performing cognitive tasks sensitive to the sensory effects of EDs whilst perceiving test samples would allow comparison of brain activation with functional and placebo EDs at rest and whilst performing cognitive tasks. It is hypothesised that greater activation would be observed in areas such as the striatum and anterior cingulate cortex (which are involved in modification of behaviour and representation of the reward value of food) in response to the functional drink compared with the placebo (based on the findings of Frank, Oberndorfer et al. 2008; Chambers, Bridge et al. 2009), and it has been hypothesised that this increase in haemodynamic response would affect performance in behavioural tasks, particularly if these effects also affect the dorsolateral prefrontal cortex (Kringelbach, de Araujo et al. 2004).

8. REFERENCES

Aarts, H., A. Dijksterhuis, et al. (2001). "On the psychology of drinking: Being thirsty and perceptually ready." *British Journal of Psychology* **92**: 631-642.

Adan, A. and J. M. Serra-Grabulosa (2010). "Effects of caffeine and glucose, alone and combined, on cognitive performance." *Human Psychopharmacology-Clinical and Experimental* **25**(4): 310-317.

Advanced Clinical Instrumentation. (2008). *"Product Range."* Retrieved 6 February 2010, 2012, from http://www.aci.eu.com/Range.html.

Alford, C., H. Cox, et al. (2001). "The effects of Red Bull Energy Drink on human performance and mood." *Amino Acids* **21**(2): 139-150.

Amiel, S. A. (1994). "NUTRITION OF THE BRAIN - MACRONUTRIENT SUPPLY." *Proceedings of the Nutrition Society* **53**(2): 401-405.

Anand, B. K., B. Singh, et al. (1964). "ACTIVITY OF SINGLE NEURONS IN HYPOTHALAMIC FEEDING CENTERS - EFFECT OF GLUCOSE." *American Journal of Physiology* **207**(5): 1146-&.

Anderson, C. and J. A. Horne (2006). "A high sugar content, low caffeine drink does not alleviate sleepiness but may worsen it." *Human Psychopharmacology-Clinical And Experimental* **21**(5): 299-303.

Anund, A., G. Kecklund, et al. (2008). "Driver sleepiness and individual differences in preferences for countermeasures." *Journal of Sleep Research* **17**(1): 16-22.

Armstrong, L. E. (2007). "Assessing hydration status: the elusive gold standard." *Journal of the American College of Nutrition* **26**(5): 575S-584S.

Arnaud, M. J. (1993). Metabolism of caffeine and other constituents of coffee. *Caffeine, coffee and health*. S. Garratini**:** 43-95.

Attwood, A. S., S. Higgs, et al. (2007). "Differential responsiveness to caffeine and perceived effects of caffeine in moderate and high regular caffeine consumers." *Psychopharmacology* **190**(4): 469-477.

Awh, E. and J. Jonides (2001). "Overlapping mechanisms of attention and spatial working memory." *Trends in Cognitive Sciences* **5**(3): 119-126.

Barone, J. J. and H. R. Roberts (1996). "Caffeine consumption." *Food and Chemical Toxicology* **34**(1): 119-129.

Barr, R. C., S. N. Young, et al. (1999). "Differential calming responses to sucrose taste in crying infants with and without colic." *Pediatrics* **103**(5): art. no.-e68.

Barr, R. G., M. S. Pantel, et al. (1999). "The response of crying newborns to sucrose: Is it a "sweetness" effect?" *Physiology & Behavior* **66**(3): 409-417.

Barr, R. G., V. S. H. Quek, et al. (1994). "EFFECTS OF INTRAORAL SUCROSE ON CRYING, MOUTHING AND HAND-MOUTH CONTACT IN NEWBORN AND 6-WEEK-OLD INFANTS." *Developmental Medicine and Child Neurology* **36**(7): 608-618.

Battram, D. S., T. E. Graham, et al. (2005). "The effect of caffeine on glucose kinetics in humans influence of adrenaline." *Journal Of Physiology-London* **569**(1): 347-355.

Bellisle, F., J. E. Blundell, et al. (1998). "Functional food science and behaviour and psychological functions." *British Journal of Nutrition* **80**: S173-S193.

Bellisle, F., J. Louissylvestre, et al. (1983). "REFLEX INSULIN-RESPONSE ASSOCIATED TO FOOD-INTAKE IN HUMAN-SUBJECTS." *Physiology & Behavior* **31**(4): 515-521.

Benton, D. (2001). "The impact of the supply of glucose to the brain on mood and memory." *Nutrition Reviews* **59**(1): S20-S21.

Benton, D. and N. Burgess (2009). "The effect of the consumption of water on the memory and attention of children." *Appetite* **53**(1): 143-146.

Biessels, G. J., E. ter Braak, et al. (2001). "Cognitive function in patients with type 2 diabetes mellitus." *Neuroscience Research Communications* **28**(1): 11-22.

Blake, R., N. J. Cepeda, et al. (1997). "Memory for visual motion." *Journal of Experimental Psychology-Human Perception and Performance* **23**(2): 353-369.

Blass, E. M. and A. Shah (1995). "PAIN-REDUCING PROPERTIES OF SUCROSE IN HUMAN NEWBORNS." *Chemical Senses* **20**(1): 29-35.

Boulenger, J. P., J. Patel, et al. (1982). "EFFECTS OF CAFFEINE AND THEOPHYLLINE ON ADENOSINE AND BENZODIAZEPINE RECEPTORS IN HUMAN-BRAIN." *Neuroscience Letters* **30**(2): 161-166.

Brice, C. and A. Smith (2001). "The effects of caffeine on simulated driving, subjective alertness and sustained attention." *Human Psychopharmacology-Clinical And Experimental* **16**(7): 523-531.

Brice, C. F. and A. P. Smith (2002). "Effects of caffeine on mood and performance: a study of realistic consumption." *Psychopharmacology* **164**(2): 188-192.

British Standards Institution (2007). Sensory Analysis - General guidance for the design of test rooms. *BS EN ISO 8589:2007*. Milton Keynes, BSI.

British Standards Institution (2007). Sensory Analysis - Methodology - Paired comparison test. *BS EN ISO 5495:2007*. Milton Keynes, BSI.

British Standards Institution (2007). Sensory Analysis - Methodology - Triangle Test. *BS EN ISO 4120:2007*. Milton Keynes, BSI.

Carpenter, R. H. S., B. A. J. Reddi, et al. (2009). "A simple two-stage model predicts response time distributions." *Journal Of Physiology-London* **587**(16): 4051-4062.

Carter, J. M., A. E. Jeukendrup, et al. (2004). "The effect of carbohydrate mouth rinse on 1-h cycle time trial performance." *Medicine And Science In Sports And Exercise* **36**(12): 2107-2111.

Carter, J. M., A. E. Jeukendrup, et al. (2004). "The effect of glucose infusion on glucose kinetics during a 1-h time trial." *Medicine And Science In Sports And Exercise* **36**(9): 1543-1550.

Castiello, U., G. M. Zucco, et al. (2006). "Cross-modal interactions between olfaction and vision when grasping." *Chemical Senses* **31**(7): 665-671.

Cedrus. (2009). *"Cedrus: The RB-830 Response Pad."* Retrieved 13/09/2009, 2009, from http://www.cedrus.com/responsepads/rb830.htm.

Chambers, E. S., M. W. Bridge, et al. (2009). "Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity." *Journal Of Physiology-London* **587**(8): 1779-1794.

Chandrashekar, J., M. A. Hoon, et al. (2006). "The receptors and cells for mammalian taste." *Nature* **444**(7117): 288-294.

Childs, E. and H. de Wit (2006). "Subjective, behavioral, and physiological effects of acute caffeine in light, nondependent caffeine users." *Psychopharmacology* **185**(4): 514-523.

Cohen, J. (1992). "A POWER PRIMER." *Psychological Bulletin* **112**(1): 155-159.

Colman, A. M. (2009). *Dictionary of Psychology*. Oxford, Oxford University Press.

Corbetta, M., J. M. Kincade, et al. (2000). "Voluntary orienting is dissociated from target detection in human posterior parietal cortex." *Nature Neuroscience* **3**(3): 292-297.

Corbetta, M. and G. L. Shulman (2002). "Control of goal-directed and stimulus-driven attention in the brain." *Nature Reviews Neuroscience* **3**(3): 201-215.

Cox, D. J., A. McCall, et al. (2007). "Effects of blood glucose rate of changes no on perceived mood and cognitive symptoms in insulin-treated type 2 diabetes." *Diabetes Care* **30**(8): 2001-2002.

D'Anci, K. E., A. Vibhakar, et al. (2009). "VOLUNTARY DEHYDRATION AND COGNITIVE PERFORMANCE IN TRAINED COLLEGE ATHLETES." *Perceptual and Motor Skills* **109**(1): 251-269.

Damak, S., M. Q. Rong, et al. (2003). "Detection of sweet and umami taste in the absence of taste receptor T1r3." *Science* **301**(5634): 850-853.

Davidson, R. A. and B. D. Smith (1991). "CAFFEINE AND NOVELTY - EFFECTS ON ELECTRODERMAL ACTIVITY AND PERFORMANCE." *Physiology & Behavior* **49**(6): 1169-1175.

de Araujo, I. E. T., E. T. Rolls, et al. (2003). "Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain." *European Journal of Neuroscience* **18**(7): 2059-2068.

Di Russo, F., A. Martinez, et al. (2002). "Cortical sources of the early components of the visual evoked potential." *Human Brain Mapping* **15**(2): 95-111.

Dolezal, V. and S. Tucek (1982). "EFFECTS OF CHOLINE AND GLUCOSE ON ATROPINE-INDUCED ALTERATIONS OF ACETYLCHOLINE SYNTHESIS AND CONTENT IN THE CAUDATE NUCLEI OF RATS." *Brain Research* **240**(2): 285-293.

Donders, F. C. (1869). "ON SPEED OF MENTAL PROCESSES." *Acta Psychologica* **30**: 412-431.

Donohoe, R. T. and D. Benton (1999). "Cognitive functioning is susceptible to the level of blood glucose." *Psychopharmacology* **145**(4): 378-385.

Donohoe, R. T. and D. Benton (2000). "Glucose tolerance predicts performance on tests of memory and cognition." *Physiology & Behavior* **71**(3-4): 395-401.

Duncan, J. (1986). "DISORGANIZATION OF BEHAVIOR AFTER FRONTAL-LOBE DAMAGE." *Cognitive Neuropsychology* **3**(3): 271-290.

Durlach, P. J., R. Edmunds, et al. (2002). "A rapid effect of caffeinated beverages on two choice reaction time tasks." *Nutritional Neuroscience* **5**(6): 433-442.

Dye, L. and J. Blundell (2002). "Functional foods: psychological and behavioural functions." *British Journal Of Nutrition* **88**: S187-S211.

Edgar, C. J., E. F. Pace-Schott, et al. (2009). "Approaches to measuring the effects of wake-promoting drugs: a focus on cognitive function." *Human Psychopharmacology-Clinical and Experimental* **24**(5): 371-389.

Edmonds, C. J. and B. Jeffes (2009). "Does having a drink help you think? 6-7-Year-old children show improvements in cognitive performance from baseline to test after having a drink of water." *Appetite* **53**(3): 469-472.

Eggleston, K., T. L. White, et al. (2010). "Adding Cocoa to Sucrose: The Effect on Cold Pain Tolerance." *Chemical Senses* **35**(4): 269-277.

Eysenck, M. W. (2006). *Fundamentals of Cognition*. Hove, Psychology Press.

Faro, D. (2010). "Changing the Future by Reshaping the Past: The Influence of Causal Beliefs on Estimates of Time to Onset." *Journal of Consumer Research* **37**(2): 279-291.

Fischer, B. and B. Breitmeyer (1987). "MECHANISMS OF VISUAL-ATTENTION REVEALED BY SACCADIC EYE-MOVEMENTS." *Neuropsychologia* **25**(1A): 73-83.

Fischer, B. and H. Weber (1993). "EXPRESS SACCADES AND VISUAL-ATTENTION." *Behavioral and Brain Sciences* **16**(3): 553-567.

Food Standards Agency. (2008, 10 October 2001). *"Food Standards Agency publishes new caffeine advice for pregnant women." Press Releases* Retrieved 15 October 2010, 2010, from http://www.food.gov.uk/news/pressreleases/2008/nov/caffeineadvice.

Frank, G. K. W., T. A. Oberndorfer, et al. (2008). "Sucrose activates human taste pathways differently from artificial sweetener." *Neuroimage* **39**(4): 1559-1569.

Fredholm, B. B. (1980). "THEOPHYLLINE ACTIONS ON ADENOSINE RECEPTORS." *European Journal of Respiratory Diseases* **61**: 29-36.

Gander, P. H., R. M. Barnes, et al. (1998). "Flight crew fatigue III: North Sea helicopter air transport operations." *Aviation Space and Environmental Medicine* **69**(9): B16-B25.

Gant, N., C. M. Stinear, et al. (2010). "Carbohydrate in the mouth immediately facilitates motor output." *Brain Research* **1350**: 151-158.

Gershon, P., D. Shinar, et al. (2009). "Evaluation of experience-based fatigue countermeasures." *Accident Analysis and Prevention* **41**(5): 969-975.

Glick, S. D. and B. Zimmerberg (1972). "AMNESIC EFFECTS OF SCOPOLAMINE." *Behavioral Biology* **7**(2): 245-&.

Gold, P. E. (1986). "GLUCOSE MODULATION OF MEMORY STORAGE PROCESSING." *Behavioral and Neural Biology* **45**(3): 342-349.

Gold, P. E. (1995). "ROLE OF GLUCOSE IN REGULATING THE BRAIN AND COGNITION." *American Journal Of Clinical Nutrition* **61**(4): S987-S995.

Goldstein, A., S. Kaizer, et al. (1969). "PSYCHOTROPIC EFFECTS FO CAFFEINE IN MAN .4. QUANTITATIVE AND QUALITATIVE DIFFERENCES ASSOCIAED WITH HABITUATION TO COFFEE." *Clinical Pharmacology & Therapeutics* **10**(4): 489-&.

Grantham-McGregor, S. M., S. Chang, et al. (1998). "Evaluation of school feeding programs: some Jamaican examples." *American Journal of Clinical Nutrition* **67**(4): 785S-789S.

Hasenfratz, M. and K. Battig (1994). "ACUTE DOSE-EFFECT RELATIONSHIPS OF CAFFEINE AND MENTAL PERFORMANCE, EEG, CARDIOVASCULAR AND SUBJECTIVE PARAMETERS." *Psychopharmacology* **114**(2): 281-287.

Haskell, C. F., D. O. Kennedy, et al. (2008). "The effects of L-theanine, caffeine and their combination on cognition and mood." *Biological Psychology* **77**(2): 113-122.

Haskell, C. F., D. O. Kennedy, et al. (2005). "Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine." *Psychopharmacology* **179**(4): 813-825.

Hayes, J. E. (2008). "Transdisciplinary Perspectives on Sweetness." *Chemosensory Perception* **1**(1): 48-57.

Heatherley, S. V., R. C. Hayward, et al. (2005). "Cognitive and psychomotor performance, mood, and pressor effects of caffeine after 4, 6 and 8 h caffeine abstinence." *Psychopharmacology* **178**(4): 461-470.

Helton, W. S. (2009). "Impulsive responding and the sustained attention to response task." *Journal of Clinical and Experimental Neuropsychology* **31**(1): 39-47.

Herness, M. S. and T. A. Gilbertson (1999). "Cellular mechanisms of taste transduction." *Annual Review of Physiology* **61**: 873-900.

Hick, W. E. (1952). "ON THE RATE OF GAIN OF INFORMATION." *Quarterly Journal of Experimental Psychology* **4**: 11-26.

Hindmarch, I., P. T. Quinlan, et al. (1998). "The effects of black tea and other beverages on aspects of cognition and psychomotor performance." *Psychopharmacology* **139**(3): 230-238.

Hofmeister, E. H., J. L. Muilenburg, et al. (2010). "Over-the-Counter Stimulant, Depressant, and Nootropic Use by Veterinary Students." *Journal of Veterinary Medical Education* **37**(4): 403-416.

Holmes, C. S., J. T. Hayford, et al. (1983). "A SURVEY OF COGNITIVE-FUNCTIONING AT DIFFERENT GLUCOSE-LEVELS IN DIABETIC PERSONS." *Diabetes Care* **6**(2): 180-185.

Hopfinger, J. B., M. H. Buonocore, et al. (2000). "The neural mechanisms of top-down attentional control." *Nature Neuroscience* **3**(3): 284-291.

Horne, J. A. and L. A. Reyner (1995). "SLEEP-RELATED VEHICLE ACCIDENTS." *British Medical Journal* **310**(6979): 565-567.

Horne, J. A. and L. A. Reyner (1996). "Counteracting driver sleepiness: Effects of napping, caffeine, and placebo." *Psychophysiology* **33**(3): 306-309.

Horne, J. A. and L. A. Reyner (2001). "Beneficial effects of an "energy drink" given to sleepy drivers." *Amino Acids* **20**(1): 83-89.

Howard, M. A. and C. A. Marczinski (2010). "Acute Effects of a Glucose Energy Drink on Behavioral Control." *Experimental and Clinical Psychopharmacology* **18**(6): 553-561.

Hoyland, A., C. L. Lawton, et al. (2008). "Acute effects of macronutrient manipulations on cognitive test performance in healthy young adults: A systematic research review." *Neuroscience and Biobehavioral Reviews* **32**(1): 72-85.

James, J. E. (1997). Psychopharmacology of Caffeine. *Understanding Caffeine*. Thousand Oaks, CA, Sage Publications: 35-57.

James, J. E. and M. A. Keane (2007). "Caffeine, sleep and wakefulness: implications of new understanding about withdrawal reversal." *Human Psychopharmacology-Clinical And Experimental* **22**(8): 549-558.

James, J. E. and P. J. Rogers (2005). "Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation." *Psychopharmacology* **182**(1): 1-8.

James, W. J. (1890). Attention. *The Principles of Psychology*. **1**.

Jay, S. M., R. M. Petrilli, et al. (2006). "The suitability of a caffeinated energy drink for night-shift workers." *Physiology & Behavior* **87**(5): 925-931.

Jenkins, D. J. A., T. M. S. Wolever, et al. (1981). "GLYCEMIC INDEX OF FOODS - A PHYSIOLOGICAL-BASIS FOR CARBOHYDRATE EXCHANGE." *American Journal of Clinical Nutrition* **34**(3): 362-366.

Jeukendrup, A. E. and E. S. Chambers (2010). "Oral carbohydrate sensing and exercise performance." *Current Opinion in Clinical Nutrition and Metabolic Care* **13**(4): 447-451.

Juliano, L. M. and R. R. Griffiths (2004). "A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features." *Psychopharmacology* **176**(1): 1-29.

Kaplan, G. B., D. J. Greenblatt, et al. (1997). "Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans." *Journal of Clinical Pharmacology* **37**(8): 693-703.

Kastner, S. and L. G. Ungerleider (2000). "Mechanisms of visual attention in the human cortex." *Annual Review of Neuroscience* **23**: 315-341.

Keane, M. A., J. E. James, et al. (2007). "Effects of dietary caffeine on topographic EEG after controlling for withdrawal and withdrawal reversal." *Neuropsychobiology* **56**(4): 197-207.

Kennedy, D. O. and A. B. Scholey (2000). "Glucose administration, heart rate and cognitive performance: effects of increasing mental effort." *Psychopharmacology* **149**(1): 63-71.

Kennedy, D. O. and A. B. Scholey (2004). "A glucose-caffeine 'energy drink' ameliorates subjective and performance deficits during prolonged cognitive demand." *Appetite* **42**(3): 331-333.

Kennedy, D. O., A. B. Scholey, et al. (2000). "The dose-dependent cognitive effects of acute administration of Ginkgo biloba to healthy young volunteers." *Psychopharmacology* **151**(4): 416-423.

Kennedy, D. O., G. Wake, et al. (2003). "Modulation of mood and cognitive performance following acute administration of single doses of Melissa officinalis (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties." *Neuropsychopharmacology* **28**(10): 1871-1881.

Knudsen, E. I. (2007). "Fundamental components of attention." *Annual Review of Neuroscience* **30**: 57-78.

Kock, K., M. Blaker, et al. (1992). "POSTNATAL-DEVELOPMENT OF VONEBNERS GLANDS - ACCUMULATION OF A PROTEIN OF THE LIPOCALIN SUPERFAMILY IN TASTE PAPILLAE OF RAT TONGUE." *Cell and Tissue Research* **267**(2): 313-320.

Kourtidou-Papadeli, C., C. Papadelis, et al. (2002). "Maximum cognitive performance and physiological time trend measurements after caffeine intake." *Cognitive Brain Research* **13**(3): 407-415.

Kringelbach, M. L., I. E. T. de Araujo, et al. (2004). "Taste-related activity in the human dorsolateral prefrontal cortex." *Neuroimage* **21**(2): 781-788.

Lapp, J. E. (1981). "EFFECTS OF GLYCEMIC ALTERATIONS AND NOUN IMAGERY ON THE LEARNING OF PAIRED ASSOCIATES." *Journal of Learning Disabilities* **14**(1): 35-38.

Lewkowski, M. D., R. G. Barr, et al. (2003). "Effects of chewing gum on responses to routine painful procedures in children." *Physiology & Behavior* **79**(2): 257-265.

Lewkowski, M. D., B. Ditto, et al. (2003). "Sweet taste and blood pressure-related analgesia." *Pain* **106**(1-2): 181-186.

Lewkowski, M. D., S. N. Young, et al. (2008). "Effects of opioid blockade on the modulation of pain and mood by sweet taste and blood pressure in young adults." *Pain* **135**(1-2): 75-81.

Li, X., D. Glaser, et al. (2009). "Analyses of Sweet Receptor Gene (Tas1r2) and Preference for Sweet Stimuli in Species of Carnivora." *Journal of Heredity* **100**: S90-S100.

Lieberman, H. R., G. P. Bathalon, et al. (2005). "Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat." *Biological Psychiatry* **57**(4): 422-429.

Lieberman, H. R., R. J. Wurtman, et al. (1987). "The Effects Of Low-Doses Of Caffeine On Human-Performance And Mood." *Psychopharmacology* **92**(3): 308-312.

Loh, S., N. Lamond, et al. (2004). "The validity of psychomotor vigilance tasks of less than 10-minute duration." *Behavior Research Methods Instruments & Computers* **36**(2): 339-346.

Loke, W. H., J. V. Hinrichs, et al. (1985). "CAFFEINE AND DIAZEPAM - SEPARATE AND COMBINED EFFECTS ON MOOD, MEMORY, AND PSYCHOMOTOR PERFORMANCE." *Psychopharmacology* **87**(3): 344-350.

Luchins, A. S. (1942). "Mechanization in Problem Solving The Effect of Einstellung." *Psychological Monographs* **54**(6): 1-95.

MacFie, H. J., N. Bratchell, et al. (1989). "Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests." *Journal of Sensory Studies* **4**(2): 129-148.

Magnussen, S., M. W. Greenlee, et al. (1991). "STIMULUS-SPECIFIC MECHANISMS OF VISUAL SHORT-TERM-MEMORY." *Vision Research* **31**(7-8): 1213-1219.

Malinauskas, B., V. Aeby, et al. (2007). "A survey of energy drink consumption patterns among college students." *Nutrition Journal* **6**(35): 1-14.

Manning, C. A., M. W. Parsons, et al. (1992). "ANTEROGRADE AND RETROGRADE ENHANCEMENT OF 24-H MEMORY BY GLUCOSE IN ELDERLY HUMANS." *Behavioral and Neural Biology* **58**(2): 125-130.

Martin, P. Y. and D. Benton (1999). "The influence of a glucose drink on a demanding working memory task." *Physiology & Behavior* **67**(1): 69-74.

Mattes, R. D. (2011). "Accumulating evidence supports a taste component for free fatty acids in humans." *Physiology & Behavior* **104**(4): 624-631.

Matthews, G., D. M. Jones, et al. (1990). "Refining the measurement of mood: The UWIST Mood Adjective Checklist." *British Journal of Psychology* **81**: 17-42.

McKenna, K. and C. Thompson (1998). "Osmoregulation in clinical disorders of thirst appreciation." *Clinical Endocrinology* **49**(2): 139-152.

McNay, E. C., T. M. Fries, et al. (2000). "Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task." *Proceedings of the National Academy of Sciences of the United States of America* **97**(6): 2881-2885.

Messier, C. (2004). "Glucose improvement of memory: a review." *European Journal of Pharmacology* **490**(1-3): 33-57.

Messier, C. and M. Gagnon (1996). "Glucose regulation and cognitive functions: Relation to Alzheimer's disease and diabetes." *Behavioural Brain Research* **75**(1-2): 1-11.

Messier, C. and N. M. White (1984). "CONTINGENT AND NON-CONTINGENT ACTIONS OF SUCROSE AND SACCHARIN REINFORCERS - EFFECTS ON TASTE PREFERENCE AND MEMORY." *Physiology & Behavior* **32**(2): 195-203.

Messier, C. and N. M. White (1987). "MEMORY IMPROVEMENT BY GLUCOSE, FRUCTOSE, AND 2 GLUCOSE ANALOGS - A POSSIBLE EFFECT ON PERIPHERAL GLUCOSE-TRANSPORT." *Behavioral and Neural Biology* **48**(1): 104-127.

Mikalsen, A., B. Bertelsen, et al. (2001). "Effects of caffeine, caffeine-associated stimuli, and caffeine-related information on physiological and psychological arousal." *Psychopharmacology* **157**(4): 373-380.

Miles, W. R. and H. F. Root (1922). "Psychologic tests applied to diabetic patients." *Archives of Internal Medicine* **30**(6): 767-777.

Miller, K. E. (2008). "Energy Drinks, Race, and Problem Behaviors Among College Students." *Journal of Adolescent Health* **43**(5): 490-497.

Miller, K. E. (2008). "Wired: Energy drinks, jock identity, masculine norms, and risk taking." *Journal of American College Health* **56**(5): 481-489.

Millot, J. L., G. Brand, et al. (2002). "Effects of ambient odors on reaction time in humans." *Neuroscience Letters* **322**(2): 79-82.

Morgan, G. A., N. L. Leech, et al. (2011). IBM SPSS for introductory statistics: use and interpretation New York, Routledge.

Mucignat-Caretta, C. (1998). "Changes in female cognitive performance after energetic drink consumption: A preliminary study." *Progress In Neuro-Psychopharmacology & Biological Psychiatry* **22**(6): 1035-1042.

Muller, N. G., O. A. Bartelt, et al. (2003). "A physiological correlate of the "zoom lens" of visual attention." *Journal of Neuroscience* **23**(9): 3561-3565.

Murphy, J. M., M. E. Pagano, et al. (1998). "The relationship of school breakfast to psychosocial and academic functioning - Cross-sectional and longitudinal observations in an inner-city school sample." *Archives of Pediatrics & Adolescent Medicine* **152**(9): 899-907.

Noorani, I. and R. H. S. Carpenter (2011). "Full reaction time distributions reveal the complexity of neural decision-making." *European Journal of Neuroscience* **33**(11): 1948-1951.

Norman, D. A. and T. Shallice (1986). Attention to action: Willed and automatic control of behaviour. *Consciousness and Self-Regulation: Advances in Research and Theory.* R. J. Davidson, Schwartz, G. E., and Shapiro, D., Plenum Press.

O'Doherty, J., E. T. Rolls, et al. (2001). "Representation of pleasant and aversive taste in the human brain." *Journal of Neurophysiology* **85**(3): 1315-1321.

Ober Consulting. (2008). *"General Features of the Saccadometer System."* Retrieved 6 February 2012, 2012, from http://www.ober-consulting.com/page/cechy_saccadometer/.

Ober Consulting. (2008). *"Technical Specification of the Saccadometer System."* Retrieved 6 February 2012, 2012, from http://www.ober-consulting.com/page/spec_saccadometer/.

Ozsungur, S., D. Brenner, et al. (2009). "Fourteen well-described caffeine withdrawal symptoms factor into three clusters." *Psychopharmacology* **201**(4): 541-548.

Papanikolaou, Y., H. Palmer, et al. (2006). "Better cognitive performance following a low-glycaemic-index compared with a high-glycaemic-index carbohydrate meal in adults with type 2 diabetes." *Diabetologia* **49**(5): 855-862.

Pavlov, I. P. (1910). *The Work of the Digestive Glands: Lectures.* London, Charles Griffen & Company.

Pierce, J. and B. P. Halpern (1996). "Orthonasal and retronasal odorant identification based upon vapor phase input from common substances." *Chemical Senses* **21**(5): 529-543.

Posner, M. I. (1980). "ORIENTING OF ATTENTION." *Quarterly Journal of Experimental Psychology* **32**(FEB): 3-25.

Prescott, J. and J. Wilkie (2007). "Pain tolerance selectively increased by a sweet-smelling odor." *Psychological Science* **18**(4): 308-311.

Ramassamy, C., F. Girbe, et al. (1995). "PEROXIDATION OF SYNAPTOSOMES ALTERS THE DOPAMINE UPTAKE COMPLEX BUT SPARES THE EXOCYTOTIC RELEASE OF DOPAMINE." *Neurodegeneration* **4**(2): 155-160.

Rao, A., H. L. Hu, et al. (2005). "The effects of combined caffeine and glucose drinks on attention in the human brain." *Nutritional Neuroscience* **8**(3): 141-153.

Raymond, J. E., K. L. Shapiro, et al. (1992). "TEMPORARY SUPPRESSION OF VISUAL PROCESSING IN AN RSVP TASK - AN ATTENTIONAL BLINK." *Journal of Experimental Psychology-Human Perception and Performance* **18**(3): 849-860.

Raz, A. (2004). "Anatomy of attentional networks." *Anatomical Record Part B:The New Anatomist* **281**(1): 21-36.

Reddi, B. A. J., K. N. Asrress, et al. (2003). "Accuracy, information, and response time in a saccadic decision task." *Journal of Neurophysiology* **90**(5): 3538-3546.

Regina, E. G., G. M. Smith, et al. (1974). "Effects Of Caffeine On Alertness In Simulated Automobile Driving." *Journal Of Applied Psychology* **59**(4): 483-489.

Reissig, C. J., E. C. Strain, et al. (2009). "Caffeinated energy drinks-A growing problem." *Drug and Alcohol Dependence* **99**(1-3): 1-10.

Reuter-Lorenz, P. A., H. C. Hughes, et al. (1991). "THE REDUCTION OF SACCADIC LATENCY BY PRIOR OFFSET OF THE FIXATION POINT - AN ANALYSIS OF THE GAP EFFECT." *Perception & Psychophysics* **49**(2): 167-175.

Reyner, L. A. and J. A. Horne (2000). "Early morning driver sleepiness: Effectiveness of 200 mg caffeine." *Psychophysiology* **37**(2): 251-256.

Reyner, L. A. and J. A. Horne (2002). "Efficacy of a 'functional energy drink' in counteracting driver sleepiness." *Physiology & Behavior* **75**(3): 331-335.

Robelin, M. and P. J. Rogers (1998). "Mood and psychomotor performance effects of the first, but not of subsequent, cup-of-coffee equivalent doses of caffeine consumed after overnight caffeine abstinence." *Behavioural Pharmacology* **9**(7): 611-618.

Roberts, H. R. and J. J. Barone (1983). "CAFFEINE - HISTORY AND USE." *Food Technology* **37**(9): 32-39.

Rogers, P. J., S. V. Heatherley, et al. (2005). "Effects of caffeine and caffeine withdrawal on mood and cognitive performance degraded by sleep restriction." *Psychopharmacology* **179**(4): 742-752.

Rollo, I., C. Williams, et al. (2008). "The Influence of Carbohydrate Mouth Rinse on Self-Selected Speeds During a 30-min Treadmill Run." *International Journal of Sport Nutrition and Exercise Metabolism* **18**(6): 585-600.

Rolls, B. J., R. J. Wood, et al. (1980). "THIRST FOLLOWING WATER-DEPRIVATION IN HUMANS." *American Journal of Physiology* **239**(5): R476-R482.

Rolls, E. T. (2007). "Sensory processing in the brain related to the control of food intake." *Proceedings of the Nutrition Society* **66**: 96-112.

Rolls, E. T., H. D. Critchley, et al. (1999). "Responses to the sensory properties of fat of neurons in the primate orbitofrontal cortex." *Journal of Neuroscience* **19**(4): 1532-1540.

Rolls, E. T., H. D. Critchley, et al. (2010). "The Representation of Information About Taste and Odor in the Orbitofrontal Cortex." *Chemosensory Perception* **3**(1): 16-33.

Rubinstein, J. S., D. E. Meyer, et al. (2001). "Executive control of cognitive processes in task switching." *Journal of Experimental Psychology-Human Perception and Performance* **27**(4): 763-797.

Rycroft, N., J. M. Rusted, et al. (2005). "Acute effects of nicotine on visual search tasks in young adult smokers." *Psychopharmacology* **181**(1): 160-169.

Saslow, M. G. (1967). "EFFECTS OF COMPONENTS OF DISPLACEMENT-STEP STIMULI UPON LATENCY FOR SACCADIC EYE MOVEMENT." *Journal of the Optical Society of America* **57**(8): 1024-&.

Sato, K., S. Endo, et al. (1995). "RECONSIDERATION OF THE SO-CALLED TONGUE TASTE MAP." *Chemical Senses* **20**(3): 366-367.

Schmitt, J. A. J., D. Benton, et al. (2005). "General methodological considerations for the assessment of nutritional influences on human cognitive functions." *European Journal of Nutrition* **44**(8): 459-464.

Scholey, A. B. and D. O. Kennedy (2004). "Cognitive and physiological effects of an "energy drink": an evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions." *Psychopharmacology* **176**(3-4): 320-330.

Scott, T. R. and J. V. Verhagen (2000). "Taste as a factor in the management of nutrition." *Nutrition* **16**(10): 874-885.

Seidl, R., A. Peyrl, et al. (2000). "A taurine and caffeine-containing drink stimulates cognitive performance and well-being." *Amino Acids* **19**(3-4): 635-642.

Serra-Grabulosa, J. M., A. Adan, et al. (2010). "Glucose and caffeine effects on sustained attention: an exploratory fMRI study." *Human Psychopharmacology-Clinical and Experimental* **25**(7-8): 543-552.

Shulman, G. L., J. M. Ollinger, et al. (1999). "Areas involved in encoding and applying directional expectations to moving objects." *Journal of Neuroscience* **19**(21): 9480-9496.

Silverman, K., S. M. Evans, et al. (1992). "WITHDRAWAL SYNDROME AFTER THE DOUBLE-BLIND CESSATION OF CAFFEINE CONSUMPTION." *New England Journal of Medicine* **327**(16): 1109-1114.

Silverman, K. and R. R. Griffiths (1992). "LOW-DOSE CAFFEINE DISCRIMINATION AND SELF-REPORTED MOOD EFFECTS IN NORMAL VOLUNTEERS." *Journal of the Experimental Analysis of Behavior* **57**(1): 91-107.

Sinha, N., J. T. G. Brown, et al. (2006). "Task switching as a two-stage decision process." *Journal of Neurophysiology* **95**(5): 3146-3153.

Small, D. M., G. Bender, et al. (2007). The role of the human orbitofrontal cortex in taste and flavor processing. *Linking Affect to Action: Critical Contributions of the Orbitofrontal Cortex.* **1121:** 136-151.

Smeets, P. A. M., P. Weijzen, et al. (2011). "Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting." *Neuroimage* **54**(2): 1367-1374.

Smit, H. J., J. R. Cotton, et al. (2004). "Mood and cognitive performance effects of "energy" drink constituents: Caffeine, glucose and carbonation." *Nutritional Neuroscience* **7**(3): 127-139.

Smit, H. J., M. L. Grady, et al. (2006). "Role of familiarity on effects of caffeine- and glucose-containing soft drinks." *Physiology & Behavior* **87**(2): 287-297.

Smit, H. J., A. Meikle, et al. (2002). "*Mood, alerting and cognitive performance effects of "energy drinks": what are the appropriate placebos?* in Abstracts - The 10th Food Choice Conference 2002 - 30 June-3 July 2002 - Wageningen, The Netherlands." *Appetite* **39**(3): 258-258.

Smit, H. J. and P. J. Rogers (2000). "Effects of low doses of caffeine on cognitive performance, mood and thirst in low and higher caffeine consumers." *Psychopharmacology* **152**(2): 167-173.

Smit, H. J. and P. J. Rogers (2002). "Effects of 'energy' drinks on mood and mental performance: critical methodology." *Food Quality And Preference* **13**(5): 317-326.

Smith, A., A. Kendrick, et al. (1994). "EFFECTS OF FAT-CONTENT, WEIGHT, AND ACCEPTABILITY OF THE MEAL ON POSTLUNCH CHANGES IN MOOD, PERFORMANCE, AND CARDIOVASCULAR FUNCTION." *Physiology & Behavior* **55**(3): 417-422.

Smith, A. P., G. Christopher, et al. (2006). "Effects of caffeine in overnight-withdrawn consumers and non-consumers." *Nutritional Neuroscience* **9**(1-2): 63-71.

Specterman, M., A. Bhuiya, et al. (2005). "The effect of an energy drink containing glucose and caffeine on human corticospinal excitability." *Physiology & Behavior* **83**(5): 723-728.

Sperling, G. and E. Weichselgartner (1990). "EPISODIC THEORY OF VISUAL-ATTENTION." *Bulletin of the Psychonomic Society* **28**(6): 482-482.

Stefurak, T. L. and D. Vanderkooy (1992). "SACCHARIN REWARDING, CONDITIONED REINFORCING, AND MEMORY-IMPROVING PROPERTIES - MEDIATION BY ISOMORPHIC OR INDEPENDENT PROCESSES." *Behavioral Neuroscience* **106**(1): 125-139.

Stewart, R. and D. Liolitsa (1999). "Type 2 diabetes mellitus, cognitive impairment and dementia." *Diabetic Medicine* **16**(2): 93-112.

Stubbs, R. J., D. A. Hughes, et al. (2000). "The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings." *British Journal of Nutrition* **84**(4): 405-415.

Suhr, J. A., J. Hall, et al. (2004). "The relation of hydration status to cognitive perfonnance in healthy older adults." *International Journal of Psychophysiology* **53**(2): 121-125.

Sunram-Lea, S. I., J. K. Foster, et al. (2001). "Glucose facilitation of cognitive performance in healthy young adults: examination of the influence of fast-duration, time of day and pre-consumption plasma glucose levels." *Psychopharmacology* **157**(1): 46-54.

Sunram-Lea, S. I., J. K. Foster, et al. (2002). "Investigation into the significance of task difficulty and divided allocation of resources on the glucose memory facilitation effect." *Psychopharmacology* **160**(4): 387-397.

Temple, J. G., J. S. Warm, et al. (2000). "The effects of signal salience and caffeine on performance, workload, and stress in an abbreviated vigilance task." *Human Factors* **42**(2): 183-194.

Thorndike, E. L. (1933). " A theory of the action of the after-effects of a connection upon it." *Psychological review* **40**(5): 434-439.

Toates, F. M. (1979). "HOMEOSTASIS AND DRINKING." *Behavioral and Brain Sciences* **2**(1): 95-101.

Tubaldi, F., C. Ansuini, et al. (2008). "Effects of olfactory stimuli on arm-reaching duration." *Chemical Senses* **33**(5): 433-440.

Tubaldi, F., C. Ansuini, et al. (2008). "The Grasping Side of Odours." *Plos One* **3**(3).

Tubaldi, F., C. Ansuini, et al. (2008). "Odours grab his hand but not hers." *Perception* **37**(12): 1886-1889.

van den Berg, J. and U. Landstrom (2006). "Symptoms of sleepiness while driving and their relationship to prior sleep, work and individual characteristics." *Transportation Research Part F-Traffic Psychology And Behaviour* **9**(3): 207-226.

Van den Eynde, F., P. Van Baelen, et al. (2008). "De effecten van energiedranken op de cognitieve prestaties [Energy drink effects on cognitive performance]." *Tijdschrift voor Psychiatrie* **50**(5): 273-281.

Villemure, C., B. M. Slotnick, et al. (2003). "Effects of odors on pain perception: deciphering the roles of emotion and attention." *Pain* **106**(1-2): 101-108.

Warburton, D. M. (1995). "Effects Of Caffeine On Cognition And Mood Without Caffeine Abstinence." *Psychopharmacology* **119**(1): 66-70.

Warburton, D. M., E. Bersellini, et al. (2001). "An evaluation of a caffeinated taurine drink on mood, memory and information processing in healthy volunteers without caffeine abstinence." *Psychopharmacology* **158**(3): 322-328.

Westenhoefer, J., F. Bellisle, et al. (2004). "PASSCLAIM - Mental state and performance." *European Journal of Nutrition* **43**: 85-117.

White, H. L., P. W. Scates, et al. (1996). "Extracts of Ginkgo biloba leaves inhibit monoamine oxidase." *Life Sciences* **58**(16): 1315-1321.

Wickelgren, W. A. (1977). "SPEED-ACCURACY TRADEOFF AND INFORMATION-PROCESSING DYNAMICS." *Acta Psychologica* **41**(1): 67-85.

Yerkes, R. M. and J. D. Dodson (1908). "The relation of strength of stimulus to rapidity of habit-formation." *Journal of Comparative Neurology and Psychology* **18**(5): 459-482.

Yeshurun, Y. and M. Carrasco (1999). "Spatial attention improves performance in spatial resolution tasks." *Vision Research* **39**(2): 293-306.

Zhao, G. Q., Y. F. Zhang, et al. (2003). "The receptors for mammalian sweet and umami taste." *Cell* **115**(3): 255-266.

LIST OF APPENDICES

APPENDIX 1: INFORMATION SHEET FOR PARTICIPANTS (FOR STUDY OUTLINED IN CHAPTER 3)

INFORMATION SHEET FOR PARTICIPANTS

An evaluation of effects of drinks on cognitive function

You are being invited to take part in a research study to evaluate effects of drinks on brain function. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. If you are pregnant, diabetic, colour blind, suffer from any disorder affecting attention or smoke you are not eligible to take part in this study. Please ask Chris Mason (stxcjm@nottingham.ac.uk) if there is anything you do not understand or if you would like further information.

This study is designed to investigate the impact of energy drink consumption on measures of mood, attention and cognitive control.

You will be asked to carry out a mood questionnaire and eye-tracking tasks using a saccadometer (a small camera that can be strapped to the forehead and monitors eye movements), and may be asked to consume a drink which may contain caffeine. The experiment will last no longer than 2 hours and you will be rewarded £10 for your time (and participants not given the test drink will be given a bottle to take home). We will send a questionnaire for you to fill out at your convenience. All information obtained during the study will be confidential.

We hope that you feel able to help us with this study. If at any time you decide that you do not want to continue to take part in the study, you are free to withdraw.

If you would like to discuss anything further, please contact me at the above email address.

Yours sincerely

Chris Mason

APPENDIX 2: CONSENT FORM (COMPLETED BY PARTICIPANTS WHO VOLUNTEERED FOR STUDY OUTLINED IN CHAPTER 3)

CONSENT FORM

Effect of drinks on cognitive function

Investigator: Chris Mason
Supervisor: Prof Stephen Jackson
School of Psychology, University of Nottingham

The participant should complete the whole of this sheet himself/herself. Please cross out as necessary

- Have you read and understood the participant information sheet YES/NO
- Have you had the opportunity to ask questions and discuss the study YES/NO
- Have all the questions been answered satisfactorily YES/NO
- Have you received enough information about the study YES/NO
- Do you understand that you are free to withdraw from the study:
    - at any time YES/NO
    - without having to give a reason YES/NO
- Do you agree to take part in the study YES/NO

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

Name (in block capitals)

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

APPENDIX 3: THE UWIST MOOD ADJECTIVE CHECKLIST AND SCORING

Instructions. This questionnaire is concerned with your current feelings. Please answer **every** question, even if you find it difficult. Answer, as honestly as you can, what is true of **you**. Please do not choose a reply just because it seems like the 'right thing to say'. Your answers will be kept entirely confidential. Also, be sure to answer according to how you feel **AT THE MOMENT**. Don't just put down how you usually feel. You should try and work quite quickly: there is no need to think very hard about the answers. The first answer you think of is usually the best.

Before you start, please provide some general information about yourself.

Age..... (years) Sex. M F (Circle one)
Occupation.....
If student, state your course.....
Date today..... Time of day now.....

Here is a list of words which describe people's moods or feelings. Please indicate how well each word describes how you feel **AT THE MOMENT**. For each word, circle the answer from 1 to 4 which best describes your mood.

	Definitely	Slightly	Slightly Not	Definitely Not
1. Happy	1	2	3	4
2. Dissatisfied	1	2	3	4
3. Energetic	1	2	3	4
4. Relaxed	1	2	3	4
5. Alert	1	2	3	4
6. Nervous	1	2	3	4
7. Passive	1	2	3	4
8. Cheerful	1	2	3	4
9. Tense	1	2	3	4
10. Jittery	1	2	3	4
11. Sluggish	1	2	3	4
12. Sorry	1	2	3	4
13. Composed	1	2	3	4
14. Depressed	1	2	3	4
15. Restful	1	2	3	4
16. Vigorous	1	2	3	4
17. Anxious	1	2	3	4
18. Satisfied	1	2	3	4
19. Unenterprising	1	2	3	4
20. Sad	1	2	3	4
21. Calm	1	2	3	4
22. Active	1	2	3	4
23. Contented	1	2	3	4
24. Tired	1	2	3	4
25. Impatient	1	2	3	4
26. Annoyed	1	2	3	4
27. Angry	1	2	3	4
28. Irritated	1	2	3	4
29. Grouchy	1	2	3	4

Scoring the UWIST Mood Adjective Checklist

The UWIST Mood Adjective Checklist (Matthews, Jones & Chamberlain, 1990) comprises three main bipolar scales supported by item factor analysis - Energetic Arousal, Tense Arousal and Hedonic Tone - plus an additional, monopolar Anger/Frustration scale. Anger/Frustration items load on the Hedonic Tone scale, but in some contexts it is useful to have a separate anger measure. Items are as follows:

Energetic Arousal (EA): 3, 5, 16, 22 (positive items), 7, 11, 19, 24 (negative items)

Tense Arousal (TA): 6, 9, 10, 17 (pos.), 4, 13, 15, 21 (neg.)

Hedonic Tone (HT): 1, 8, 18, 23 (pos.), 2, 12, 14, 20 (neg.)

Anger/frustration (AF): 25, 26, 27, 28, 29 (pos.)

Scale scores are obtained by summating item scores, but, on the UMACL, positive items are 'reverse-scored'. These items must be recalculated before item scores are added together. To do this, subtract each positive item score from 5.

For example, to score the Energetic Arousal scale, take the scores on items 3, 5, 16 and 22, and subtract each one from 5. Then add together all eight item scores to obtain the scale score. Alternatively, the following formulae may be used:

EA = 20 + I7 + I11 + I19 + I24 - I3 - I5 - I16 - I22

TA = 20 + I4 + I13 + I15 + I21 - I6 - I9 - I10 - I17

HT = 20 + I2 + I12 + I14 + I20 - I1 - I8 - I18 - I23

AF = 25 - I25 - I26 - I27 - I28 - I29,

where I1, I2 etc. refer to the raw, uncorrected item scores.

Reference

Matthews, G., Jones, D.M., & Chamberlain, A.G. (1990) Refining the measurement of mood: The UWIST Mood Adjective Checklist. British Journal of Psychology, 81, 17-42.

APPENDIX 4: EXAMPLE CASE REPORT FORM (AS USED IN STUDY OUTLINED IN CHAPTER 4).

Sensory & postprandial impact of energy drinks on cognitive function.

CASE REPORT FORM

Participant		
Number:		
Name:		
Age:		
Sex:		

Trial	Visit 1	Visit 2
Date		
Time		
Drink code		
Comments about trial/data		

Trial	Visit 3	Visit 4
Date		
Time		
Drink code		
Comments about trial/data		

Trial Visit 5

EXPERIMENTAL SCHEDULE

The schedule for the test session is shown in the table below. After arrival for the familiarisation session, participants will be given the information sheet to read and consent form to complete. Participants will be given instructions for each task and sat down at a computer to complete a block of familiarisation trials for each cognitive task (approx 5 minutes for each task) before measurement of baseline performance (20 minutes).

In the sensory part of the experiment, 80ml of the test drink will be delivered to the participant's mouth over the course of the 20 minute test period. The drink will be delivered using a programmable pump, controlled by the computer programme running the cognitive tasks. In the familiarisation session, still mineral water will be delivered through the pump to allow the participant to become accustomed to performing the tasks whilst consuming a test drink.

300ml of the test drink will be given to the participant after completion of the sensory part of the experiment (therefore a total of 380ml will be administered, the volume of a standard bottle of Lucozade Energy Original), and given a 20 minute break before repeating the two tasks a final time (measurement of postprandial effects).

Participants will be paid on completion of the fifth and final session.

The impact of four different treatment drinks will be investigated, one in each experimental test session. There will be two energy drink treatments and two placebo treatments, Evian still mineral water (which will be coloured orange, the same colour as the energy drinks) and no-treatment.

Time	Trial Type	Task	Duration (min)
~10.00		Participant arrives, reads information sheet and signs consent form.	1
10.00	Familiarisation	Choice RT task	5
10.05		Attentional Blink task.	5
10.10	Baseline	Choice RT task	7
10.17		Attentional Blink task	13
10.30	Sensory	Choice RT task	7
10.37		Attentional Blink task	13
10.50	Break	Participant receives drink	
10.50		Break	20
11.10	Postprandial	Choice RT task	7
11.17		Attentional Blink Task	13
11.30		End	91 (total)

Screening Questions

Please answer the following questions to assess your ability to take part in this study:

CRITERIA	Yes/No?
Do you suffer from diabetes?	
Is there any possibility that you are pregnant?	
Do you smoke?	
Do you suffer from any condition you feel may affect you ability to take part? (i.e. allergies to ingredients shown in the information sheet)	

CRITERIA	OK
No medication prior to testing on the trial day	
No Caffeine drink i.e. Tea, Coffee, Cola, Chocolate on the trial day	
No chocolate confectionery prior to testing on the trial day	
No alcohol prior to testing on the trial day	
No energy drinks prior to testing on the trial day	

Do you regularly consume caffeine in any form (energy drinks, coffee, tea, chocolate)?

_____

If so, how much would you say you consume in an average week?

_____

If all the information contained in the questionnaire is accurate to the best of your knowledge, please sign below:

Signed: _____

CONSENT FORM

Effect of drinks on cognitive function

Investigator: Chris Mason
Supervisor: Prof Stephen Jackson
School of Psychology, University of Nottingham

The participant should complete the whole of this sheet himself/herself. Please cross out as necessary

- Have you read and understood the participant information sheet YES/NO
- Have you had the opportunity to ask questions and discuss the study YES/NO
- Have all the questions been answered satisfactorily YES/NO
- Have you received enough information about the study YES/NO
- Do you understand that you are free to withdraw from the study:
  - at any time YES/NO
  - without having to give a reason YES/NO
- Do you agree to take part in the study YES/NO

Visit 1

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

Visit 2

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

Visit 3

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

Visit 4

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

Visit 5

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

ADVERSE EVENT RECORDING – CONFIDENTIAL

1. Participant number

2. Adverse event

Description (signs and symptoms)	Severity: 1:Mild 2:Moderate 3:Severe	Time of onset	Outcome: 1:Resolved 2:Improved 3:Unchanged 4:Worsened	Duration (if resolved)

Overall diagnosis (where possible)

Was this event life threatening for the participant?

Yes		No	

3. Treatment of Adverse event

(Please give times where relevant)	Yes	No	Date/Time
Participant withdrawn from study?			
Did symptoms resolve?			
Did the event require hospitalisation?			

Comments

4. Causality

	Almost certainly	Probably	Possibly	Unlikely	Not related
Could the participant's original condition or other illness account for the adverse event?					
Do you think the adverse event was related to the study?					

5. Comments

6. Action taken

7. Research associate

Name (print)	
Signature	
Date	

APPENDIX 5: EXAMPLE CASE REPORT FORM (AS USED IN STUDY OUTLINED IN CHAPTER 5).

Sensory & postprandial impact of energy drinks on cognitive function.

CASE REPORT FORM

Participant		
Number:		
Name:		
Age:		
Sex:		

Trial	Visit 1	Visit 2
Date		
Time		
Drink code		
Comments about trial/data		

Trial	Visit 3	Visit 4
Date		
Time		
Drink code		
Comments about trial/data		

Screening questions

Please answer the following questions to assess your ability to take part in this study:

CRITERIA	Yes/No?
Do you suffer from diabetes?	
Is there any possibility that you are pregnant?	
Do you smoke?	
Do you suffer from any condition you feel may affect you ability to take part?	

CRITERIA	OK
No medication prior to testing on the trial day?	
Nothing to eat or drink since midnight the previous night?	
No caffeine drink i.e. tea, coffee, cola, chocolate on the trial day?	
No alcohol prior to testing on the trial day?	
No energy drinks prior to testing on the trial day?	

Do you regularly consume caffeine in any form (energy drinks, coffee, tea, chocolate)?

_____

_____

If so, how much would you say you consume in an average week?

_____

_____

If all the information contained in the questionnaire is accurate to the best of your knowledge, please sign below:

Signed: _____

CONSENT FORM

Effect of drinks on cognitive function

Investigator: Chris Mason
Supervisor: Prof Stephen Jackson
School of Psychology, University of Nottingham

The participant should complete the whole of this sheet himself/herself. Please cross out as necessary

- Have you read and understood the participant information sheet YES/NO
- Have you had the opportunity to ask questions and discuss the study YES/NO
- Have all the questions been answered satisfactorily YES/NO
- Have you received enough information about the study YES/NO
- Do you understand that you are free to withdraw from the study:
    - at any time YES/NO
    - without having to give a reason YES/NO
- Do you agree to take part in the study YES/NO

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

ADVERSE EVENT RECORDING – CONFIDENTIAL

1. Participant number

2. Adverse event

Description (signs and symptoms)	Severity: 1:Mild 2:Moderate 3:Severe	Time of onset	Outcome: 1:Resolved 2:Improved 3:Unchanged 4:Worsened	Duration (if resolved)

Overall diagnosis (where possible)

Was this event life threatening for the participant?

Yes ☐ No ☐

3. Treatment of Adverse event

(Please give times where relevant)	Yes	No	Date/Time
Participant withdrawn from study?			
Did symptoms resolve?			
Did the event require hospitalisation?			

Comments

4. Causality

	Almost certainly	Probably	Possibly	Unlikely	Not related
Could the participant's original condition or other illness account for the adverse event?					
Do you think the adverse event was related to the study?					

5. Comments

6. Action taken

7. Research associate

Name (print)	
Signature	
Date	

APPENDIX 6: VOLUNTEER INFORMATION SHEET AND EXAMPLE CASE REPORT FORM (AS USED IN STUDY OUTLINED IN CHAPTER 6)

The University of Nottingham

S Sensory
S Science
C Centre

Title of Project: The impact of sensory properties of energy drinks on brain function.

Name of Investigator: Chris Mason, Joanne Hort

Volunteer Information Sheet

Invitation paragraph
You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. If you decide to take part you may keep this leaflet. Thank you for reading this.

What does the study involve?
We ask that you attend one session, which will last approximately one hour. The session will involve carrying out a computer based task whilst tasting sweet tasting samples.

The samples will be non-carbonated and will contain glucose and artificial sweeteners. You will be asked to consume no more than 100 mL of test samples.

Ingredients: glucose syrup, aspartame and acesulfame k.

All ingredients are food grade and the samples were prepared by Chris Mason. The specific task detail will be explained at the start of the session. Anybody who completes the session will be given £5 inconvenience allowance.

Do you have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?
You will be asked to arrive at 9, 10 or 11 am, and you should refrain from eating or drinking in the 2hrs prior to a session, and abstain from caffeinated food and drink on the morning of the session. No perfume/aftershave should be worn on the day of the session.

Screening questions

Please answer the following questions to assess your ability to take part in this study:

CRITERIA	Yes/No?
Do you suffer from diabetes?	
Is there any possibility that you are pregnant?	
Do you smoke?	
Do you suffer from any condition you feel may affect you ability to take part?	

CRITERIA	OK
No medication prior to testing on the trial day	
No Caffeine drink i.e. Tea, Coffee, Cola, Chocolate on the trial day	
No chocolate confectionery prior to testing on the trial day	
No alcohol prior to testing on the trial day	
No energy drinks prior to testing on the trial day	

Do you regularly consume caffeine in any form (energy drinks, coffee, tea, chocolate)?

_____

_____

If so, how much would you say you consume in an average week?

_____

_____

_____

If all the information contained in the questionnaire is accurate to the best of your knowledge, please sign below:

Signed: _____

CONSENT FORM

Effect of drinks on cognitive function

Investigator: Chris Mason
Supervisor: Prof Stephen Jackson
School of Psychology, University of Nottingham

The participant should complete the whole of this sheet himself/herself. Please cross out as necessary

- Have you read and understood the participant information sheet YES/NO
- Have you had the opportunity to ask questions and discuss the study YES/NO
- Have all the questions been answered satisfactorily YES/NO
- Have you received enough information about the study YES/NO
- Do you understand that you are free to withdraw from the study:
    - at any time YES/NO
    - without having to give a reason YES/NO
- Do you agree to take part in the study YES/NO

"This study has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

Signature of the Participant: Date:

I have explained the study to the above participant and he/she has agreed to take part.

Signature of researcher: Date:

ADVERSE EVENT RECORDING – CONFIDENTIAL

1.	**Participant number**				
2.	**Adverse event**				
	Description (signs and symptoms)	Severity: 1:Mild 2:Moderate 3:Severe	Time of onset	Outcome: 1:Resolved 2:Improved 3:Unchanged 4:Worsened	Duration (if resolved)
	Overall diagnosis (where possible)				
	Was this event life threatening for the participant?				
	Yes		No		
3.	**Treatment of Adverse event**				
	(Please give times where relevant)	Yes	No	Date/Time	
	Participant withdrawn from study?				
	Did symptoms resolve?				
	Did the event require hospitalisation?				
	Comments				
4.	**Causality**				
		Almost certainly	Probably	Possibly	Unlikely	Not related
	Could the participant's original condition or other illness account for the adverse event?					
	Do you think the adverse event was related to the study?					
5.	**Comments**				
6.	**Action taken**				
7.	**Research associate**				
	Name (print)				
	Signature				
	Date				