Neurocognitive and gluco-regulatory effects of *Panax ginseng*

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Author's declarations / contributions

This work has not been submitted for any other award. In all experimental chapters of this thesis the author had sole responsibility for the data collection, analysis, interpretation and writing. The author and supervisors worked together on the methodology making each experimental chapter.

The writing of this thesis is the sole work of the author.

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ABSTRACT

Complementary and Alternative Medicine (CAM) has long been used in the Far East to aid in the recovery and prevention of illness. Ginseng, an over-the-counter herbal product in the UK, is amongst these herbal CAMs currently available to the general public. Ginseng is renowned for its rejuvenating properties and its purported ability to aid cognitive function and well-being. Despite the huge global market for ginseng there is little in the way of human research, utilising standardised ginseng extracts and well controlled methodology to support many of these claims. Additionally, ginseng's underlying mechanisms of action are poorly understood. The present thesis documents 5 double-blind, placebo-controlled, cross-over trials investigating the effects of *Panax ginseng*, following acute and chronic ingestion, on behaviour, mood and indices of glucose regulation in young healthy volunteers. The results of the five studies making this thesis suggest that both acute and chronic dosing with *Panax ginseng* is capable of modulating mood and cognitive performance in healthy young volunteers. Chapters 2 and 3 also demonstrate, for the first time, *Panax ginseng's* ability to modulate blood glucose levels following a single acute dose in overnight fasted healthy volunteers. In chapters 2 and 3, significant reductions in blood glucose levels and concomitant improvements in mental arithmetic (working memory) performance were reported. Chapter 4 revealed for the first time *Panax ginseng's* positive effects on traditional measures of working memory, thus posing the suggestion that previous failures to report working memory effects (using traditional working memory tasks) may have been due to poor task selection. Chapter 5 revealed an unexpected superimposed relationship between chronic and acute ingestion of *Panax ginseng.* The pattern of results suggests that following chronic dosing, an acute dose can further modulate cognition and mood (suggestive of a psychological dependence). The final chapter documents a different profile of cognitive and mood effects following a non-standardised *Panax ginseng* extract, thus highlighting the need for caution when generalising results across ginseng types and beyond the specific parameters of the methodologies utilised in any given study. Methodological differences between studies may go some way in explaining the inconsistent data patterns reported between studies, research groups and ginseng extracts. These data further highlight the need for well-controlled studies utilising standardised ginseng extracts and the need for the integration of 'theory driven' research in order to fractionate any behavioural effect. Such methodologies will inevitably lead to greater consistency between behavioural studies, at least in the first instance within the restricted population of volunteers utilised in the present thesis.

CHAPTER 1. INTRODUCTION

1.1. Complementary and Alternative Medicine

Complementary and Alternative Medicine (CAM) has long been used in the Far East to aid in the recovery and prevention of illness. More recently the use of CAM has become an integral part of western society. For instance, a number of American studies employing probability samples have found that between 40-45% of the adult population have used CAM to deal with health problems over a twelve-month period (Eisenberg et al., 1998; Nahleh et al., 2003). In one of the most recent of these reports, Barnes et al (2004) suggested that this figure had risen to 62%. The estimate of CAM usage rises further, to as much as 100%, amongst those who suffer from chronic problems and life threatening diseases (e.g. Boon et al., 2004; Cherkin et al., 2002, Goldstein et al., 2005).

The self-medication and consumption of herbal products (arguably the fastest growing area of CAM) was documented as making up a significant proportion of the total consumption of medicines and other health products in the UK (6th Report, 'Complementary and Alternative Medicine', 2000). In line with this, Ernst and White (2000) estimated that herbal medicines constituted 34% of CAM usage in the UK and it was estimated that herbal retail reached $16.5 billion worldwide (Scimone, 1997; Scimone and Scimone, 1998).

Ginseng, an over-the-counter herbal product in the UK, is amongst these herbal CAMs currently available to the general public. Ginseng is renowned for its rejuvenating properties and its purported ability to aid cognitive function and well-being (see Kennedy and Scholey, 2003). Ginseng has recently been estimated to be the most frequently taken psychoactive herbal product in the US (Barnes et al., 2004).

Despite the huge global market for ginseng there is little in the way of human research, utilising standardised ginseng extracts and well controlled methodology to support many of these claims. Additionally, ginseng's underlying mechanisms of action are poorly understood.

The remainder of this introduction includes a review of the evidence, across a number of disciplines, pertaining to *Panax ginseng's* purported effects.

1.2 Ginseng

1.2.1 Background

The term 'ginseng' is often used as an umbrella term covering a number of different ginseng plants, ginseng extracts and commercial products. For instance, several family members of the plant *genus Panax* (Araliaceae family) are referred to as 'ginseng'. *Panax ginseng,* which is of Asian derivation (most notably Eastern China and Korea), is the most popularly used member; however, other members of the genus include *Panax quinquefolius* (American ginseng), *Panax notoginseng* (Chinese ginseng) and *Panax japonicus* (Japanese ginseng). Wild ginseng or ginseng tonics have been used traditionally, for several millennia, as a restorative or prophylactic agent in Chinese medicine (Bahrke and Morgan, 1994; 2000). The first recorded prescription of *Panax ginseng* as a medicinal agent appears in Shen-Neng Pharmacopoeia (AD 456-536) and during the Koryo Dynasty (AD 918-1392) ginseng was renowned as 'the best medicine with miraculous potency'. This 'general tonic' belief, along with physical performance enhancers, 'adaptogenic' and aphrodisiac properties of ginseng have survived to the present day. Additionally, ginseng (species of the genus Panax) products are reported to be the most popular self-administered psychoactive herbal products (Barnes et al., 2004) with many consumers taking it to aid 'memory loss' and 'absentmindedness' (see Kennedy and Scholey, 2003).

1.2.2 The active constituents of ginseng

Triterpenoid glycosides or saponins, also known as ginsenosides are thought to be the active constituents of ginseng. At least 30 individual examples have been

isolated but many only exist in minute amounts (Tachikawa et al., 1999). Other minor components include amino acids, peptides and minerals (Gillis, 1997). The sugar moieties found are glucose, maltose, fructose and saccharine (Gillis, 1997). The chemical structure of ginseng's sapogenins (aglycones) can be used to classify its ginsenosides into three groups: the protopanaxadiol (PD) group (e.g. Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, Rh_2, Rs_1); the protopanaxatriol (PT) group (e.g. Re, Rf, Rg_1, Rg_2, Rh_1); and the Oleanolic acid group (e.g. Ro) (Gillis, 1997). Previous studies have found that the ginsenoside content in ginseng can vary substantially. For example, differences in the ginsenoside content have been reported between species, and been shown to vary with age, preservation method, the session of harvest and the extraction method employed (Liberti and Der Manderosian, 1978; Phillipson and Anderson, 1984). Indeed, huge variations in the ginsenoside content of commercially available ginseng products have recently been reported (Cui et al., 1995; Russo, 2001). As ginsenosides have been implicated in the underlying mechanisms of ginseng's effects, it is of great concern that the ginsenoside levels themselves vary so greatly. The practical dilemmas brought about by these variations are obvious (see Kennedy et al., 2003; Scholey et al., 2004), however, the most widely used ginseng extract, both commercially and for research purposes is a concentrated aqueous extract of *Panax ginseng* contained in the marketed product Ginsana®. While this extract is standardised by rigorous extraction and manufacturing processes to contain an invariable 4% total ginsenosides (Soldati and Sticher 1980) it is still possible that there may be small variations in individual ginsenosides and differences in the ratio of PD : PT between samples.

1.2.3 Ginsenoside metabolism

Any compound that has been through a biochemical modification in living organisms and cells has been subjected to metabolism. Metabolism usually consists of a sequence of enzymatic steps, also called metabolic pathways. At each step, in this metabolic pathway both the end-product and the by-products are potentially 'active'. In order to gain an understanding, of a compound's biological mechanism, it is important to know what these active constituents are (the original and their degraded forms); the metabolic pathway taken and the bioavailability of these compounds (how much reaches systemic circulation). These issues are central to gaining an understanding of the biological mechanisms given the potentially huge number of active constituents that make up ginseng. Another important issue to consider is the route of administration. This last point should not be overlooked as studies that have utilised different administration methods (for example, oral vs. non-oral methods) and reported similar results (or different results) may be oversimplifying the mechanisms. For example, the active constituents of a compound in vitro (out side the body) may undergo several changes when inside the body (in vivo) as they come into contact with gastric juice or digestive and bacterial enzymes following oral ingestion, which may lead to different biological effect or even the same biological effects but by a different chemical.

It is believed that the ginsenosides are ginseng's active principles (see section 1.2.2 of this thesis). Indeed, in vitro studies have demonstrated the various pharmacological activities of these ginsenosides on numerous cells (Hasegawa, 2004). Additionally, a huge animal literature base has demonstrated a plethora of physiological effects of these individual ginsenosides following intravenous

absorption of these metabolites has been demonstrated (Hasegawa et al., 2002) (see Table 1).

1.2.4 Pharmacokinetics

While there is evidence of the pharmacological actions of these individual ginsenosides (see Kennedy and Scholey, 2003) and there is now an understanding of the degrading process, we must now consider the evidence for the absorption of either the intact ginsenosides or the intestinal metabolites. Odani et al, (1983) reported, in rats, that Rb_1 was absorbed rapidly from the upper digestive tract, reached peak serum and tissue concentrations at 30 minutes and was widely distributed throughout the body but was not found in the brain. Others have reported that Rb_1 could not be detected in the urine of rats when 50 mg was administered orally, although compound –K (the degraded intestinal metabolite of Rb_1) was detected (Park et al., 2005). Conversely, Rb_1 and compound –K were identified in the plasma and urine of humans up to 24 hours following a single oral 700 mg dose (Tawab et al., 2003). Cui et al, (1997) demonstrated that about 1.2% of an orally ingested dose of protopanaxatriol ginsenosides (3 mg) and considerably smaller amounts of protopanaxadiol ginsenosides, not exceeding 0.2% of the 7 mg administered dose could be recovered in human urine. However, neither the individual ginsenoside nor their metabolites could be identified. Tawab et al (2003) took blood and urine samples over a 24hr period following an orally administered single dose of 700 mg of *Panax ginseng* (G115) to human volunteers. Results demonstrated that the same hydrolysis products, which are not originally present in the extract ingested, were identified in plasma and urine. They suggested that M8 and M11 (two intestinal metabolites of the

protopanaxatriol group) might reach systemic circulation. In addition, they report the presence of M1 in plasma and urine. However, they also report the presence of the whole protopanaxadiol original ginsenoside Rb_1 in the plasma and urine of one subject. These were detectable up to 24 hours after dosing. Cui et al, (1996) reported the uptake of ginsenosides in humans as metabolites were found in the urine of athletes that had consumed ginseng within the last 10 days. Additionally, Hasegawa et al (2004) identified EM1 (esterified M1) in the liver and EM4 (esterified M4) in numerous organs in the body.

These studies suggest that both the intact original ginsenosides and the degraded intestinal metabolites may be acting upon the tissues in the body. Indeed, Kobashi et al, (1992; 1997) proposed the concept that plant glycoside (ginseng's ginsenosides are an example) are metabolised to their active form by intestinal bacterial.

Table 1. The major ginsenosides of the protopanaxadiol and protopanaxatriol groups and their metabolic pathways.

	Major ginsenoside	**Metabolic Pathway**
Protopanaxadiol type	Rb1	Rb1 [M10, M5 or M9,] M1
	Rb2	Rb2 [M6, M2] M1
	Rc	Rc [M7, M3] M1
	Rd	Rd [M5 or M9] M1
Protopanaxatriol type	Re	Re [Rg1, M11 or M8] M4
	Rg1	Rg1 [M11 or M8] M4

Table was adapted from Hasegawa , H (2004) Proof of the mysterious efficacy of ginseng basic and clinical trials: metabolic activation of ginsenoside:deglycosylatio by intestinal bacteria and esterification with fatty acid

injection (see Kennedy and Scholey, 2003). Although these studies provide evidence of the pharmacological action of individual ginsenosides it is less clear whether they are absorbed into the blood stream in their original intact form, after coming into contact with gastric juice or digestive and bacterial enzymes following oral ingestion. The implications for understanding ginseng's mechanisms are obvious. For instance, any degradation in the chemical structure of ginsenosides may impact upon their pharmacological action. Thus, this may have implications for the conclusions that can be drawn from studies that have utilised non-feeding methods of administration as a tool to understanding ginseng's biological and behavioural affects.

It has been reported that gastric juices appear not to decompose ginsenosides, with the exception of slight oxygenation (Karikura et al., 1991). However, the bioavailability (the amount that reaches systemic blood flow) of these intact ginsenosides had been shown to be very low (Takino, 1994: Xu et al., 2003). More recently it has been demonstrated that intestinal bacteria cleave the oligosaccharides connected to the C-3 or C-6 hydroxyl group of the aglycone stepwise from the terminal sugar (Hasegawa et al., 1996; Bae et al., 2002). These reactions result in the individual ginsenosides following two main metabolic pathways. Ginsenosides of the protopanaxadiol-type (PD) are gradually hydrolyzed to the intestinal metabolite known as M1 (M1 is then gradually hydrolyzed to M12). Ginsenosides of the protopanaxatriol type (PT) are gradually hydrolyzed to the intestinal metabolite know as M4 (See Table 1 for a summary of the metabolism of ginsenosides). These intestinal bacterial metabolites have also been shown to undergo esterification with fatty acids and subsequent

1.2.5 Mechanisms of action

In vitro and *in vivo* studies have reported that whole ginseng, ginseng extracts or its individual ginsenosides can exert numerous and often opposing effects (for example; Joo et al., 2005) on many physiological parameters. These physiological effects may have some relevance for understanding the mechanisms underlying ginseng's behavioural effects (see Kennedy and Scholey, 2003).

To further complicate matters it has recently been suggested that the actions of the individual ginsenosides may be explained by their biotransformation to their hydrolyzed active intestinal metabolites (Bae et al., 2002; Lee et al., 2005). Indeed, *in vitro* and *in vivo* studies have demonstrated that the intestinal bacterial metabolites of the Protopanaxadiol type may have many of the same effects as the ginsenosides, for example, anti-inflammatory activity (Bae et al., 2002), anti-cancer effects (Kobashi et al., 1997; Suda et al., 2000; Wakabayashi et al., 1998; Bae et al., 2002) and regulation of the Hypothalamic pituitary adrenal (HPA) axis (Kim et al., 2003). Similarly, intestinal metabolites of the Protopanaxatriol type have been shown to have anti-cancer effects (Wakabayashi et al., 1997) and interact with neurotransmitters (Tachikawa et al., 2003). Additionally, the fatty acid ester of intestinal bacterial metabolites of the Protopanaxadiol type have been shown to have anti-cancer effects and immunomodulatory properties (Hasegawa et al., 2000; 2000b).

Despite a growing understanding of the physiological effects of whole ginseng, ginseng extracts, individual ginsenosides and intestinal metabolites it is still far from clear as to mechanisms underlying the behavioural effects. This uncertainty is partly due to the inconsistent (and lack of) behavioural data obtained in the animal and human literature (see Kennedy and Scholey 2003).

As the underlying mechanisms are not known, what follows is an attempt to outline some of the physiological effects that may directly or indirectly impact upon behavioural performance.

1.2.5.1 Effects on the cardiovascular system

The cardiovascular /circulatory system transports food, hormones, metabolic wastes, and gases (oxygen, carbon dioxide) to and from all cells. The cardiovascular/circulatory system is made of two systems: the pulmonary and systemic systems. The pulmonary system carries blood from the heart (right side) to the lungs (where oxygenation and carbon-dioxide removal occur) then from the lungs back to the heart (left side). Systemic circulation, driven by the left side of the heart, carries blood to the rest of the body. Food products enter the system from the digestive organs into the portal vein. The liver and kidneys remove waste products. All systems ultimately return to the right side of the heart via the inferior and superior vena cavae. The lymphatic system is a specialised component of the circulatory system. This system consists of a moving fluid (lymph/interstitial fluid); vessels (lymphatics); lymph nodes and organs (bone marrow, liver, spleen, and thymus). The body is able to eliminate the products of cellular breakdown and bacterial invasion through the flow of blood in and out of arteries, and into the veins, and through the lymph nodes and into the lymph.

Any compound that can modulate the cardiovascular system (a system responsible for the efficient and constant delivery of all nutrients and antibodies to all living cells around the body) could in all practicality have secondary effects on human behaviour and mood.

Modulation of the cardiovascular /circulatory system by ginseng has been demonstrated in the animal research. For example, whole ginseng and individual ginsenosides have been shown to reduce and increase blood pressure (Woods *et al.*, 1964; Lei and Chiou, 1986), increase and decrease total peripheral resistance and cause vasodilatation and bradycardia (Lee *et al.*, 1981). However, recent human studies have revealed a neutral acute effect (Stavro et al., 2005) and no effect of American ginseng following a 12 week chronic administration regimen on blood pressure in hypotensive individuals (Stavro et al., 2006). Additionally, a systematic review of ginseng's effects on the cardiovascular system concluded that '*current evidence does not support the use of ginseng to treat cardiovascular risk factors. Some studies suggest a small reduction in blood pressure*' (Buettner, 2005).

Despite recent literature suggesting negligible modulation of the cardiovascular system there is evidence to suggest that ginseng and its individual ginsenosides have cardio-protective properties. For example, ginseng was shown to protect the endothelial function in aortic rings (Gillis, 1997) and partially protect against adriamycin-induced heart failures in rats following just 30 days of ginseng treatment (cumulative dose 150 g/kg) (You et al, 2005). Additionally, Fu et al (2006) demonstrated that saponins from the stem and leaf of *Panax notoginseng* alleviated the degree and area of myocardial ischemia and increased the myocardial blood flow of the ischemia section in anaesthetised dogs. Similarly, pre-treatment with an American ginseng berry extract has been shown to protect cardiomyocytes from injury induced by exogenous or endogenous oxidants and reduced cell death up to 63% (Shao et al., 2004). The individual ginsenoside Re has also been shown to promote angiogenesis, which led to enhanced tissue

regeneration in the pericardium of bovine species (Huang et at., 2005). Ohashi et al., (2005) reported that Rb_1 blocked homocysteine (Hcy) inhibition of endothelial cell proliferation (both animal and human) and also ameliorated the Hcy increase in superoxide anion production – suggesting that Rb_1 is an effective antagonist for Hcy, which is an independent risk factor for cardiovascular disease. This was further supported by Zhou et al, (2005) demonstrating that when Rb_1 is administered in conjunction with Hcy, it resulted in the blocking of Hcy endothelium dependent relaxation and the blocking of superoxide anion production in the coronary artery of a pig. Rb_1 also led to the protection against Hcy down regulation of endothelial nitric oxide synthase, mRNA and protein level. It has been suggested that these cardio-protective properties of ginseng may be mediated by the release of Nitric Oxide (NO) (see Gillis,1997)

1.2.5.2 Neuro-protective properties

Injury, infection, cancer, infarction, toxins and inflammation can cause accidental cell death (known as necrosis). Unlike in apoptosis (programmed cell death), cells that die by necrosis may release harmful chemicals that damage other cells. If the original cell damage was severe enough it could lead to substantial accidental cell damage/death. Ginseng has been shown to have some neuro-protective properties. It seems plausible to suggest that a compound that can protect against the accidental death of cells may lead to benefits in behavioural performance. Both *in vitro* and *in vivo* studies have demonstrated ginseng's neuro-protective properties. For example, following ischemia in rodents, ginseng led to the protection of hippocampal CA_1 neurons (Wen et al., 1996) reductions of infarct area (Fu et al., 2006) and the preservation of local cerebral glucose utilisation (Fu et al., 2006).

Individual ginsenosides have also be shown to protect against excitotoxic-induced cell damage but failed to protect against oxidative stress-induced cell damage (Bao et al, 2005). Individual ginsenosides have also been shown to protect against irradiation induced cell death (Lee et al., 2006). The mechanism underlying ginseng's apparent neuro-protective properties is not fully understood at present. However, some potential mechanism could include a defence against the over production of NO, glutamate and kainic acid-induced excitotoxicity and the protection against free radical-mediated lipid peroxidation and a blockage of calcium over influx into neurons (see Kennedy and Scholey 2003).

1.2.5.3 Ginseng's regulation of the Hypothalamic-Pituitary-Adrenal axis

The hypothalamic-pituitary-adrenal axis (HPA axis) refers to a complex homeostatic relationship between the hypothalamus, the pituitary gland and the adrenal gland. This axis controls the reactions to stress and regulates various body processes including digestion, the immune system, mood and sexuality, and energy usage. Dys-regulation of stress hormones (e.g. cortisol) has been related to cognitive dysfunction in the young, elderly and dementia patients (for review, see Magri *et al.*, 2006). Therefore, any compound that can impact upon the HPA axis (i.e. modulate the biological response to a stressor) could in all practicality have an impact upon behavioural performance. In line with this, an *in vitro* study demonstrated that a saponin-rich fraction of *Panax ginseng*, as well as its metabolites, inhibited Acetylcholine (ACh)-stimulated catecholamine secretion from bovine adrenal chromaffin cells (Tachikawa and Kudo, 2004). Additionally, total saponins, and the individual ginsenoside Rg_3 and Rb_1 attenuated the rise in Putrescine (PUT) levels in immobilisation-stressed gerbil mice (Lee et al., 2006).

Additionally, both increases and decreases in corticosterone levels (which is a steroid hormone of the corticosteroid type produced by the adrenal glands. Such hormones are involved in a range of systems including stress response) have also been reported following ginseng ingestion (Filaretov et al., 1998; Hiai et al., 1983) and others have demonstrated that the stress-induced increase in corticosterone levels can be inhibited by total saponins (e.g. Kim et al., 1998b). However, whilst total root saponins and Rb_1 have been shown to inhibit the rise in stress induced corticosterone levels, in rats, the same dosage/ kilogram produced an opposite effect in mice (Kim et al 1998c). The apparent biphasic effects of ginseng are not well understood at present, however, one suggestion is that catalytic enzymes are inhibited by the individual ginsenosides which result in the up-regulation of both positive and negative feedback in stress hormone receptors, resulting in an increased existing stress response in either direction (see Kennedy and Scholey 2003).

1.2.5.4 Ginseng's effects on neurotransmission

Neurotransmitters are integral to the efficient communication between neurons. Any modulation of the synthesis, release, breakdown, uptake or actions of these chemical messengers may have a direct influence on behaviour and mood. Numerous studies have identified cholinergic properties of whole ginseng extracts and individual ginsenosides. For example, *Panax ginseng* extracts and *Panax quinquefolium* extracts have been reported to improve scopolamine induced memory impairments in rodents, with the latter extract increasing choline uptake in synaptosomal preparations (Bao et al., 2005). Additionally, *Panax ginseng* has been shown to exhibit an affinity for both nicotinic and muscarinic

receptors in human brain cerebral cortex membranes (Lewis et al., 1999). Wang et al., (2006) demonstrated that beta-amyloid memory impairments were reversed by 5 days pre-treatment with total ginseng saponins (80 mg/kg/day) and that this pre-treatment completely protected the animal against beta-amyloid-induced reduction of hippocampal Acetylcholine (ACh) release. Conversely, *Panax ginseng* was shown to inhibit ACh-stimulated release of catecholamine *in vitro* (Tachikawa and Kudo, 2004).

With regards to cholinergic modulation by single ginsenosides it has been reported that Rg_2 has a direct interaction with nicotinic receptor subtypes (Sala et al., 2002; Choi et al., 2002), whilst, Rb_1 modulated ACh release and uptake, and also up-regulated the number of uptake sites in the hippocampus, and the cortex (Benish, 1992). Additionally, increased acetyltransferase levels (in the rodent brain) have been reported following both Rg_1 and Rg_2 (Zhang et al 1990; Salim et al., 1997).

Ginseng and its constituent parts have also been reported to modulate a number of monoamines, for example, increased levels of dopamine, noradrenalin and serotonin were reported in the cortex following 50 mg/kg ginseng administration (Petkov, 1997). Others have reported both facilitated and inhibited monoamine metabolism dependent on the period of dosing (100 mg/kg for 2 or 7 weeks), the monoamine under investigation and the discrete brain area involved (Itoh et al., 1989). The total saponin content of ginseng has been shown to inhibit serotonin receptor subtypes (Min et al., 2003) and modulate dopamine activity at both pre-synaptic and post-synaptic dopamine receptors and block behavioural sensitisation induced by psychostimulants, for example, nicotine (Kim et al., 2005). See Kennedy and Scholey , 2003. It has been suggested that these effects

are mediated by the inhibition of drug-related dopamine release by the action of ginseng total saponins on presynaptic dopamine terminals (Shim et al., 2000) and postsynaptically by the binding to DA D(2) receptors (Kim et al 2005).

1.2.5.5 Nitric Oxide synthesis

Nitric oxide (NO) is one of only a few gaseous signaling molecules known. It is synthesized from arginine and oxygen by various nitric oxide synthase (NOS) enzymes. NO is involved and integral to many biological functions in the body, for example, it is a secondary messenger in blood vessel dilatation and neurotransmission. The enhanced synthesis of NO has been documented following ginseng administration in a number of cells and tissues throughout the body that may have direct relevance to behavioural performance (see Kennedy and Scholey 2003). This enhanced synthesis of NO has previously, and repeatedly, been suggested to partially explain many of ginseng's physiological effects (Gillis, 1997). A number of authors have suggested that the enhanced synthesis of NO is the mechanism for ginseng's antioxidant and cardiovascular effects (Gillis, 1997; Kang et al., 2006; 2006b), its cardio (Maffei-Facino et al., 1999), and neuro–protective properties (Kim et al., 1998d), its gluco-regulatory effects (Vuksan et al., 2000a,b), its immune defensive function (Friedl et al., 2001; Kim et al., 2005) and its anti-inflammatory effect (Joo et al., 2005; Park et al., 2005). It has been speculated that ginseng may exert its effects on behaviour and mood through the same pathway i.e. the enhanced synthesis of NO (see Kennedy and Scholey, 2003).

1.2.5.6 Ginseng's gluco-regulatory effects

The human brain is completely dependent upon the efficient and constant delivery of glucose (the body's main source of 'fuel') and oxygen through its rich capillary network (for review see Messier, 2004). Fluctuations in blood glucose levels have been demonstrated to impact upon behavioural performance (for review see Messier, 2004). It is therefore tempting to speculate that any change in blood glucose levels may partially explain any acute behavioural change by ginseng.

The long term and acute hypoglycaemic effects of a number of different types of ginseng have been demonstrated both in rodents (Xie *et al.*, 2002; Ohnishi *et al.*, 1996; Bae and lee, 2004; Xie et al., 2005) and humans (Sotaniemi *et al.*, 1995; Tetsutani *et al.*, 2000; Vuksan *et al.*, 2000; Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001). As an example, Xie et al (2005) demonstrated that both oral (150 and 300 mg/kg) and intraperitoneal injection (100 and 200 mg/kg) of total ginsenosides resulted in a significant reduction in fasting blood glucose levels after 12 days of treatment and also improved glucose tolerance in diabetic mice. With regards to human participants, a reduction in fasting blood glucose levels and glycated haemoglobin (the condensation of haemoglobin with hexoses gives rise to five glycated haemoglobin derivatives, designated as: HbA1a1, HbA1a2, HbA1b, HbA1c, and HbA1d. The level of glycated haemoglobin can be used as an indication of glucose regulation over a 4 – 6 week period) were reported following 8 weeks administration of 100 mg and 200 mg/day of an unspecified extract in 18 participants with type 2 Diabetes Mellitus (Sotaniemi *et al.*, 1995). Similarly, Tetsutani (2000) reported that 24 months of treatment with 3 - 4.5g/day of Korean red *Panax ginseng* decreased HbA_{1c} (an index of average

blood glucose levels over approximately the previous month) in 34 type 2 diabetics compared with controls. With regards *Panax quinquefolius*, a decrease in fasted blood glucose and HbA_{1c}, has been reported in 24 type II diabetic patients following 8 weeks administration of 1g of a proprietary ginseng extract, taken 40 minutes before each meal (Vuksan *et al.*, 2000).

Of particular relevance to the present thesis, the acute hypoglycaemic effects of *Panax quinquefolius* have also been demonstrated in a series of randomised, placebo-controlled studies. Reductions in blood glucose levels, following a 25g glucose challenge, have been reported during a 120 minute oral glucose tolerance test in both diabetic patients who had ingested 3g, 6g and 9g (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b), and healthy participants administered 1g, 2g and 3g of *P. quinquefolius* (Vuksan *et al.*, 2000a; Vuksan *et al.*,2001).

The acute human studies have direct relevance to the forthcoming experimental chapters (chapter 2, 3, 4, 5 and 6) so will be outlined in some detail: In the first of these studies Vuksan et al (2000) investigated the glucose response to the ingestion of 3g, 6g and 9g of American ginseng (AG) and placebo ingested 120 minutes before, 80 minutes before, 40 minutes before, and simultaneously (0 minutes) with a 25g glucose drink. Blood glucose levels were measured at baseline (before treatment) at 0 minutes (before the glucose drink) and thereafter at 15, 30, 45, 60, and 90 minutes post drink. Results revealed that blood glucose concentrations did not differ from fasting blood glucose levels for any dose/time administration of AG, when measured at time point 0 (i.e. before the ingestion of the glucose drink). Conversely, a post-prandial hypoglycaemic effect was revealed at 30, 45 and 60 minutes following 3g, 6g, and 9g and at 90 minutes following 3g and 9g of AG compared to placebo. There were also significant

reductions in the area under the curve (AUC) for all doses. Although such results look impressive, a closer inspection of the statistics reveals some problems. For example, each dose was analysed independently of its pre-prandial administration time. Therefore the reported results are the average (pooled) post-prandial glucose response to a 25g glucose drink for each dose (3g, 6g, and 9g of AG) administered at 120, 80, 40 minutes before and simultaneously with the 25g glucose drink. Similarly, the authors suggest that there is no effect of pre-prandial ginseng administration time on post-prandial glucose levels. However, again closer inspection of the results reveals some problems. The authors analysed this factor independently of AG dose. Therefore, the reported results are once again the average (pooled) post-prandial response to a 25g glucose drink of all AG's doses (including placebo) for each pre-prandial administration time. The authors fail to mention if there was a significant (or non-significant) dose x time interaction. Although the results can be used to suggest AG's glucose modulating properties, there are problems with such analyses and interpretation (especially when the interaction results are missing). For example, it is impossible to determine which time point is most effective for each dose. This question needs to be answered as the results, as they stand, give no indication to the practical application in the use of AG (unless the authors are trying to suggest that administration time is unimportant).

In the second of these acute studies Vuksan et al (2000b) investigated the glucose response to a 25g glucose drink, in healthy and diabetic (type 2) volunteers. AG (3g) was ingested 40minutes before and simultaneously with a 25g glucose drink. Blood glucose levels were measured at baseline (before ginseng treatment) at 0 minutes (before the glucose drink) and thereafter at 15, 30, 45, 60, and 90 minutes

post drink. For healthy participants the result indicated that AG, when ingested simultaneously with the glucose drink, did not affect glucose levels post drink. However, when the ginseng was administered 40 minutes prior to the glucose drink the results revealed significant reductions in glucose levels at 45 and 60 minutes post drink as compared to placebo. Similarly, with regards to those with diabetes the results revealed significant reduction in glucose levels at 30 and 45 minutes post drink, compared to placebo, when AG was ingested 40 minutes prior to the glucose drink. However, there were significant reductions in blood glucose levels at 45 and 60 minutes post drink, as compared with placebo, when AG was ingested simultaneously with the glucose drink. AUC was also significantly reduced when treatment was taken before and simultaneously with the 25g glucose drink.

In the third of these acute studies Vuksan et al (2000c) investigated the effect of different AG doses (3g, 6g and 9g) ingested at different times (120, 80, and 40 minutes before, and simultaneously with a 25g glucose drink) on blood glucose in 10 diabetic (type 2) volunteers. The authors failed to report if at time point 0 (i.e. before the ingestion of the glucose drink) there were any differences in blood glucose levels for any dose/time modality of AG. However the results suggested that 3g, 6g and 9g of AG could significantly lower blood glucose levels at 30, 45 and 120 minutes post drink and additionally at 60 and 90 minutes post drink following 3g. The authors report no difference in area under the curve for any dose. The reported results also suggest that there is no effect of AG administration time (i.e. whether ginseng was administered 120, 80, or 40 minutes before or simultaneously with the glucose drink) on post drink glucose levels or area under the curve. These results look impressive and could be taken

as firm evidence of AG's gluco-regulatory efficacy. However, a closer inspection of the results and chosen statistical approach reveals similar problems to those discussed above. Firstly, again no interaction term is reported as to whether any interaction effects were found (significant or non-significant). Secondly, the authors once again analyse the effect of dose independent of administration time but fail to mention if the analysis for administration time was independent of dose. The results would be more valuable, interpretable and have clear significant practical applications if the results were presented separately for each dose administered at specific times.

In the fourth of these acute studies, Vuksan et al (2001) investigated the effect of three different doses of AG (1g, 2g and 3g) ingested at different times (40, 20, and 10 minutes before and simultaneously with a 25g glucose drink) on blood glucose levels in 12 healthy participants. There were no differences in glycaemic effect at time point 0 (i.e. before the ingestion of the glucose drink) for any dose administered at any time. However, the reported results did suggest that all doses of AG (1g, 2g and 3g) significantly reduced blood glucose levels at 60 minutes post drink. Additionally, 1g and 2g significantly reduced blood glucose levels 90 and 45 minutes post drink, respectively. Area under the curve was also significantly reduced for all three doses. With regards to the effect of administration time the authors report a significant reduction in blood glucose levels at 30 and 45 minutes post drink if AG was ingested 40 minutes before the glucose drink as compared to an administration time of less than 40 minutes (i.e. 20, 10 and 0 minutes). Additionally, there were significant reductions in blood glucose levels at 60 and 90 minutes post drink when AG is ingested 40 minutes before as compared to the administration time points of 10 and 0 minutes or 20

and 0 minutes, respectively. However, again there are problems with the chosen statistical approach utilised. For example, the analysis used for dose was independent of administration time. Similarly, the analysis utilised for administration time was independent of dose. Results would be more informative if the effect of each dose, administered at each time point was compared to that of its equivalent placebo.

In a fifth acute study Sievenpiper et al (2002) investigated the effect of 6g of AG administered 40 minutes prior to and simultaneously with a 75g glucose drink on blood glucose levels in 12 healthy volunteers. The AG was of the same type and source but was from a different batch to that used in the four earlier studies. The results demonstrated no effect on blood glucose levels post drink. The authors speculate that the failure to find an effect may be explained by the disparity in ginsenoside profiles found between this batch and the previously utilised AG batches. The authors speculate that the later ineffective batch had a reduced ginsenoside profile as compared to the previously studied batch of AG (e.g. Vuksan 2000; 2000b; 2000c; 2001).

1.2.5.6.1 Summary of ginseng's gluco-regulatory effect in humans

The five studies outlined above have reported significant reductions in blood glucose levels, following a 25g glucose challenge, during a 120 minute oral glucose tolerance test in both healthy and diabetic patients who had ingested 3g, 6g and 9g (Vuksan *et al.*, 2000; Vuksan *et al.*, 2000b; Vuksan *et al.*,2000c), and healthy participants administered 1g, 2g and 3g of *P. quinquefolius* (Vuksan *et al.*, 2000b; Vuksan *et al.*,2001) but no effect following a 75g glucose challenge (Sievenpiper et al., 2002). However, caution should be taken when interpreting

these studies as the authors used pooled (over dose and time) data (e.g. Vuksan *et al.*, 2000; Vuksan *et al.*, 2000c; Vuksan *et al.*, 2001). Results would have been better represented if the data documented the post-prandial response to the glucose challenge, of each dose administered at each different pre-prandial time point as compared to its placebo control.

1.2.5.7 Ginseng's glycaemic mechanisms of action

The mechanisms responsible for ginseng's glycaemic effects are not clear at present; however, the animal data may support three possible mechanisms that could potentially account for the modulation in blood glucose levels.

1.2.5.7.1 *Modulation of glucose transport*

Glucose, galactose, fructose (collectively referred to as hexose molecules) are simple carbohydrate and the basic molecules that serve as fuel for all living cells. These hexose molecules cannot diffuse across cellular membranes and therefore require specific transporter proteins to facilitate entry into and exit from the cells. Two groups of transporters have been identified. The first transports hexose down a concentration gradient (known as GLUT1, GLUT2, GLUT3, GLUT4 and GLUT5) allowing facilitated diffusion from the blood stream into the cell (and vice-versa). The second group transport hexose against a concentration gradient (known as SGLUT1), which is important in the absorption of hexose molecules from the small intestines and in the kidney. Given that glucose is essential for the survival of the cell (and indeed any metabolic function) and has been implicated in memory formation itself, it seems logical to suggest that any compound (e.g. ginseng) that can influence the transport of glucose or impact upon the glucose

transport system may result in secondary behavioural effects, and may also partially explain the mechanism for ginseng's glycaemic effects. Lai et al (2006) showed Rh_2 led to reductions in plasma glucose levels and an over expression of GLUT4 in rats. Similarly, saponin fractions and individual ginsenosides were shown to increase 2-Deoxy-D-[2-^{3}H] glucose (2-DG) uptake in isolated sheep erythrocytes by GLUT 1 in a dose dependent manner (Hasegawa et al., 1993). Additionally, a water extract of Chinese ginseng administered to normal and hypoglycaemic mice caused an up-regulation of GLUT2 in the liver (Ohnishi et al., 1996). *Panax ginseng* (G115) has also been shown to increase 2-DG uptake in a dose dependent manner in rabbit brain (Samira et al., 1985). Wang et al., (2003) provided evidence that ginseng increases aerobic glycolysis through enhancing the activities of citrate synthetase, malate dehydrogenase, succinate dehydrogenase, cytochrome oxidase (4 rate-limiting enzymes in aerobic glycolysis). They suggest that this modulation must be through secondary messenger cAMP. However, the net result of the enhanced activities of these enzymes would be the speeded utilisation of glucose, which will results in a reduction in plasma glucose levels. Conversely, there have been reports of an opposing glycaemic effect of ginseng and specific ginsenosides. Hong et al (Hong *et al.*, 2000) reported that a water extract of Asian ginseng significantly inhibited insulin stimulated 2-DG uptake. Additionally, Kimura et al (1981) showed that an extract containing Rb and Rc increased glycaemia at 100mg/kg.

1.2.5.7.2 Modulation of glucose disposal

In the gut, the process of digestion reduces carbohydrates to glucose. Glucose is then taken up by active transport by intestinal cells and passed into the blood by

facilitated diffusion. Once in the blood, glucose can be metabolised in cells as an energy source (known as glycolysis) or can be converted into glycogen (this process is especially true in the liver and muscles) and stored for later use (known as glycogenesis). When glucose levels fall (i.e. during a fast) and there is no exogenous glucose available, glycogen is converted back to glucose, therefore raising circulating glucose levels, thus, allowing free glucose to enter into glycolysis. Additionally, liver cells can also synthesise glucose from other substances (e.g. Pyruvate) if needed. The path that glucose takes (glycolysis or glycogenesis) depends on a number of parameters (e.g. current energy need, presence of hormones etc.) but is ultimately dependent on a series of very complex reactions catalysed by enzymes, some of which are rate limiting. Therefore, if ginseng can affect the reactions responsible for glucose homeostasis then this may underlie ginseng's glycaemic effect. In line with this 200 mg/ml of *Panax ginseng* (G115) increased the glycolytic enzyme, phosphohexose isomerase, and several isozymes of pyruvate lactate shunt enzyme, lactate dehydrogenase, although no change in intracellular or extracellular glucose concentrations or glucose uptake accompanied these changes (Shia et al., 1982). Chung et al (1993) reported that 50 mg/kg of an aqueous *Panax ginseng* root body and rootlets administered for 28 days decreased the activity of the rate limiting gluconeogenic enzyme glucose- 6 – phosphatase (G6Pase) in liver preparations in diabetic mice by 46% and 20%. The individual ginsenoside Rb_2 increased the activity of the rate limiting glycolytic enzymes phosphofructokinase and pyruvate kinase, while decreasing the activity of the rate limiting gluconeogenic enzyme G6Pase in liver (Yokozawa et al., 1984) and increased the

activity of glucokinase while decreasing the activity of G6Pase in the rats liver (Yokozawa et al., 1985).

1.2.5.7.3 Modulation of insulin secretion

Insulin is a hormone that is essential for glucose homeostasis (the other hormone being glucagon). The main effect of insulin is to lower blood glucose levels by stimulating glycogenesis (converting glucose to glycogen) in the liver and muscles and inhibiting gluconeogenesis (conversion of glycogen to glucose) in the liver (the opposite being true for glucagon). Insulin is also important in glucose transport as the glucose transporter GLUT4 is activated by insulin. Therefore, if ginseng can modulate insulin secretion this could underlie ginseng's glycaemic mechanism. In line with this ginseng has been shown to modulate insulin secretion. For example, intraperitoneal injection of whole ginseng (10 – 50 mg/kg) increased insulin secretion in alloxan diabetic mice (Waki et al., 1982). Total ginsenosides from *Panax ginseng* increased glucose and non-glucose stimulated insulin release from pancreatic islets in a dose dependent manner at doses between 0.10 – 0.25 mg/ml (Li et al., 1987). Liu et al., (2005) provided evidence that the oral ingestion of a *Panax ginseng* root improved insulin sensitivity that resulted in the modulation (i.e. lowering) of circulating glucose levels. The single ginsenoside Rg_1 has been shown to increase insulin binding with a 2 fold increase in the total number of binding sites in rat liver and brain 3 days and 5 day after 3 consecutive days of intraperitoneal injections at 10 mg/kg (Tchilian et al., 1991). Lee et al (2006) demonstrated that 1 mg/kg of Rh_2 administered by intravenous injection to healthy Wistar rats decreased plasma glucose in a dose dependent manner as well as increasing plasma insulin levels.

The authors conclude that the gluco-regulatory mechanism for Rh_2 is via increased insulin secretion as a result of the release of ACh from nerve terminal that then stimulate muscarinic M_3 receptors in pancreatic cells. However, others have suggested that Rh_2 lowers plasma glucose based on an increase in beta-endorphin secretion that activates opioid μu-receptors thereby resulting in an increased expression of GLUT 4 in diabetic rats (Lai et al., 2006). Glucose transport and insulin secretion may well be influenced by increased nitric oxide (NO) production (see Kennedy and Scholey 2003).

1.3 Glucose and human behaviour

Circulating blood glucose levels have been implicated as an important factor in human behaviour (for review see Messier, 2004). As a result it is tempting to speculate that ginseng's acute behavioural effects may be partially explained by its acute modulation of circulating blood glucose levels. It has been established that fluctuations in the level of circulating blood glucose can modulate cognitive performance (for review see Messier, 2004). For example, participants in a hypoglycaemic state (low blood glucose levels) have shown behavioural impairment in a number of domains (Holmes *et al.*, 1984; Gold *et al.*, 1985). Similarly, supra-hypoglycaemic states have also led to impairments (De Feo *et al.*, 1988; Tylor and Rachman, 1988). Conversely, acute improvements in behavioural performance have been reported following a glucose drink. These improvements have been reported across a variety of tasks, assessing a number of different behavioural processes (e.g. Messier *et al.*, 2003; Donohoe and Benton, 2000; Kennedy and Scholey, 2000; Scholey *et al.*, 2001; Sünram-Lea *et al.*, 2001; 2002; 2002b; Benton 1990; Martin and Benton 1999; Foster *et al.*, 1998). One

study that may have particular relevance to the present thesis is Kennedy and Scholey (2000). In this study a positive association was reported between the rate at which a person's blood glucose levels returned back to baseline, following an initial peak, and the level of cognitive performance. This relationship was particularly evident during periods of higher 'cognitive demand' (those tasks that are more effortful as reported subjectively and measured objectively). For example, it was reported that a 25g glucose drink improved performance on a more difficult mental arithmetic task (but not on an easier version), with the rate at which blood glucose levels returned to baseline positively correlating with behavioural performance on that task (Kennedy and Scholey, 2000). Additionally compared with placebo, a glucose drink improved performance during intense mental processing, which, in turn, led to a measurable reduction in blood glucose levels (Scholey *et al.*, 2001).

One explanation for such findings is that increased uptake of blood glucose results in better performance and therefore a measurable reduction in circulating blood glucose level. Indeed, Stone et al (2005) demonstrated, utilising fMRI, significantly greater activation (therefore uptake of glucose) of specific brain regions during a verbal encoding task following glucose administration as compared to a placebo control. One interpretation of this result could be that of a greater utilisation of an energy source during encoding; however, in this study there was no difference in behavioural (memory) performance. It follows that any intervention which modulates glucose transport may also affect cognitive performance. There is some support for this notion from the finding that insulin administration can improve memory in sufferers from Alzheimer's disease (Watson and Craft, 2004). However, insulin's role in glucose utilisation is only

one possible mechanism that could account for the improved memory performance. Insulin has also been shown to modulate neurotransmitters, affect membrane potentials, neuronal physiology and long-term potentiation (see Craft, 2005).

The behavioural effects of ginseng

1.4.1 Animal evidence

1.4.1.1 Ginseng as an anti-stress and anxiolytic agent

Stress, especially chronic stress, has been associated with disease pathology and accelerated aging (Solomon et al., 1984). Ginseng, with its adaptogenic properties, has long been considered as an anti-stress and anxiolytic agent. A number of studies have demonstrated that crude ginseng extracts and individual ginsenosides can protect against or attenuate the concomitant behavioural and physiological indicators of stress and anxiety in animals. For example, a crude *Panax ginseng* extract (100mg/kg, p.o) administered 1hour before an unpredictable foot shock everyday for 21 days attenuated the stress-induced cognitive deficits and physiological dysfunction and inhibited the significant increase in plasma corticosterone levels induced by chronic stress, in rats (Bhattacharya and Muruganandam, 2003). Similarly, treatment with Rb_1 (2.5, 5 and 10mg/kg, i.p) prior to repeated hanging stress procedure reduced the stress-induced insult to the reproduction-endocrine system (Lian et al., 1998) and reduced the neuronal damage and cognitive impairments following 60 consecutive days of stress (Lian et al., 2001). Additionally, Rg_1 was shown to reduce the severity of the stress induced behavioural disorders commonly associated with social isolation (Yoshimura et al., 1998). Churchill et al, (2002) reported that Rb_1 (0, 0.25, 2.5 or 5 mg/kg, i.p) produced a change in separation

distress (both negative and positive) depending on the dose and environmental condition. Other examples include the suppression of psychological and foot shock stress-induced anti-nociception in mice (Nguyen et al., 1995); an attenuation of the disruption of pentobarbital induced sleep produced by 30 min of psychological stress in mice, with no change in sleep duration in unstressed mice (Nguyen et al., 1996); protection against psychological stress-induced gastric lesions in mice (Huong et al., 1998); and an inhibition of intra-cerebro-ventricular injection stress-induced increases in plasma corticosterone levels in mice (Kim et al., 1998b).

With regards to ginseng's anxiolytic properties, Park et al, (2005) reported the anxiolytic effects of a red ginseng extract (100mg/kg) and sun ginseng extract (heat processed ginseng) (50mg/kg) in mice using the elevated plus-maze model. Similarly, Car et al, (2006) demonstrated that red ginseng powder (300, 600, 1200 mg/kg, o.p); the saponin fraction (50, 100, and 200 mg/kg, i.p); and Rb_1 had potent anxiolytic effects in rats.

A common physiological response of such psychological stress, in animals, is often an enhancement of lipid peroxidation activity. Yobimoto et al. (2000) demonstrated the protection against oxidative damage to brain membranes as a result of a stressful experience following Vietnamese ginsengs. Another common physiological consequence is that various forms of stress result in the alteration of polyamine metabolism (Hayashi et al 2004; Sohn et al., 2002). Lee et al (2006) demonstrated that the pre-treatment with total ginseng saponins led to the homeostasis of polyamine metabolism, which is essential for cellular growth, proliferation, regeneration and differentiation (Rao et al., 1998), during immobilization stress.

1.4.1.2 Ginseng as an anti-fatigue agent

The administration of ginseng or its active components has been shown to attenuate the physical indicators of fatigue and modulate the concomitant physiological responses. For example, a single administration of ginseng was shown to significantly increase endurance time to exhaustion on a treadmill running test, with a concomitant increase in the basal level of ACTH and corticosteroids (Filaretov et al., 1988). Similarly, Min et al (2003) reported that treatment with red ginseng (10, 25, 50, 100mh/kg, i.p, once per day for 5 days) increased the time to exhaustion for treadmill running in a dose-dependent manner and inhibited exercise-induced increases in 5 HT synthesis. However, Martinez and Staba (1984) found no increase in endurance times and no effects of ginseng extract on plasma lactic acid, glucagon, insulin, or liver glycogen levels in rested or exercised rats. Furthermore, Jung et al (2004) reported that the oral administration of a *Panax ginseng* tonic (500mg/kg/d in a volume of 250μL for 4 weeks) actually led to significantly decreased forced swimming time compared with a control group, despite a concomitant rise in plasma glucose levels in the ginseng group (however, the authors suggest that this results is an artifact of the shorter swim duration for the ginseng group) and no difference in liver and muscle glycogen stores. However, Voces et al (2004) demonstrated that 3 months treatment with *Panax ginseng* (G115) at 100mg/kg per day protected against a 20% reduction in exercise-induced mitochondrial function (following 80min exercise per day) and also protected against the exercise-induced lipid peroxidation (unfortunately performance data is not reported). Several authors suggest that this variability in results may possibly be attributed either to the quality of the extract of ginseng used and/or to the dose investigated, and a

possible habituation either to the effects of ginseng or exercise (see Kennedy and Scholey 2003).

1.4.1.3 Ginseng as a cognition enhancer

Various memory-impairment models have been used to evaluate how ginseng and its active ingredients affect behavioural performance. Consequently, the animal literature is replete with claims that ginseng can effectively improve performance in healthy animals and attenuate learning deficits due to brain damage and ageing. As an example, Nishijo et al (2004) reported the administration of red ginseng (100mg/kg/d, o.p) for four days significantly ameliorated spatial learning deficits in young rats with hippocampal lesions and reversed aged associated deficits in spatial learning, in aged animals. Interestingly, Kurimoto et al (2004) reported that treatment with the non-saponin fraction of red ginseng (50mg/kg/d, o.p) also led to improvements in spatial learning in aged rats. Additionally, Bao et al (2005) reported that individual ginsenosides orally administered to mice for four days significantly ameliorated ethanol and scopolamine induced memory impairments, utilising passive avoidance. Moreover, they reported the latency period for ginsenoside treated mice was 1.2 times longer than that of controls (no amnesia) group.

Chronic administration of ginseng extracts or some of its fractions have been reported to improve learning and memory in several hippocampal / amygdala-dependent behavioural tasks (e.g. Nishijo *et al.*, 2004; Kurimoto *et al.*, 2004; Zhong *et al.*, 1998; Yoshimura *et al.*, 1998). The mechanisms underlying this effect are unknown, but Qiao et al (2005) provided evidence that ginseng's efficacy is due to its ability to enhance the survival of newly generated neurons in the hippocampus. In line with this, are reports of total ginsenosides (25 – 100

mg/kg, i.p) and ginsenoside Rg_1 (10 and 100nmol/L, i.c.v.) improving indices (basic synaptic transmission and amplitude) of long term potentiation (LTP) in the hippocampus, however, Rg_1 was shown to exert an inhibitory effect on these indices (Zhang *et al.*, 2000; Wang and Zhang, 2001). Additionally, in gerbils, ischemia-induced hippocampal CA_1 neuronal death was shown to be rescued following 7 days pre-treatment with red ginseng powder, crude ginsenosides, and ginsenoside Rg_1. Additionally, the concomitant memory deficits that would be associated with such cell death were ameliorated (Wen *et al.*, 1996).

With regards to the individual ginsenoside, Rg_1 (25 – 100 mg/kg) has been shown to ameliorate the anisodine, cyclohexiamide and alcohol-induced memory impairments in rats and restore memory deficits in elderly animals (see Chang *et al.*, 2005). Churchill et al (2002) reported that a low dose of Rb_1 (0,25mg/kg, i.p) improved performance of a visual discrimination task, however, higher doses (2.5, 5 mg/kg, i.p) resulted in impaired performance when tested 72hrs later. Ginseng-related improvements in the learning and memory of normal and young rats tend to be both dose dependent and sensitive to the nature of the task (See Kennedy and Scholey 2003). The above literature suggests that ginseng may have the potential to benefit cognitive performance.

1.4.2 Human evidence

1.4.2.1 Ginseng's physical properties

The physical or 'ergogenic' properties of ginseng have been investigated extensively (see Kennedy and Scholey 2003). Although these properties may not be directly relevant to behavioural performance, it seems plausible to suggest that if ginseng can modulate this domain there may well be concomitant changes to

psychological domains. Over the last few decades the findings in this domain have been very inconsistent which has rendered interpretation very difficult (see Kennedy and Scholey, 2003). For example, Ziemba (2002) summarises ginseng's ergogenic effects from 28 human studies published between 1986 and 2002 and notes that 16 fail to report any beneficial effects of ginseng administration. However, these studies have used vastly different methodologies and investigated different ginseng extracts. Such fundamental differences between studies have led Bahrke and Morgan (1994; 2000) to conclude that the lack of robust ergogenic effect of ginseng treatment may result from inadequate research design. For example, studies have often investigated different ginseng extracts (hindering interpretation and generalization as the actives in each extract are fundamentally different); others have measured inappropriate variables; while others have had inadequate sample sizes and often failed to use double blind and placebo controlled methodologies. Indeed, when an attempt is made to interpret the pattern of results for only one standardised ginseng extract (i.e. *Panax ginseng* G115 – the most popular ginseng extract used both commercially and in research and is rigorously standardised to include an invariable 4% total ginsenoside content), it is still difficult to come to any firm conclusions. For instance, of the 28 studies outlined by Ziemba (2002), 5 specifically state the use of *Panax ginseng* (G115) and a further 2 state using a standardised 4% *Panax ginseng* extract (which we may presume is G115). The other 21 studies are comprised of a mix of American ginseng, Siberian ginseng, Chinese/Russian ginseng, and unspecified *Panax ginseng* extracts (that may be G115). In the studies that have used *Panax ginseng* (G115) the results show that a 200mg dose administered daily for 9 weeks improves aerobic capacity, reduces lactate levels, reduces heart

rates in young healthy elite athletes (Forgo and Kirchdofer et al., 1981; 1982; 1983) and increases VO_2 (Forgo and Schimertet al., 1985). However, other studies have reported that G115 has no effects in young females (19 – 26 yrs) who ingested the same 200mg dose daily for 8weeks on VO_2, heart rate, lactate levels, sub and max-work (Engels et al., 1995; 1996) or following the ingestion of 400mg per day for 8weeks on supramaximal exercise performance and short-term recovery (Engels et al., 2001). Still others have reported that G115 led to a loss of fitness in young (24 – 36yrs) female triathletes after 11 – 20 weeks of ingesting 400mg per day (Van Shedael et al., 1993). The evidence concerning the effects of G115 still appears to be mixed even when strict methodologies are followed as suggested by Bahrke and Morgan (1994; 2000) (except for Forgo and Kirchdofer 1981; 1982, where no controls were implemented). However, there are still differences between studies in samples, dosing and outcome parameters, which makes interpretation difficult. When consideration is taken of the most recent reports the picture is no clearer. Kim et al (2005) reported in a placebo-controlled trial that the ingestion of 6g of *Panax ginseng* per day for 8 weeks significantly increased exercise duration until exhaustion by 1.5 minutes and attenuated the concomitant increase in malondialdehyde (the end product of lipid peroxidation), which facilitated recovery. Similarly, Liang et al (2005) reported that an extract of *Panax notoginseng* (1,350 mg per day for 30 days) improved endurance time to exhaustion, and lowered mean blood pressure and VO_2 during endurance exercise in young (20 – 35 yrs) untrained adults. However, Cheng et al (2005) reported that 4 weeks pre-treatment with American ginseng (1,200 mg / d) to male college students did not enhance aerobic work capacity in an exhaustive running exercise. Additionally, Goulet and Dionne (2005) reviewed 5 studies (all

of which utilised rigorous research protocols) that investigated the efficacy of Siberian ginseng (SE) as an ergogenic aid and concluded that SE shows no benefit on cardio-respiratory fitness, fat metabolism or endurance performance. Concluding that 'SE supplementation (1000 to 1200 mg/d for 1 to 6 weeks) offers no advantage during exercise ranging in duration from 6 to 120 minutes' (it should be noted that SE does not contain ginsenosides).

It appears that interpretation of the ergogenic benefits of ginseng is still hindered by methodological differences between studies.

1.4.2.2 Ginseng as a mood enhancer

Experimentally induced stress *per se* has not been the subject of any research. However, a number of studies have considered the more generalised notion of 'quality of life' or 'well-being.' *Panax ginseng's* effect on these parameters has been investigated in a number of placebo controlled trials administered alone (Wiklund et al., 1999; Sotaniemi et al., 1995; Ellis and Reddy 2002; Cardinal and Engles, 2001) and in conjunction with vitamins and minerals (Wiklund et al., 1994; Neri et al., 1995; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000). Ginseng's effects have been evaluated using doses ranging from 80 to 400 mg in patient (Wiklund et al., 1994, Sotaniemi et al., 1995, Neri et al., 1995) and healthy populations of various ages and stress levels (Wiklund et al., 1994; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000; Ellis and Reddy 2002; Cardinal and Engles, 2001). Study duration has spanned from 2 to 9 months (See Coleman et al., 2003 for review). Improvements in the measures pertaining to 'quality of life' or 'well-being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996;

Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations have been demonstrated, although findings of this nature are by no means unequivocal (see Kennedy and Scholey 2003). Even when considering studies that have utilised 'pure' *Panax ginseng* supplements, there is still an equivocal pattern. For example, improvements have been reported following 16 weeks administration of ginseng to 394 symptomatic postmenopausal women in several subscales of the Psychological General Well Being Index (Wiklund et al., 1999); additionally, improvements have been reported following two doses of ginseng (100 and 200 mg per day for 8 weeks) administered to 36 non-insulin dependent diabetics on self-ratings of mood, vigour, and well-being (Sotaniemi et al., 1995). Conversely, Cardinal and Engels (2001) reported no significant differences on the Positive Affect–Negative Affect Scale (PANAS) or Profile of Mood States (POMS) 8-weeks following 200 mg G115 or 400 mg G115 ingested daily as compared to placebo, in a cohort of 83 healthy young adults (however, where ginseng has been effective in the above mentioned studies there may have been underlying negative mood states associated with the medical conditions that are not apparent in the healthy population).

In relation to non-panax species, Cicero et al., (2004) investigated the effects of Siberian ginseng (non-panax species) on quality of life in older adults. In this placebo controlled trial 20 elderly (>65 years) hypertensive volunteers were administered either 300mg per day or placebo for 8 weeks. Results showed positive results after 4 weeks, however these effects attenuated with continued use (i.e. at the 8 week testing session).

Additionally, a retrospective study (ginseng species and type not documented) conducted by Cui et al (2006) evaluated the associations of ginseng use with

quality of life in a cohort of 1,455 breast cancer patients between 1996 – 1998 and through 2002. The results revealed that ginseng use after cancer diagnosis, particularly current use, was positively associated with quality of life scores, with the strongest effect in the psychological and social well-being domains. Additionally, quality of life improved as cumulative ginseng use increased.

1.4.2.3 Ginseng as a cognitive enhancer

Despite ginseng's purported ability to modulate behavioural performance and the abundance of experimental studies (animal and human) suggesting a plethora of physiological effects that could, in all practicality, results in behavioural change (directly or indirectly), it still remains the fact that studies of this nature are few and empirical evidence pertaining to the behavioural modulation by ginseng is weak. What follows is a summary of those studies that have attempted to document ginseng's effects on human behaviour. For ease and clarity these studies will be summarised in two sections. The first section will deal with the evidence relating to chronic use of ginseng. The second will outline those studies that have investigated the acute effects of ginseng.

1.4.2.3.1 Evidence from chronic studies

With regards to the evidence pertaining to the behavioural modulation following prolonged (chronic) administration of ginseng only three studies have attempted to investigate this directly.

The first; D'Angelo et al (1986) reported the effects, in healthy young volunteers, of 12 weeks administration of *Panax ginseng* (200 mg G115 per day) on cognitive and psychomotor performance measures. Utilising a between subject,

placebo controlled, double blind, balanced, experimental design, thirty-two participants (mean age 21.8 years) were randomly allocated to a ginseng or placebo condition. Cognitive and psychomotor assessments were conducted at a pre-treatment baseline and then during the 12th week of treatment. Within (change from baseline) and between (treatment vs. placebo) groups comparisons were made. There were both within and between group effects on a mental arithmetic task. It was reported that ginseng led to significantly more correct responses per second, as compared to the placebo group. Additionally, ginseng led to significant within group improvements as compared to initial baseline. The only other significant effect was revealed for a cancellation task, with significant within group improvements in the ginseng group only. However, at 12 weeks, no between group difference (placebo vs. ginseng) was revealed on this task, which may suggest a learning effect.

The second study was that of Soerensen and Sonne (1996) who implemented an independent measures, double blind, randomised design to investigate the effects of 8 to 9 weeks of ginseng ingestion (400 mg standardised Gerimax ginseng extract; Dansak Droge A/S, Denmark) on cognitive performance in 112 healthy middle aged participants (mean age 51 yrs). Participants completed a 60 to 90 minute cognitive test battery at a pre-treatment baseline and then again following 8 to 9 weeks of ginseng ingestion (battery included psychomotor tasks, attention and concentration tests, learning and memory tests and the central executive Wisconsin card sorting task). The results revealed that ginseng led to significantly faster performance in only the most rapid auditive reaction times test (10^{th} percentile). This tasks involved measuring the time taken to press a key in response to a high, clear sound coming at irregular intervals. Accuracy in

performing the Wisconsin card sort test was also reported to be superior following ginseng, however, there was no baseline completion of this task, as there are no parallel versions, therefore this result is confounded.

A third empirical investigation was that of Labadorf et al (2004) who examined the mood, memory and attentional effects following 14 days of ginseng treatment (200mg G115) in 18 healthy young volunteers. An independent measures (8 volunteers receiving ginseng and 10 volunteers receiving placebo), placebo controlled, double blind, randomised design was utilised. Volunteers were required to ingest 2 x 100 mg capsules (G115) or placebo every morning for a 3-week period. Cognitive assessment was conducted using the CDR computerised assessment battery (CDR limited, UK) at a pre-dose baseline, and thereafter at 1hr, 3hrs and 6hrs post treatment on days 1, 7 and 14. Results revealed that ginseng led to better performance on all four factors: speed of attention; quality of attention; speed of memory; quality of memory; derived from the CDR research battery. It should be noted that Labadorf et al (2004) was presented as a poster at the annual BPS conference. Although such abstracts are still peer-reviewed caution should be taken as the exact methodologies and statistics utilised are not scrutinised to the same extent, as they would be for peer-reviewed journal publication.

A further investigation, involving analysis of data from a small cohort of self-reported users of ginseng products drawn from a large prospective study concluded that ginseng has no beneficial effects to cognition. Persson et al, (2004) analysed the results/responses from 3500 self-reporting participants, from a database of Swedish dwelling adults. Participants were in *The Betula prospective cohort study: memory, health and aging.* Selection was based on two

questions: "(i) Do you regularly use any of the herbal products (Ginkgo biloba, Ginseng)? You must have used the product for two years. (ii) If you use a herbal product, but if you have not yet used it regularly for a period of two years, mark the product (Ginkgo biloba, Ginseng) and specify for how long a time you have used it". Of the 3500 participants, 40 reported they were currently using, and had been using Ginkgo biloba regularly. Of these, 19 reported using Ginkgo biloba for two years or more, and the mean intake time for the remaining 21 participants was 5.3 months. Eighty-six participants reported that they were currently using, and had been using ginseng regularly. Of these, 51 had been using ginseng for more than two years, and the mean intake time for the remaining 35 participants was 5.6 months. The study implemented two control groups: The first consisted of participants who used, neither, ginkgo or ginseng but did use nutritional supplements. The second consisted of participants who did not use ginkgo, ginseng or nutritional supplements. Episodic and semantic memory was assessed using eight memory tests. The results revealed no significant difference between ginseng and control groups or ginkgo and control groups. The Persson study has been criticised on a number of methodological issues that could account for the lack of effect. For example: (1) no attempt was made to assess the level or frequency of dose, (2) there was a huge variability in the period of herbal use, and (3) there was no pre-treatment cognitive performance data reported or even available (for critical discussion see: Scholey et al., 2004).

1.4.2.3.1.1 Summary of the empirical evidence – chronic ingestion

Three experimental studies and one further prospective study have investigated the behavioural effects of chronic ginseng ingestion in humans. These studies

have revealed improved speed of performing a mental arithmetic task in young healthy participants who received 200 mg (G115) per day for 12 week (D' Angelo et al, 1986), faster reaction times on the most rapid auditory reaction time task, following 8-9 weeks of a standardised Gerimax ginseng extract (400 mg per day), in a healthy middle age cohort (Soerensen and Sonne, 1996), and global memory and attention improvements, following 200 mg (G115) per day for 14 days in young healthy volunteers (Labadorf et al., 2004). A further investigation, involving analysis of data from a small cohort of self-reported users of ginseng products drawn from a large prospective study found no effect on episodic or semantic memory (Persson et al., 2004).

1.4.2.3.1.2 Conclusions – chronic evidence

No conclusive interpretations can be elicited from the above-mentioned chronic studies as they have investigated different cohorts (i.e. healthy young and middle aged), administered different ginseng extracts (G115 or Gerimax) and doses (200 mg and 400 mg), for different durations (2 to 12 weeks) and implemented different assessment tools designed to assess different aspects/domains of cognitive functioning. The literature would benefit greatly from the use of a standardised ginseng extract, standardised cognitive assessments tools and administration protocols.

1.4.2.3.2 Evidence from acute studies

Despite the behavioural benefit associated with chronic ginseng use being somewhat debatable there is however a series of recent acute experimental trials that have implemented the same placebo-controlled, double-blind, balanced,

crossover design and utilised an identical computerised assessment battery to investigate the acute behavioural effects. These studies have demonstrated some consistent patterns and are allowing interpretations and conclusions to be reached regarding ginseng's acute behavioural effects.

In the first of these studies Kennedy et al (2001a) investigated the acute changes in cognitive performance and mood following single doses of *Panax ginseng* (G115) in 20 healthy young volunteers (mean age 21years) utilising a repeated measures, placebo controlled, double blind, balanced, randomised, cross over design. Participants ingested 200 mg, 400 mg, and 600 mg of ginseng, on separate days (each testing day was separated by a 7-day washout period). On each testing day participants completed a tailored version of the Cognitive Drug Research (CDR) computerised assessment battery at a pre-dose baseline and thereafter at 1hr, 2.5hrs, 4hrs, and 6hrs post-dose. The CDR computerised assessment battery is a collection of nine individual tasks (see Kennedy et al 2001a). Each task is computerised and the participant responds by simply pressing either a 'yes' or 'no' button located on an external button box. The outcome for each task is reported (reaction time and accuracy). However, each task has been shown to load (factor analysis) on 4 primary factors (speed of attention; accuracy of attention; speed of memory; accuracy of memory) and 2 secondary domains (working memory; secondary memory). Results were reported for the four "primary cognitive outcome measures" (quality of memory, speed of memory, speed of attention, and accuracy of attention), the two "secondary cognitive outcome measures" (working memory and secondary memory) and finally for all individual tasks. With regards to the four primary outcome measures the results revealed that 200 mg (G115), as compared with

placebo, led to a significant slowing in the 'speed of memory' 4hrs post-dose and also significant slowing in 'speed of attention' 4hrs and 6hrs post-dose. However, the same 200 mg dose led to improvements in the 'accuracy of attention' 6hrs post-dose. The 400 mg dose led to significant improvements in the 'quality of memory' at 1hr, 2.5hrs, 4hrs, and 6hrs post-dose, in comparison to placebo. The 600 mg dose led to significant improvements, as compared to placebo, in 'quality of memory' at 2.5hrs post-dose, however, the same dose led to a significant slowing in the 'speed of attention' at 4hrs and 6hrs post-dose. For the 'secondary outcome measures' results revealed that there was no significant effects of any dose, at any post-dose testing session, in comparison to placebo, on 'working memory'. However, 200 mg (G115) led to significant improvements in 'secondary memory' at 4hrs post-dose. Similarly, 400 mg and 600 mg led to significant improvements in 'secondary memory' performance at 1hr, 2.5hrs and 4hrs 6hrs (400 mg only) post-dose. With regards to the individual tasks that load the 'secondary outcome factors' the results revealed no effect on any dose on those tasks that load on 'working memory'. However, when examining the individual tasks that load on "secondary memory" (i.e. Immediate Word Recall, Delayed Word Recall, Delayed Word Recognition, Delayed Picture Recognition), the results revealed that all doses improved performance of the four individual tasks, as compared to placebo. The effect was most pronounced for the 'Immediate Word Recall' task, with a significant greater number of words recalled, as compared to placebo, at 1hr, 2.5hrs, 4hrs, and 6hrs post-dose following the 400 mg (G115) dose. Similarly, 600 mg was associated with a significant greater number of recalled words at 1hr, 2.5hrs and 6hrs post-dose. There was also a single significant improvement 4hrs post-dose following 200

mg. A consistent pattern also emerged on the 'Delayed Word Recognition' task with significant improvements revealed following both 400 mg and 600 mg (G115) at 1hr (400 mg only), 2.5hrs and 6hrs post-dose. On the 'Delayed Picture Recognition' 200 mg and 400 mg led to significantly improved performance at 1hr post-dose. Whereas a greater number of words were recalled in the 'Delayed Word Recall' task following 400 mg at 2.5hrs post-dose. When the individual tasks (i.e. simple reaction time, choice reaction time and digit vigilance reaction time) that load on the 'Speed of Attention' factor are examined in more detail it is apparent that 400 mg and 600 mg (G115) led to a significant slowing in 'simple reaction time' 4hrs (600 mg only) and 6hrs post-dose, while 200 mg led to significant slowing of 'choice reaction time' at 1hr, 4hrs and 6hrs post-dose. Additionally, both, 200 mg and 600 mg led to significant slowing in 'Digit Vigilance' at 4hrs and 6hrs post-dose. Inspection of the individual tasks that load on the "Accuracy of Attention" factor, results revealed that both 200 mg and 600 mg led to significant poorer accuracy in performing 'digit vigilance' at 2.5hrs post-dose testing session. In contrast, 400mg resulted in improved accuracy of performing the same task at 1hr and 4hrs post-dose, as compared to placebo. With regards to subjective self-reported mood, both, 200 mg and 400 mg (G115) were associated with significant reductions in 'alertness' (as measured by the bond-Lader, 1974) 6hrs post-dose.

In a second study Kennedy et al (2002) investigated the mood and cognitive effects of single doses of *Ginkgo biloba, Panax ginseng* and a ginkgo / ginseng combination in 20 (mean age 21.2 years) young, healthy volunteers utilising a repeated measures, placebo controlled, double blind, randomised, crossover trial. Participants received 4 treatments: placebo, 360 mg of ginkgo, 400 mg ginseng,

and 960mg of the ginkgo / on separate testing days separated by a 7 day washout period. Behavioural and mood assessment comprised a tailored version of the CDR computerised test battery, two serial subtraction mental arithmetic tasks and the Bond-Lader visual analogue mood scale. Assessments were made at a pre-dose baseline and thereafter at 1hr, 2.5hrs, 4hrs and 6hrs post-dose. Results were reported for the 'primary' and 'secondary' outcome measures and for the individual tasks that loads these factors. Accuracy and speed of performing the serial subtraction mental arithmetic tasks was also reported, as was participant's subjective, self-reported mood. Results revealed significant improvements in 'quality of memory' for all three active doses, in comparison to placebo. These improvements were restricted to single post-dose time point improvement at 4hrs and 6hrs following ginseng and ginkgo treatments respectively. However, following the combination treatment performance was significantly improved at 1hr, 2.5hrs and 4hrs post-dose. When 'quality of memory' is broken down into its component parts: 'working memory' and 'secondary memory', the results revealed that all three active treatments improved 'secondary memory' but had no affect on 'working memory'. Improvements were reported, in 'secondary memory'; following ginkgo at 1hr and 6hrs post-dose; following ginseng at 4hrs and 6hrs post-dose; and following the combination at 1hr and 2.5hrs post-dose. Through inspection of the individual tasks that load 'secondary memory' the results revealed that it is the performance on the Immediate and Delayed Word Recall tasks that was improved by all three treatments. Results also revealed 'speed of memory' was significantly speeded, following ginseng treatment, as compared to placebo, at the 4hrs post-dose. Inspection of the individual tasks that load 'speed of memory', results revealed significant speeded performance,

following the ginseng treatment, as compared to placebo, on spatial memory performance and word recognition performance at 2.5hrs and 4 hrs post-dose (word recognition only). With regards to serial subtraction performance, results revealed that the combination led to significant speeded performance of the serial three's task 6hrs post-dose whereas ginkgo led to significant improvements in accuracy 4hrs post-dose, as compared to placebo. Similarly, ginkgo and the combination treatment led to speeded performance of the Serial Seven's task 4hrs and 6hrs post-dose (combination only) whereas, ginkgo and combination treatment led to improvements in accuracy 6hrs post-dose testing. In relation to self-report subjective mood it was revealed that participants rated themselves as becoming progressively more alert following ginkgo at 1hr, 2.5hrs, 4hrs and 6hrs post-dose. Participants also rated themselves as more content following both ginkgo and the combination at 1hr, 2.5hrs (combination only), 4hrs and 6hrs post-dose.

In a third study Scholey and Kennedy (2002) reported the results of three separate placebo controlled, double blind balanced cross over trials investigating the effect of *Ginkgo biloba* (study 1), *Panax ginseng* (study 2) and their combination (study 3) on the performance of Serial Three and Serial Seven subtraction tasks. Twenty healthy young volunteers participated in each study. Participants were assessed at a pre-dose baseline and thereafter at 1hr, 2.5hrs, 4hrs and 6hrs post-dose (It should be noted that the subtraction tasks were part of a larger, 35 minute computerised assessment battery and were the last tasks to be completed in the battery). Results concerning study 1 revealed that all three active doses of *Ginkgo biloba* (120 mg, 240 mg and 360 mg) led to significantly speeded performance of the Serial Three's task at 4hrs and 6hrs (240 mg only) post-dose. In study 2 it was

shown that 200 mg of *Panax ginseng* led to a significant slowing in the performance of the Serial Seven's task at 1hr, 2.5hrs and 6hrs post-dose. However, at the 4hrs post-dose 200 mg was associated with a significant improvement in accuracy. Similarly, 400 mg of *Panax ginseng* was associated with significant improvements in accuracy of performing the serial seven's task, as compared to placebo, at 4hrs and 6hrs post-dose. In study 3 it was reported that 640 mg of a ginkgo/ginseng combination (comprising 240 mg ginkgo and 400 mg ginseng) led to significant speeded performance of the Serial Three's task at the 1hr, 2.5hrs, 4hrs and 6hrs post-dose. Similarly, 320mg (comprised of 120mg ginkgo and 200mg ginseng) led to a significant speeded performance at 4hrs post-dose. The most striking results were found when considering the performance of the Serial Seven's task. 320 mg of the combination led to a significant speeded performance, as compared to placebo, at 1hr, 2.5hr, 4hr and 6hrs post-dose and significant improved accuracy at 2.5hrs and 6hrs post-dose. Similarly, 640 mg led to significant speeded performance at 4hrs post-dose and significant improvements in accuracy at 2.5hrs and 6hrs post-dose whereas 960mg led to significant improved accuracy at 2.5hrs and 6hrs post-dose.

In a fourth study Kennedy et al (2001b) investigated the acute changes in mood and cognitive performance of 3 *Ginkgo biloba / Panax ginseng* combination treatments and placebo, in 20 healthy young volunteers (mean age 20.6 years). Utilising a repeated measures, placebo controlled, double blind, balanced, cross over design participants received 320mg (120 mg ginkgo / 200 mg ginseng), 640mg (240 mg ginkgo / 400 mg ginseng) and 960 mg (360 mg ginkgo / 600mg ginseng) of the ginkgo / ginseng combination and placebo in a counterbalanced order on four testing days separated by a 7 day washout period. Participants

completed a tailored version of the CDR computerised battery at a pre-dose baseline and thereafter at 1hr, 2.5hrs, 4hrs and 6hrs hours post-dose. Results revealed that 960 mg led to significant improvements in 'quality of memory' at 1hr and 6hrs post dose, as compared to placebo. When 'quality of memory' is broken down into its component parts: 'working memory' and 'secondary memory' results revealed that there were no significant effects, of any treatment, in comparison to placebo, on 'working memory'. However, 'secondary memory" performance was improved following 960 mg at 1hr, 4hrs and 6hrs post-dose. With regards to attentional performance, the results revealed that 320 mg and 640 mg led to significantly slowed 'speed of attention', in comparison to placebo, at 4hrs and 6hrs (320 mg treatment only) post-dose. Mood was not affected, at any post-dose time point, by any treatment.

In a fifth study Kennedy et al (2003) investigated electroencephalograph (EEG) effects of single doses of both *Ginkgo biloba* (360mg GK501) and *Panax ginseng* (200mg G115) in 15 (mean age 26.6) healthy young volunteers. Utilising a double-blind, placebo-controlled, balanced, crossover design, participants were assessed on three separate occasions 4hr post-dose. Results revealed that ginseng led to a significant shortening of the P300 latency component of auditory evoked potential. Additionally, both ginseng and ginkgo led to significant reductions in the frontal 'eyes closed' theta and beta activity, with additional reductions in the alpha waveband for ginseng. Despite the modulation in EEG there was no relationship between this and any behavioural measure.

In a sixth study Sünram-Lea et al (2004) assessed the acute mood and cognitive effects of single doses of *Panax ginseng* (400 mg) in 30 healthy, young (mean age 20years) volunteers. Utilising a placebo controlled, double blind, balanced

cross over design participants received either 400 mg *Panax ginseng* (G115) or placebo. Participants completed the CDR computerised assessment battery and Bond Lader visual analogue mood scale at a pre-dose baseline and then at 90 minutes post-dose. Results revealed that 400 mg ginseng, as compared to placebo, led to significant improvements in 'speed of attention' 90 minutes post-dose. When considering the individual tasks that load the 'speed of attention' (i.e. simple reaction time, choice reaction time, digit vigilance reaction time) it was revealed that ginseng led to significant speeded choice reaction times at 90 minutes post-dose, as compared to placebo. This study was the first to report speeded attentional performance by ginseng and may indicate the complex dose x time x domain relationship of ginseng (highlighting the importance of multiple dosing, multiple testing experimental designs). For example, Sünram-Lea's study demonstrated speeded attentional performance at 90 minutes post dose (a time not previously assessed) whereas Kennedy et al (2001) has documented slowing in attention performance following 600mg; however, Sünram-Lea failed to report any effect on secondary memory performance 90 minutes post dose whereas previous studies have shown this to be a consistent domain for ginseng improvement following the same 400mg dose (Kennedy et al., 2003).

In a seventh study Kennedy et al (2004) investigated the mood and cognitive effects of guaraná (*Paullinia cupana*), *Panax ginseng* and their combination in 28 healthy young (mean age 21.4years) volunteers. A repeated measures, placebo controlled, double blind, balanced, crossover trial was utilised. All participants received, in a counterbalanced order, 75 mg of a dried ethanolic extract of guaraná (Pharmaton, Lugano Switzerland), 200 mg *Panax ginseng* (G115) and their combination. Treatments were separated by a 7-day washout period.

Cognitive performance and subjective mood was assessed, using a tailored version of the CDR computerised assessment battery, Serial Subtraction mental arithmetic tasks and the Bond-Lader visual analogue mood scale, at a pre-dose baseline and thereafter at 1hr, 2.5hrs, 4hrs and 6hrs post-dose. Results were reported for the 'primary' and 'secondary' outcome measures as well as the individual tasks that load these factors. The effect on the 'primary' outcome measure of 'quality of memory' is not reported, however, the results for the 'secondary' outcome measures of 'working' and 'secondary' memory are reported. There was no effect found, of any treatment, at any post-dose testing session on "working memory" performance. However, both guaraná and ginseng led to significant improved 'secondary memory' performance at 2.5hrs post-dose. Inspection of the individual tasks that load 'secondary memory' revealed that performance was significantly improved, on the picture recognition task, by both treatments at 1hr, 2.5hrs and 6hrs post-dose. With regards to 'speed of memory', the results revealed that ginseng and the combination treatment led to significantly improved 'speed of memory' at 1hr (ginseng only), 4hrs and 6hrs (combination only) post-dose. Inspection of the individual tasks that load 'speed of memory' revealed that following ginseng performance was speeded on the numeric working memory task at the 2.5hrs, 4hrs and 6hrs post-dose; on delayed word recognition at 1hr and 4hrs post-dose; and on the delayed picture recognition task at 4hrs post-dose. Similarly, following guaraná, performance was speeded on the picture recognition task at 1hr, 2.5hrs and 4hrs post-dose. Following the combination treatment was significantly speeded on both delayed word recognition and delayed picture recognition at the 4hrs post-dose. With regards to 'speed of attention', results revealed improvements following all three

treatments, as compared to placebo; guaraná led to significant improvements at 1hr, 4hrs and 6hrs post-dose; ginseng led to significant improvements at 4hrs and 6hrs post-dose; the combination led to significant improvements at 2.5hrs, 4hrs and 6hrs post-dose. Inspection of the individual tasks that load 'speed of attention' revealed significantly faster reaction times on the digit vigilance task, as compared to placebo, following guaraná at 1hr, 4hrs and 6hrs post-dose; following ginseng at 6hrs post-dose; and following the combination treatment at 4hrs and 6hrs post-dose. Similarly, significantly speeded performance of choice reaction times was revealed following guaraná and ginseng at 1hr (guaraná only), 4hrs and 6hrs (ginseng only) post-dose. However, the speeded choice reaction time performance, following guaraná, was associated with a concomitant production of more errors, implying a simple speed accuracy trade off. The results for sentence verification and serial subtraction tasks are also reported. With regards to sentence verification, results revealed that all three treatments led to speeded performance of this task: ginseng and guaraná led to improvements at 1hr (ginseng only), 2.5hrs, 4hrs and 6hrs post-dose. Whereas, the combination treatment only resulted in speeded performance at 2.5hrs post-dose. The improvements in the speed of performance were not combined with any effect on accuracy of performance. With regards to serial subtractions the results revealed that both guaraná and the combination treatment led to a significant improvements in the accuracy of performing serial 3's at the 2.5hrs, 4hrs and 6hrs (combination only) post-dose. Inspection of the Serial Sevens subtraction task revealed that all three treatments resulted in improved serial 7's performance: ginseng and guaraná led to significant speeded performance at 1hr, 2.5hrs (guaraná only), 4hrs (guaraná only), and 6hrs post-dose. Whereas, the

combination treatment led to significant improved accuracy at 1hr and 2.5hrs post-dose. However, in contrast, guaraná led to significant decrements in accuracy at 4hrs post-dose. There was no effect of any treatment on any subjective mood outcome at any post dose testing session.

1.4.2.3.2.1 Summary of the empirical evidence – acute ingestion

Taken together these acute studies have identified both positive, and to a lesser extent, negative, cognitive and mood effects of single doses of *Panax ginseng* (standardised extract G115) in healthy young (mean age <25 years) adults. The most consistent finding is of improved secondary memory performance following G115 alone (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004), and in combination with both *Ginkgo biloba* (Kennedy et al., 2001b; Kennedy *et al.*, 2002) and guaraná (*Paullinia cupana*) (Kennedy *et al.*, 2004). Whilst these mnemonic effects appear to be robust, particularly following a single dose of 400 mg, in one instance both lower (200 mg) and higher (600 mg) doses led to significantly slower performance of attentional tasks (Kennedy *et al.*, 2001). Similarly, in the same cohort, whilst 400 mg improved accuracy of performing a serial subtraction task, 200 mg led to modest, but significant, reductions in the speed of performing the same task (Scholey and Kennedy, 2002). These decrements in speed of task performance contrast with recent findings for the same 200 mg dose of improved speed of information retrieval, attention and arithmetical performance (Kennedy *et al.*, 2004), and significantly shortened latency of the P300 component of auditory evoked potentials (Kennedy *et al.*, 2003), and following 400 mg G115, faster responses on an attentional task 90 minutes post-dose (Sünram-Lea., 2004).

1.5 Rationale

The above evidence pertaining to a plethora of physiological, cognitive and mood effects of ginseng is overwhelming. It is clear that ginseng is capable of modulating a number of physiological parameters. It is also reasonable to suggest that these physiological changes underlie ginseng's behavioural effects. However, the precise mechanism is not known and it is more probable that ginseng's behavioural effects be explained by a combination of the reported physiological changes.

One physiological effect that can be linked to behaviour is that of changes in blood glucose levels. Behaviour has previously been shown to be sensitive to acute changes in circulating blood glucose levels. Members of the *Panax genus* have been shown to modulate blood glucose levels; however, no study has reported any relationship between changes in glucose levels, as a result ginseng ingestion, and behavioural performance. Chapters 1 and 2 in this thesis will investigate ginseng's acute modulation of blood glucose levels and whether any relationship is evident between the physiological modulation and behaviour.

The evidence pertaining to the acute behavioural effects of a standardised ginseng extract are somewhat consistent; however, they are by no means unequivocal. A dose specific pattern seems to be emerging on two gross performance indicators (the first being that of the accuracy of behavioural performance and the second being the speed of behavioural performance); however, there is still limited consistency between studies in the specific task/domain affected by ginseng. The most consistent pattern pertaining to the gross performance indicators (i.e. accuracy and speed of performance) suggests that 200 mg G115 affects speed of

performance (not always in a positive direction) and 400 mg affects accuracy of performance (most consistent in secondary memory performance). However, as stated above, such findings are not true for all studies. An additional consistent finding amongst the acute studies has been the recurring failure to report any effect on working memory. The present thesis will address this issue (i.e. the failure to report any modulation of working memory) by exploring working memory processes in more detail as it could be suggested that the a validity of the conclusion reached by the previous acute studies (i.e. ginseng does not affect working memory) may be problematic. This problem may arise simply through the sensitivity of the previously utilised working memory tasks. The previously utilised task may not load all fractionated working memory resources, which may if further investigated prove to be sensitive to effects of ginseng.

The available data pertaining to the chronic use of ginseng is somewhat limited making interpretation difficult. Interpretation of the available data is further complicated as methodological differences between the available studies make explain inconsistent data patterns. The current thesis will investigate the effects of chronic ginseng use utilising standardised methodologies that have been shown to be sensitive in previous acute ginseng studies.

The present thesis will therefore address three general research questions:

- What are the effects of acute, sub-chronic and chronic ingestion of *Panax ginseng* on memory, attention and mood in young healthy volunteers?
- What are the acute gluco-regulatory effects of *Panax ginseng* in healthy volunteers?
- Is there a relationship between the acute modulation of glucose levels and the acute change in memory, attention and mood in young healthy volunteers?

The studies that make up this thesis include the first scientific investigations of the acute anti-fatiguing properties of *Panax ginseng* associated with extended periods of mental effort; further reports of the acute modulation of circulating blood glucose levels and the first investigations of the behavioural and mood effect of chronic ingestion of *Panax ginseng* utilising standardised methodologies.

CHAPTER 2. THE EFFECT OF PANAX GINSENG ON COGNITIVE PERFORMANCE AND BLOOD GLUCOSE LEVELS DURING SUSTAINED MENTAL ACTIVITY

2.1 Introduction

Ginseng (species of the genus Panax) products are amongst the most popular self-administered herbal products for 'memory loss' and 'absentmindedness' (see: Kennedy and Scholey, 2003). Despite an extensive literature documenting the effects of ginseng on potentially relevant physiological parameters, studies that have addressed the behavioural effects of chronic (e.g. the daily ingestion of ginseng over an extended period of time) administration of ginseng in humans has produced little evidence of behavioural benefits (see Kennedy and Scholey, 2003). This lack of evidence of efficacy may be accounted for by the methodological shortcomings. For instance, few studies into the effects of ginseng in humans have used adequately standardised ginseng extracts, and many fail to adopt double blind or placebo controls (Kennedy and Scholey, 2003; Bahrke and Morgan, 1994; Bahrke and Morgan, 2000; Vogler *et al.*, 1999). As a recent example, Persson et al (2004) reported a lack of positive behavioural effects of ginseng in a group who self-reported taking the extract either for over two years or an average of six months. However, the study used too small a sample for their chosen statistical analysis, utilised non-standardised psychometric instruments, no placebo, and made no attempt to standardise either extract or dosing regime (see Scholey et al., 2004).

However, a recent series of placebo-controlled, double-blind, balanced crossover studies has identified both positive, and to a lesser extent, negative, cognitive and

mood effects of acute doses of *Panax ginseng* (standardised extract G115) in young healthy humans. The most consistent finding is of improved memory performance following G115 alone (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004), and in combination with both *Ginkgo biloba* (Kennedy et al., 2001b; Kennedy *et al.*, 2002) and guaraná (*Paullinia cupana*) (Kennedy *et al.*, 2004). Whilst these mnemonic effects appear to be robust, particularly following a single dose of 400 mg, in one instance both lower (200 mg) and higher (600 mg) doses led to significantly slower performance of attentional tasks (Kennedy *et al.*, 2001). Similarly, in the same cohort, whilst 400 mg improved accuracy of performing a serial subtraction task, 200 mg led to modest, but significant, reductions in the speed of performing the same task (Scholey and Kennedy, 2002). These decrements in speed of task performance contrast with recent findings for the same 200 mg dose of improved speed of information retrieval, attention and arithmetical performance (Kennedy *et al.*, 2004), and significantly shortened latency of the P300 component of auditory evoked potentials (Kennedy *et al.*, 2003), and following 400 mg G115, faster responses on an attentional task 90 minutes post-dose (Sünram-Lea et al., 2004).

The mechanisms by which ginseng might modulate human cognitive performance are not yet well understood, but they may involve several central and peripheral physiological effects that are potentially relevant to human cognitive performance. These include effects on the cardiovascular system, platelet aggregation, the Hypothalamic-Pituitary-Adrenal system, neurotransmission, and nitric oxide synthesis (see Kennedy and Scholey, 2003; see Section 1.2.5 of this thesis).

The long term and acute hypoglycaemic effects of ginseng have been demonstrated both in rodents (Xie *et al.*, 2002; Ohnishi *et al.*, 1996) and humans (Sotaniemi *et al.*, 1995; Tetsutani *et al.*, 2000; Vuksan *et al.*, 2000; Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001). With regards to *Panax ginseng,* a reduction in fasted blood glucose levels and glycated haemoglobin were reported following 8 weeks administration of 100 mg and 200 mg/day of an unspecified extract in 18 participants with type 2 Diabetes Mellitus (Sotaniemi *et al.*, 1995). Similarly, Tetsutani et al (2000) reported that 24 months of treatment with 3 - 4.5g/day of Korean red *Panax ginseng* decreased HbA_{1c} (an index of average blood glucose levels over approximately the previous month) in 34 type 2 diabetics compared with controls. With regards *Panax quinquefolius* (American ginseng), a decrease in fasted blood glucose and HbA_{1c}, has been reported in 24 type 2 diabetic patients following 8 weeks administration of 1g of a proprietary ginseng extract, taken 40 minutes before each meal (Vuksan *et al.*, 2000). Of particular relevance to the present study, acute hypoglycaemic effects of *Panax quinquefolius* have also been demonstrated in a series of randomised, placebo-controlled studies. Reductions in blood glucose levels, following a 25g glucose challenge, have been reported during a 120 minute oral glucose tolerance test in both diabetic patients who had ingested 3g, 6g and 9g (Vuksan *et al.*, 2000b; Vuksan *et al.*, 2000c), and healthy participants administered 1g, 2g and 3g of *Panax quinquefolius* (Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001). Prior to this study, the hypoglycaemic effects of single doses of *Panax quinquefolius* or *Panax ginseng* have not been assessed in the absence of a concomitant glucose load.

It has been established that fluctuations in the level of circulating blood glucose can modulate cognitive performance (for review see Messier, 2004). For

example, participants in a hypoglycaemic state (low blood glucose levels) have shown behavioural impairment in a number of domains (Holmes *et al.*, 1984; Gold *et al.*, 1985). Similarly, supra-hypoglycaemic states have also led to impairments (De Feo *et al.*, 1988; Tylor and Rachman, 1988). Conversely, acute improvements in behavioural performance have been reported following a glucose drink. These improvements have been reported across a variety of tasks, assessing a number of different behavioural processes (e.g. Messier *et al.*, 2003; Donohoe and Benton, 2000; Kennedy and Scholey, 2000; Scholey *et al.*, 2001; Sünram-Lea *et al.*, 2001; 2002; 2002b; Benton 1990; Martin and Benton 1999; Foster *et al.*, 1998). One study that may have particular relevance to the present thesis is Kennedy and Scholey (2000). In this study a positive association was reported between the rate at which a person's blood glucose levels returned back to baseline, following an initial peak, and the level of cognitive performance. This relationship was particularly evident during periods of higher 'cognitive demand' (those tasks that are more effortful as reported subjectively and measured objectively). For example, it was reported that a 25g glucose drink improved performance on a more difficult mental arithmetic task (but not on an easier version), with the rate at which blood glucose levels returned to baseline positively correlating with behavioural performance on that task (Kennedy and Scholey, 2000). Additionally compared with placebo, a glucose drink improved performance during intense mental processing, which, in turn, led to a measurable reduction in blood glucose levels (Scholey *et al.*, 2001).

One explanation for such findings is that increased uptake of blood glucose results in better performance and therefore a measurable reduction in circulating blood glucose level. Indeed, Stone et al, (2005) demonstrated, utilising fMRI,

significantly greater activation (therefore uptake of glucose) of specific brain regions during a verbal encoding task following glucose administration as compared to a placebo control. One interpretation of this result could be that of a greater utilisation of an energy source during encoding; however, in this study there was no difference in behavioural (memory) performance. It follows that any intervention which modulates glucose transport may also affect cognitive performance. There is some support for this notion from the finding that insulin administration can improve memory in sufferers from Alzheimer's disease (Watson and Craft, 2004). However, insulin's role in glucose utilisation is only one possible mechanism that could account for the improved memory performance. Insulin has also been shown to modulate neurotransmitters, affect membrane potentials, neuronal physiology and long-term potentiation (see Craft, 2005).

Panax ginseng, in common with *Panax quinquefolius*, has been shown to modulate blood glucose levels in healthy humans; however, the post-prandial modulation of blood glucose levels by these two different members of the genus *Panax* may be in opposite directions, following a glucose challenge. It is possible that the acute glucose modulating properties of ginseng may partially explain ginseng's acute behavioural effect. In particular, ginseng's hypoglycaemic properties may increase the transport and / or disposal of glucose as an energy source. It is possible that ginseng administration may increase glucose disposal (through its effects on rate limiting enzymes) or drive cellular glucose uptake (through the up regulation of GLUT transporters), which, at least acutely, may be available for metabolism during periods of effortful processing and thus improve performance. In previous research both 200 mg and 400 mg of *Panax ginseng*

(G115) have led to enhanced cognitive performance (in some instances impairments have also been documented) following a single dose in healthy volunteers (see Kennedy and Scholey, 2003). In line with this there is *in vitro* evidence reporting the presence of ginsenoside metabolites in systemic circulation 1 hours post ingestion (see section 1.2.3 and 1.2.4 of this thesis). Therefore, it was decided to use both 200 mg and 400 mg of *Panax ginseng* in the current study. Additionally, given that 'cognitively demanding' tasks may be the most sensitive to the changes in blood glucose levels, the present placebo-controlled, double-blind, balanced-crossover study investigated the effects of two separate single doses of *Panax ginseng* (200mg and 400mg G115) on changes in fasted blood glucose levels and performance during sustained 'mentally demanding' tasks. This experimental paradigm has previously been used to demonstrate positive effects of a caffeine / glucose energy drink (Kennedy and Scholey, 2004).

2.2. SUBJECTS AND METHODS

2.2.1 Participants

Sixteen female and 14 male undergraduate volunteers (mean age 22.6 years, S.D. 5.46) participated in the study, which was approved by the Northumbria University Division of Psychology Ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation each participant gave informed consent and completed a medical health questionnaire (see appendix 1). All participants reported that they were in good health, and that they were free from heart disorders, high blood pressure, respiratory disorders, epilepsy, panic attacks and diabetes. Additionally, they reported being free from 'over the-counter' treatments, illicit social drugs and prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) and pregnant females were excluded from the study. Of the 30 participants 3 were light smokers and they agreed to abstain from smoking on the days of testing. All participants were overnight fasted, were alcohol free for 12 hours prior to baseline measure, and abstained from products containing caffeine on the days of testing. Volunteers were paid £60 for participation. Participants were randomly allocated a position on a Latin Square counterbalancing the treatment order by the computerised generation of random numbers (see appendix 2).

2.2.2 Blood Glucose measurement

Blood glucose levels were monitored using a Reflotron Plus diagnostic machine and Reflotron test sticks (Roche Diagnostics, Germany). The reliability of the test has previously been confirmed (Price and Koller, 1988).

On each of the three active study days, blood glucose levels were measured via capillary finger prick at baseline, one-hour post treatment (before commencement of the first post-dose battery completion), and after the third (i.e. mid-point of testing) and sixth (i.e. end of testing) completions of the demand battery (see Diagram 1).

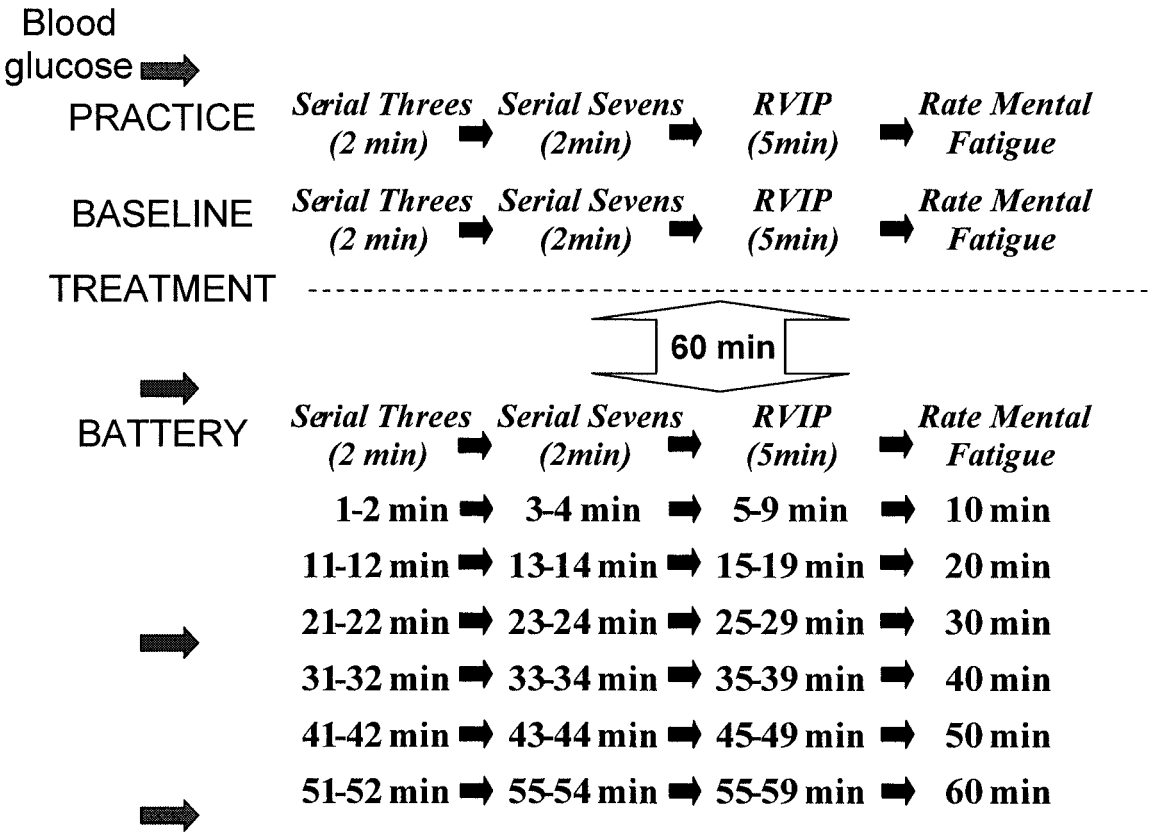

Diagram 1. Timeline and running order for each of the three study days. Participants received one treatment (placebo, 200 mg G115, 400 mg G115) on each study day. Study days were separated by not less than a 7 day washout period.

2.2.3 Cognitive Demand Battery

A 10 minute, computerised 'cognitive demand battery' was utilised comprising the Serial Threes subtraction task (2 mins), Serial Sevens subtraction task (2 mins), a Rapid Visual Information Processing task (RVIP – 5 mins), and a

'mental fatigue' visual analogue scale. Tasks within this battery have been shown to be sensitive to the effects of *ginkgo biloba* and *Panax ginseng* (Scholey and Kennedy, 2002), and a glucose drink (Scholey *et al.*, 2001). The overall experimental paradigm has been used to demonstrate positive effects of a caffeine/glucose energy drink (Kennedy and Scholey, 2004). The individual tasks are described below (see Diagram 1)

2.2.3.1 *Serial Sevens*

A modified computerised version of the Serial Sevens test was utilised. The original verbal Serial Sevens test (Hayman, 1942) has appeared in a number of forms, including as part of the Mini-Mental State Examination for dementia screening (Folstein *et al.*, 1975). It has been used to assess cognitive impairment during hypoglycaemia (Hale *et al.*, 1982; Taylor and Rachman 1988), and has also been used to investigate the relationship between blood glucose levels and cognitive performance (Kennedy and Scholey, 2000; Scholey *et al.*, 2001; Scholey, 2001) and the acute effects of ginkgo and ginseng (Scholey and Kennedy, 2002). In the current study, computerised versions of serial subtraction tasks were implemented (see Scholey *et al.*, 2001 for details), using tests of 2 minutes duration. For the Serial Sevens task a standard instruction screen informed the participant to count backwards in sevens from the given number, as quickly and accurately as possible, using the keyboard's linear number pad to enter each response. Participants were also instructed verbally that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each three-digit

response was entered via the linear number pad with each digit being represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. The task was scored for total number of subtractions and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

2.2.3.2 *Serial Threes*

The Serial Threes task was identical to Serial Sevens, except that it involved serial subtraction of threes.

2.2.3.3 *Rapid Visual Information Processing task (RVIP)*

This task has been widely used to study the cognitive effects of psychotropic drugs, and has been shown to be sensitive to augmented blood glucose levels (Donohoe and Benton, 1999). The participant monitors a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits are presented on the computer screen at the rate of 100 per minute in random order and the participant responds to the detection of a target string by pressing the space bar as quickly as possible. The task lasts for 5 minutes, with 8 correct target strings being presented in each minute. Scores are computed for number of target strings correctly detected, the average reaction time for correct detections, and number of false alarms.

2.2.3.4 *'Mental fatigue' visual analogue scale*

Participants rate their subjective feelings of mental fatigue on a 100mm visual analogue scale with the left and right end-points labelled 'not at all' and 'very much so' respectively (see appendix 3).

2.2.4 Treatments

Active treatments and placebo capsules (containing Soya-bean oil, Partly hydrogenated Soya-bean oil, Yellow Beeswax, Lecithin, Yellow iron oxide), matched for size, colour, opacity and odour were provided by the manufacturer. Prior to the commencement of the study, a disinterested third party, who had no other involvement in the study, prepared the three treatments for each of the individual participants (in accordance with the study's Latin Square) and sealed them in containers marked only with the participant code and study day number. On each study day, participants received four capsules. The individual capsules contained either an inert placebo, or 100mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland). Depending on the condition to which the participant was allocated on that particular day, the combination of capsules corresponded to a dose of 0mg (placebo), 200mg, or 400mg of G115 (see appendix 2).

2.2.5 Procedure

Each participant was required to attend a practice day and three active study days that were conducted not less than seven days apart to ensure a sufficient washout period between conditions. Testing took place in a suite of research-dedicated laboratories with participants visually isolated from each other. On arrival on the

practice day, participants were randomly allocated to a treatment regime according to a Latin Square that counterbalanced the order of treatments across the three active days of the study (see appendix 2). The practice day was identical to the three active study days with the exception that no treatment was offered, nor was analysis of the resulting data undertaken. On the three remaining study days, after an initial practice run through the 10 minute 'cognitive demand battery' (Serial Threes – 2 mins, Serial Sevens- 2 mins, RVIP – 5 mins, Mental fatigue rating scale) on arriving at the laboratory (data not analysed), each participant completed the baseline10-minute cognitive demand battery pre-dose, followed immediately by ingestion of the treatment. Commencing 60 minutes after consuming the day's treatment the participants completed the demand battery six times in succession (i.e. a total of 60 minutes task performance). Participants' blood glucose levels were measured pre-dose, one hour post-dose (i.e. before commencing the cognitive tasks), after three completions (i.e. the mid point of the post-dose tasks) and after the six completions of the 'cognitive demand battery'.

2.2.6 Statistics

2.2.6.1 Planned Comparisons

'Change from baseline' scores on the serial subtractions, RVIP, subjective mental fatigue and blood glucose levels were analysed using the Minitab statistical package version 13.1. Following an initial repeated measures ANOVA (participant x treatment x demand battery completion) (see appendix 4), *a priori* planned comparisons were made between placebo and each of the active treatments (200 mg and 400 mg G115) at each time point utilising t tests with

MSError as an error term (Keppel, 1991) (see appendix 5). To ensure the overall protection level against Type I errors, comparisons were strictly planned prior to commencement of the study, only probabilities associated with planned comparisons were calculated, all planned comparisons were two-tailed, and the reporting and interpretation of results was restricted to measures that showed a pattern of results commensurate with a genuine treatment effect (i.e. in the event that a measure generated only a single significant planned comparison for a specific dose, the comparison was not interpreted).

2.2.6.2 Post-hoc correlation analysis

Pearson's Product-Moment Correlation Coefficients were carried out to investigate any relationship between cognitive performance and blood glucose levels. 'Change from baseline' blood glucose levels at pre test, mid test and final were correlated with 'change from baseline' task performance at the nearest post dose completion of the demand battery (i.e. the first, fourth and sixth completions). Correlations were conducted separately for each condition (see appendix 6).

2.3. RESULTS

2.3.1 Baseline scores

Prior to analysis of change from baseline data, raw baseline scores for all three conditions (placebo, 200mg, 400mg) for each of the primary outcome measures (Blood glucose levels, Mental fatigue, RVIP, Serial Threes, and Serial Sevens) were subject to one-way, repeated-measures ANOVAs There were no significant differences in baseline performance on any measure (see appendix 7). Mean pre-dose baseline raw scores and change from baseline scores, for each condition at each post-dose time point on blood glucose levels and the individual cognitive tasks, are represented in Table 2.1 and Table 2.2 respectively.

Table 2.1. Effects of Ginseng (G115) on blood glucose levels. Mean baseline glucose level and mean change from baseline glucose level at one-hour post treatment (pre-test) and after three (mid-test) and six (final) post-dose completions of the battery, with standard errors in italics. Significance (planned comparisons) is indicated (^{C}, $p<0.005$; ^{D}, $p<0.001$; ^{E}, $p<0.0005$)

			Post-dose change from baseline					
N=30	Pre-dose baseline level		pre-test		mid-test		final	
blood glucose levels (mmol/l)								
Placebo	5.336	*0.211*	-0.076	*0.248*	0.034	*0.258*	0.013	*0.216*
200mg	5.681	*0.159*	-0.646^{C}	*0.176*	-0.594^{D}	*0.198*	-0.432^{B}	*0.182*
400mg	5.939	*0.196*	-0.664^{C}	*0.214*	-0.580^{D}	*0.212*	-0.933^{E}	*0.213*

Table 2.2. Effects of Ginseng (G115) on subjective fatigue and cognitive outcome measures. Mean baseline and mean change from baseline scores, at each completion (1 to 6), are presented, with standard errors in italics. Significance (planned comparisons; treatment vs. placebo) is indicated (^{A}, $p<0.05$; ^{B}, $p<0.01$; ^{C}, $p<0.005$; ^{D},$p<0.001$; ^{E}, $p<0.0005$).

N=30			Post dose change from baseline score											
Measure	Pre dose baseline score		1		2		3		4		5		6	
Mental fatigue (mm)														
Higher score = increased fatigue														
Placebo	33.03	*4.360*	2.033	*2.942*	10.833	*3.569*	20.067	*4.535*	15.133	*4.975*	26.533	*4.675*	31.433	*4.638*
200mg	37.90	*3.540*	-3.033	*2.710*	3.533^{A}	*2.910*	10.633^{C}	*3.078*	6.200^{D}	*3.663*	17.300^{C}	*3.456*	20.667^{C}	*3.802*
400mg	35.26	*3.812*	0.400	*2.776*	6.200	*3.215*	12.600^{B}	*3.717*	10.100	*4.036*	22.367	*4.566*	26.767	*5.012*
RVIP reaction time (sec)														
Placebo	0.522	*0.015*	-0.015	*0.011*	-0.001	*0.011*	-0.009	*0.012*	-0.013	*0.012*	0.003	*0.015*	-0.015	*0.011*
200mg	0.521	*0.011*	0.001	*0.008*	0.003	*0.012*	-0.002	*0.011*	0.009	*0.013*	0.032^{A}	*0.019*	-0.001	*0.009*
400mg	0.529	*0.013*	-0.014	*0.010*	0.001	*0.011*	-0.007	*0.010*	0.007	*0.014*	0.018	*0.013*	0.026^{D}	*0.019*
RVIP (accuracy)														
Placebo	19.80	*1.223*	1.067	*0.783*	-0.967	*0.726*	-1.933	*0.798*	-1.667	*1.043*	-2.600	*1.112*	-2.900	*0.789*
200mg	19.60	*1.363*	1.100	*0.703*	-0.900	*0.746*	-0.567	*0.718*	-1.433	*0.702*	-1.600	*0.887*	-0.700^{B}	*0.819*
400mg	19.67	*1.214*	0.200	*0.680*	0.100	*0.808*	-0.533	*0.702*	-1.367	*0.701*	-0.433^{B}	*1.027*	-1.700	*0.739*
Serial 3's (No. correct)														
Placebo	42.30	*2.585*	1.067	*0.978*	2.867	*0.926*	0.300	*2.123*	-0.433	*1.575*	3.000	*1.571*	3.467	*1.352*
200mg	42.67	*3.010*	0.900	*1.186*	2.600	*1.857*	2.400	*1.477*	3.400^{B}	*1.331*	3.567	*1.399*	3.833	*1.662*
400mg	41.27	*2.367*	1.933	*1.033*	1.000	*0.922*	1.167	*1.685*	-1.100	*1.799*	0.467	*1.478*	-1.033^{C}	*1.915*
Serial 3's (errors)														
Placebo	2.80	*0.632*	0.300	*0.458*	-0.600	*0.386*	0.700	*0.735*	0.467	*0.321*	-0.700	*0.422*	-0.300	*0.476*
200mg	1.93	*0.437*	0.567	*0.467*	0.433^{A}	*0.475*	0.567	*0.401*	0.567	*0.507*	1.133^{E}	*0.609*	-0.133	*0.299*
400mg	2.30	*0.681*	0.067	*0.453*	0.533^{A}	*0.415*	0.233	*0.465*	0.500	*0.462*	-0.033	*0.452*	0.367	*0.484*
Serial 7s (No. correct)														
Placebo	24.37	*1.564*	0.467	*0.625*	-0.533	*1.069*	0.633	*1.072*	0.533	*1.032*	1.767	*0.926*	1.100	*1.151*
200mg	22.50	*1.676*	2.533^{A}	*0.948*	2.667^{C}	*0.936*	3.433^{B}	*1.067*	2.667^{A}	*0.996*	2.600	*1.202*	3.200^{A}	*1.261*
400mg	22.57	*1.334*	-0.300	*1.042*	0.667	*0.930*	1.867	*0.942*	1.467	*1.145*	2.000	*1.055*	2.200	*1.159*
Serial 7's (errors)														
Placebo	1.73	*0.322*	0.167	*0.497*	0.767	*0.444*	0.633	*0.439*	0.533	*0.587*	0.733	*0.453*	1.000	*0.699*
200mg	1.80	*0.341*	-0.100	*0.459*	0.567	*0.538*	0.733	*0.551*	1.367	*0.714*	0.367	*0.585*	0.800	*0.648*
400mg	1.80	*0.430*	0.167	*0.363*	-0.033	*0.481*	0.467	*0.392*	0.733	*0.359*	0.333	*0.419*	0.500	*0.418*

2.3.2 Blood glucose levels

Planned comparison revealed that, relative to placebo, there were significant reductions in blood glucose levels for both active treatments at all post-dose time points. As shown in Figure 1 (a) and Table 2.1, 200mg led to reductions at one-hour post-dose [t (116) = 3.31, P = 0.001; d=0.4], and after three [t (116) = 3.65, P = 0.0003; d=0.4] and six [t (116) = 2.58, P = 0.01; d=0.4] post-dose completions of the demand battery. The 400mg treatment also led to reductions at one-hour post dose [t (116) = 3.42, P = 0.0007; d = 0.4], and after three [t (116) = 3.57, P = 0.0004; d = 0.4] and six [t (116) = 5.50, P = 0.0000001; d = 0.8] completions of the demand battery.

2.3.3 Serial threes (Number correct)

A significantly fewer number of Serial three subtractions were made following 200 mg on the fourth completion of the cognitive demand battery [t (290) = 2.47, P = 0.014; d=0.4]. Additionally, significantly fewer number of Serial three subtractions were made following 400 mg on the sixth completion [t (290) = 2.75, P = 0.002; d=0.6] (see Table 2.2).

2.3.4 Serial threes (Error)

A significantly greater number of errors were made on the Serial three subtraction task following 400 mg on the second completion of the cognitive demand battery [t (290) = 2.250, P = 0.0025; d= 0.5]. Additionally, a greater number of errors were made on the Serial three task following 200 mg on the second [t (290) = 2.052, P = 0.041; d= 0.4] and the fifth [t (290) = 3.640, P = 0.0003; d=0.8] completion of the cognitive demand battery (see Table 2.2).

There were no interpretable significant differences in performance of the Serial Threes subtraction task (Table 2.2).

2.3.5 Serial sevens (Number correct)

A greater number of correct subtractions were made on the Serial Sevens task following 200 mg. Participants made more correct responses on the first [t (290) = 2.056, P = 0.041; d=0.6], second [t (290) = 3.18, P = 0.002; d=0.5], third [t (290) = 2.78, P = 0.006; d=0.5], forth [t (290) = 2.12, P = 0.035; d=0.4], and sixth post-dose completions [t (290) = 2.09, P = 0.038; d=0.3] of the cognitive demand battery (see Table 2.2 and Figure 1 (b)).

2.3.6 Serial sevens (Error)

There were no significant differences between placebo and treatment at any of the post dose completions of the cognitive demand battery (see Table 2.2).

2.3.7 Rapid visual information processing task (Reaction time)

A significantly slower reaction time was recorded following the 200 mg on the fifth completion of the cognitive demand battery [t (290) = 2.307, P = 0.022; d=0.3] and following 400 mg on the sixth completion [t (290) = 3.25, P = 0.001; d=0.7] of the cognitive demand battery (see Table 2.2).

2.3.8 Rapid visual information processing task (Total correct)

A significantly fewer number of targets were identified following 400 mg on the fifth completion of the cognitive demand battery [t (290) = 2.71, P = 0.007;

d=0.4]. However, a significantly greater number of targets were identified following 200mg on the sixth completion of the cognitive demand battery [t (290) = 2.76, P = 0.006; d=0.5] (see Table 2.2).

2.3.9 Rapid visual information processing task (False alarms)

A significantly greater number of false alarms were found following 400 mg on the sixth completion of the cognitive demand battery [t (290) = 2.195, P = 0.029; d=0.5] (see Table 2.2).

There were no significant differences in the performance of the RVIP task (see Table 2.2).

2.3.10 Mental fatigue

An attenuation of the increase in subjective ratings of mental fatigue suffered as a consequence of extended task completion was revealed for both active treatments. 400mg led to reductions in subjective ratings of mental fatigue during the third [t (290) = 2.62, P = 0.009; d=0.3] completion of the demand battery, whilst 200mg led to reductions on the second [t (290) = 2.56, P = 0.011; d=0.4], third [t (290) = 3.31, P = 0.001;d=0.4], fourth [t (290) = 3.14, P = 0.002; d=0.3], fifth [t (290) = 3.24, P = 0.001; d=0.4], and sixth [t (290) = 3.78, P = 0.0002; d=0.4] completions of the demand battery (see Table 2.2 and Figure 1 (c)).

2.3.11 Correlations

There were no interpretable significant correlations between the change in blood glucose levels evinced in any of the three conditions and 'change from baseline' task performance at the completion of the battery nearest to the blood glucose reading (see appendix 5).

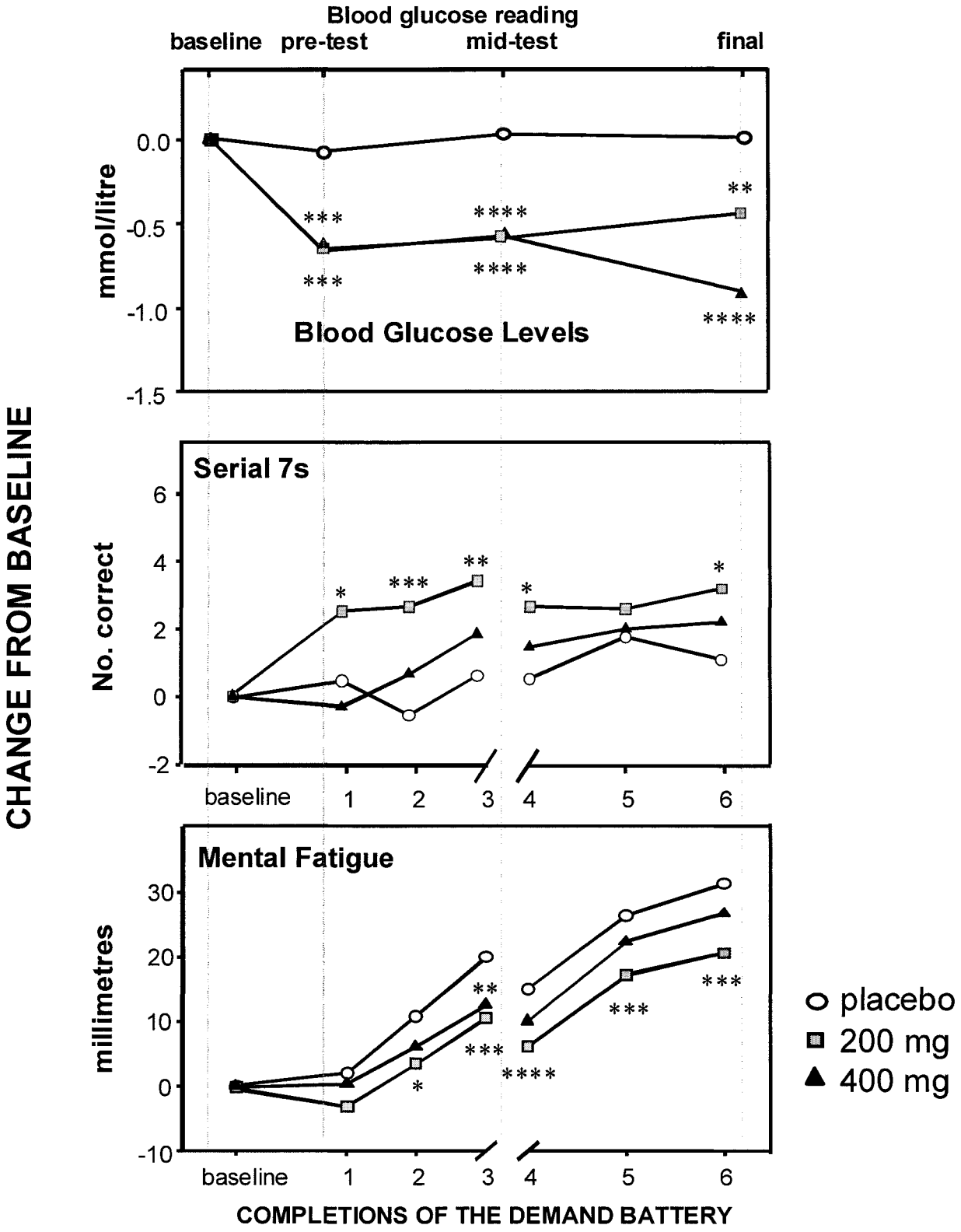

FIGURE 2.1. Effects of ginseng on blood glucose levels (top) Serial Subtractions (middle) and subjective ratings of mental fatigue (bottom). Top graph depicts mean change from baseline glucose level at one-hour post treatment (pre-test) and after three (mid-test) and six (final) post-dose completions of the battery. Middle and bottom graphs depict mean change from baseline scores at each post-dose completion (1 to 6) of the battery, for the Serial 7s subtraction task and for participants' subjective ratings of mental fatigue (lower scores indicate reduced mental fatigue). (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****,$p<0.001$; *****, $p<0.0005$). Significance is compared with placebo.

2.4 DISCUSSION

The results of the present placebo-controlled study demonstrate that single doses of *Panax ginseng* (G115) administered to healthy young volunteers can lower circulating blood glucose levels, enhance cognitive performance of a mentally demanding task (Serial Sevens), and ameliorate the increase in subjective feelings of mental fatigue experienced by participants during sustained intense cognitive processing.

Both 200mg and 400mg of *Panax ginseng* (G115) led to significant reductions in circulating blood glucose levels at all three post-treatment time points measured. To my knowledge this is the first demonstration of *Panax ginseng's* acute hypoglycaemia-inducing properties in healthy, overnight fasting human volunteers. Research has previously addressed the chronic effects of both *Panax ginseng* (Sotaniemi *et al.*, 1995; Vuksan *et al*, 2000) and *Panax quinquefolius* (American ginseng) (Tetsutani *et al.*, 2000) and the acute effects of the latter on the glycaemic response to a glucose challenge (Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001; Sievenpiper *et al.*, 2003). The acute hypoglycaemic effect of *American ginseng,* following a 25g glucose load, has been reported in both diabetic patients who had ingested 3g, 6g and 9g *Panax quinquefolius* (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b), and non-diabetics administered 1g, 2g and 3g (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2001). This effect, however, was restricted to a batch of *Panax quinquefolius* with a specific total ginsenosides content (3.54%) and protopanaxadiol: protopanaxatriol ratio (2:4) (Sievenpiper *et al.*, 2003). Additionally Sievenpiper *et al* (2003) reported that there was no effect of eight other widely-used *ginseng* types (*Sanchi, Siberian, American, Asian, Korean red, Japanese, wild American, and Vietnamese*) on indices of glycaemic control

following a 75g oral glucose tolerance test. However, an extract of *Panax ginseng* was associated with increased blood glucose levels and insulin response following the glucose load (Sievenpiper *et al.*, 2003). The effects of *Panax ginseng* in the absence (as here) and presence of a glucose load may reflect the differential working of a single gluco-regulatory mechanism, and this possibility requires further investigation.

With regards cognitive performance, the only meaningful significant effects of *Panax ginseng* were seen during performance of the most difficult task (Serial 7s). 200mg of ginseng extract led to a significantly greater number of correct subtractions being carried out on 5 of the 6 post dose battery completions (the exception being the fifth post dose completion). This improved speed of performance was not associated with more errors, precluding the possibility of any treatment specific "speed/accuracy trade-off". The improved performance following a 200 mg dose of *Panax ginseng* is consistent with the recent findings of faster memory, attention, and serial subtraction task performance (Kennedy et al., 2004), and decreased latency of the P300 component of auditory evoked potentials following the same dose (Kennedy *et al.*, 2003). The accumulation of findings of positive effects on the speed of task performance does suggest that the original observation of slower attention (Kennedy *et al.*, 2001) and Serial 7s (Scholey and Kennedy, 2002) task performance may have been anomalous. The most parsimonious explanation for this is that of a simple cohort effect (both studies reported data from the same cohort). Alternatively, whilst the extract used is standardised to total ginsenoside content, it is possible that even minor differences in the levels of single ginsenosides, or groups of ginsenosides (e.g. the ratio of panaxadiols to panaxatriols), may have exerted an effect.

In relation to the reported subjective feelings of mental fatigue it was found that the 200mg treatment led to a significant amelioration in the participants' subjective feelings of mental fatigue at all post dose time points (except the first post-dose battery completion). The 400mg dose led to a significant reduction in ratings of mental fatigue after the third battery completion only. This is the first reported human study that has examined the effect of *Panax ginseng* on subjective feelings of mental fatigue associated with intense cognitive processing. A number of studies have, however, demonstrated that ginseng or its active components can attenuate the effects of fatigue in night nurses (Wesnes *et al.*, 2003), and improve measures pertaining to 'quality of life' or 'well being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations [although findings of this nature are by no means unequivocal (Kennedy and Scholey, 2003)]. The relationship between acute and chronic effects merits further investigation.

Since both improvements in performance and amelioration of mental fatigue were associated with the same 200 mg dose, it is possible that the effects on either measure were secondary to those on the other. Unfortunately, the current study was not designed to address this potential cause and effect issue. Similarly, studies of ginseng's acute effects have not directly measured performance related fatigue (although they have included measures of mood), and it is possible that the previously demonstrated cognitive effects are as a consequence of altered fatigue levels.

The mechanisms responsible either for ginseng's hypoglycaemic effect or its cognitive effects are not clear at present. With regards the former, the animal

literature may suggest three possible mechanisms that could account for modulation in blood glucose levels. These include modulation of glucose disposal, glucose transport or insulin secretion. The latter two may well be mediated by increased nitric oxide (NO) production (Roy *et al.*, 1998; Spinas *et al.*, 1998). The involvement of ginsenosides in this proposed mechanism is supported from a number of studies. For example, there is evidence of enhanced NO synthesis by total ginsenosides (Chen and Lee, 1995; Chen *et al.*, 1997), the PPT fraction (Kim *et al.*, 1992), and single ginsenosides such as Rg_1 (Kang *et al.*, 1995; Kim *et al.*, 1992), Re (Kim *et al.*, 1992), Rg_3 (Kim *et al.*, 1998) and Rc (Kim *et al.*, 1998). These effects have been seen in nervous tissue (Kim *et al.*, 1998; Vuksan and Sievenpiper 2000), kidney (Han and Kim, 1996) and aorta (Kang et al., 1995). Increased plasma NO concentrations have also been demonstrated following 8 weeks administrating of *American ginseng* in type 2 diabetics (Xu *et al.*, 2000). This increase in NO correlated with improvements observed in HbA_{1c}. Certain ginsenosides have also shown effects consistent with cholinergic stimulation (Salim *et al.*, 1997; Yamaguchi *et al.*, 1997), and adrenergic blockade (Tachikawa *et al.*, 1999; Kudo *et al.*, 1998), with such changes in either system potentially resulting in increased glucose uptake (Lekas *et al.*, 1999; Xie and Lautt 1996). The Asian ginseng extract G115 has also been shown to increase 2- Deoxy-D-[2-^{3}H] glucose (2 – DG) uptake in a dose dependent manner (Samira *et al.*, 1985). Opposing glycaemic effects of ginseng and specific ginsenosides have also been documented. Hong et al (2000) showed that a water extract of Asian ginseng significantly inhibited insulin stimulated 2-DG uptake, whereas Kimura et al (Kimura *et al.*, 1981) showed that an extract containing Rb and Rc increased glycaemia at 100mg/kg. It is possible that in the

current study ginseng improved performance by modulating some aspect of the mechanisms responsible for the reciprocal relationship between falling blood glucose and improved performance during cognitive demand. One (highly speculative) possibility is that *Panax ginseng* promotes the transport of glucose (including into active cells) and thus facilitates metabolism in task-sensitive structures. This is consistent with the findings reported here, showing a combination of reduced blood glucose, improved performance and reduced mental fatigue. Further studies are needed to elucidate any such processes, including examining the cognitive effects of co-administration of glucose with ginseng.

Whatever the outcome of such studies, it is clear that ginseng's ability to modulate human cognitive performance could be attributable to a single effect, or a combination of effects, on a wide number of physiological parameters. One conclusion that might be drawn from the current results, given the lack of any correlational relationship between the modulation of glucose level and cognitive performance, is that the mechanism underlying the improved performance on the most difficult task seen here is unlikely to be a direct modulation of blood glucose levels. Indeed, whilst little research has directly addressed the correlation between cognitive effects and the manipulation of blood glucose levels, previous research would suggest that reduced blood glucose levels, as here, would have led to a decline in task performance (e.g. Holmes *et al.*, 1984; Gold *et al.*, 1985; De Feo *et al.*, 1988; Taylor and Rachman, 1988; Scholey et al., 2006). The possibility remains that both effects are as a consequence of improved utilisation and metabolism of circulating glucose, but it seems equally likely, in the absence of a cognitive effect for 400 mg, that they reflect differing mechanisms. Whilst, the

lack of a cognitive effect following 400 mg may appear curious, previous ginseng research, both in humans and animals, is replete with dose-specific effects and non-linear dose response profiles (for review, see Kennedy and Scholey, 2003).

The importance of the observed hypoglycaemic effect in the present study should not be understated. Diabetes Mellitus, and the treatment of diabetes, remains a major and growing health problem. Vuksan *et al* (2001) has provided evidence to suggest that *Panax quinquefolius* (American ginseng) may be an effective alternative therapy for patients suffering from type 2 diabetes. The present findings suggest that *Panax ginseng* (G115) may have a similar therapeutic value. Additionally the cognitive deficits in a number of conditions may be related in part to poor gluco-regulation, for example, aging (Hall et al., 1989), Alzheimer's disease (Hoyer, 2000), and Schizophrenia (Schultz, 1999). It is possible that ginseng may have a positive (or negative) effect on this aspect of such disorders. However, until further research has delineated the mechanisms underlying the demonstrated gluco-regulatory effects, caution should be exercised in the use of this product by sufferers from diabetes.

In conclusion, both doses of *Panax ginseng* utilised here led to reduced levels of circulating blood glucose. The lower (200 mg) dose also led to improved task performance, and reduced mental fatigue as a consequence of extended task performance. These latter effects were not directly related to the modulation of blood glucose levels. Given the possibility that members of the Panax genus may eventually provide a natural, well tolerated, treatment for diabetes (Vuksan *et al.*, 2001) the mechanisms underlying these effects require further investigation.

CHAPTER 3. THE EFFECT OF PANAX GINSENG, CONSUMED WITH AND WITHOUT GLUCOSE, ON BLOOD GLUCOSE LEVELS AND COGNITIVE PERFORMANCE DURING SUSTAINED 'MENTALLY DEMANDING' TASKS

3.1. Introduction

A recent series of placebo-controlled, double-blind, balanced crossover studies have demonstrated that single doses of *Panax ginseng* (standardised extract G115) can improve cognitive performance in young healthy humans both alone and in combination with other products (e.g. Kennedy et al., 2001a; 2001b 2002a; 2004; Sünram-lea 2004). The mechanisms by which ginseng might modulate human cognitive performance are not yet well understood, but they may involve several central and peripheral physiological effects that are potentially relevant to human cognitive performance. These include effects on the cardiovascular system, platelet aggregation, the Hypothalamic-Pituitary-Adrenal system, neurotransmission, and nitric oxide synthesis (see: Kennedy and Scholey, 2003). Ginseng's acute behavioural effects may also be partially explained by ginseng's acute glycaemic effects (see chapter 2). Ginseng extracts have been shown to have gluco-regulatory properties. For instance, the long term and acute hypoglycaemic effects of ginseng have been demonstrated both in rodents (Ohnishi et al., 1996; Xie et al., 2002) and humans (Sotaniemi et al., 1995; Tetsutani et al., 2000; Vuksan et al., 2000a; Vuksan et al., 2001; Vuksan et al., 2000b; Vuksan et al., 2000a). It has previously been established that fluctuations in levels of circulating blood glucose can modulate cognitive performance (see Messier, 2004 for review).

Of particular relevance to the present study is the unexpected pattern of results revealed in chapter 2. In chapter 2 the results revealed that a single dose of either 200 mg or 400 mg of *Panax ginseng* (G115) caused significant reductions in blood glucose levels, with concomitant speeded performance on a serial subtraction task and amelioration of mental fatigue following the 200 mg dose. Despite the unexpected relationship of lowered blood glucose levels and improved cognitive performance, one explanation for such findings is that of increased cellular uptake of blood glucose. However, it should be noted that there was no correlational evidence revealed between the changes in behavioural performance and the changes in blood glucose levels following any dose (see appendix 6).

Given the above speculation into the mechanistic explanation of this unexpected relationship, it seems expedient to investigate the relationship between the administration of both glucose and ginseng (in combination) on behavioural performance and blood glucose levels. If ginseng's behavioural effects (under 'demanding' conditions) can indeed be partially explained through its ability to 'drive' the uptake of glucose into the cell (by some unknown mechanism) than one might expect that there be a synergistic relationship between glucose and ginseng administration which would lead to further enhanced behavioural performance following the combined treatment (however, whatever the results of such a study the extraneous variables such as administration time and dose would need to be further investigated). Given that the 'cognitive demand' battery was shown to be a sensitive measurement tool (see Chapter 2) and 200 mg (G115) of ginseng was shown to lower blood glucose levels and improve aspects of cognitive performance (whereas 400 mg had a reduced effect on cognitive

performance) suggesting that 200 mg may be the most beneficial dose it was therefore decided to use the 'cognitive demand battery' and the 200 mg (G115) dose in the current study. Additionally, the ginsenoside metabolites have been shown to be present within one hour of ingestion (see introduction section 1.13 and 1.14) and ginseng has been shown to modulate behavioural performance one hour post ingestion (see Kennedy and Scholey 2003; Chapter 2) . Similarly, a 25g glucose drink has been shown to modulate both glucose levels and behavioural performance (see Messier 2004 for a review) and it is know to take between 20 – 40 minutes for plasma glucose levels to reach peak concentration after the oral ingestion of a glucose drink of this type. Therefore, The present placebo-controlled, double-blind, balanced-crossover study investigated the effects of single doses of *Panax ginseng* (200mg G115); glucose (25g); and a combination of *Panax ginseng* and glucose (200mg G115 + 25g glucose) on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks.

3.2. SUBJECTS AND METHODS

3.2.1 Participants

Seventeen male and 10 female undergraduate volunteers (mean age 21.89 years, S.D. 4.64) participated in the study, which was approved by the Northumbria University Division of Psychology Ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation each participant gave informed consent and completed a medical health questionnaire (see appendix 8). All participants reported that they were in good health, and that they were free from heart disorders, high blood pressure, respiratory disorders, epilepsy, panic attacks and diabetes. Additionally, they reported being free from 'over the-counter' treatments, illicit social drugs and prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) and pregnant females were excluded from the study. Of the 27 participants 1 was a light smoker (< 3 per day), and this participant agreed to abstain from smoking on the days of testing. All participants were overnight fasted, were alcohol free for 12 hours prior to baseline measure, and abstained from products containing caffeine on the days of testing. Volunteers were paid £80 for their participation. Participants were randomly allocated a position on a Latin Square counterbalancing the treatment order by the computerised generation of random numbers (see appendix 9).

3.2.2 Blood Glucose measurement

Blood glucose levels were monitored using a Reflotron Plus diagnostic machine and test sticks (Roche Diagnostics, Germany). The reliability of the test has previously been confirmed (Price and Koller, 1988). On each of the three active

study days, blood glucose levels were measured via capillary finger prick at baseline, one-hour post treatment (before commencement of the first post-dose battery completion), and after the sixth (i.e. end of testing) completions of the demand battery (see diagram 2).

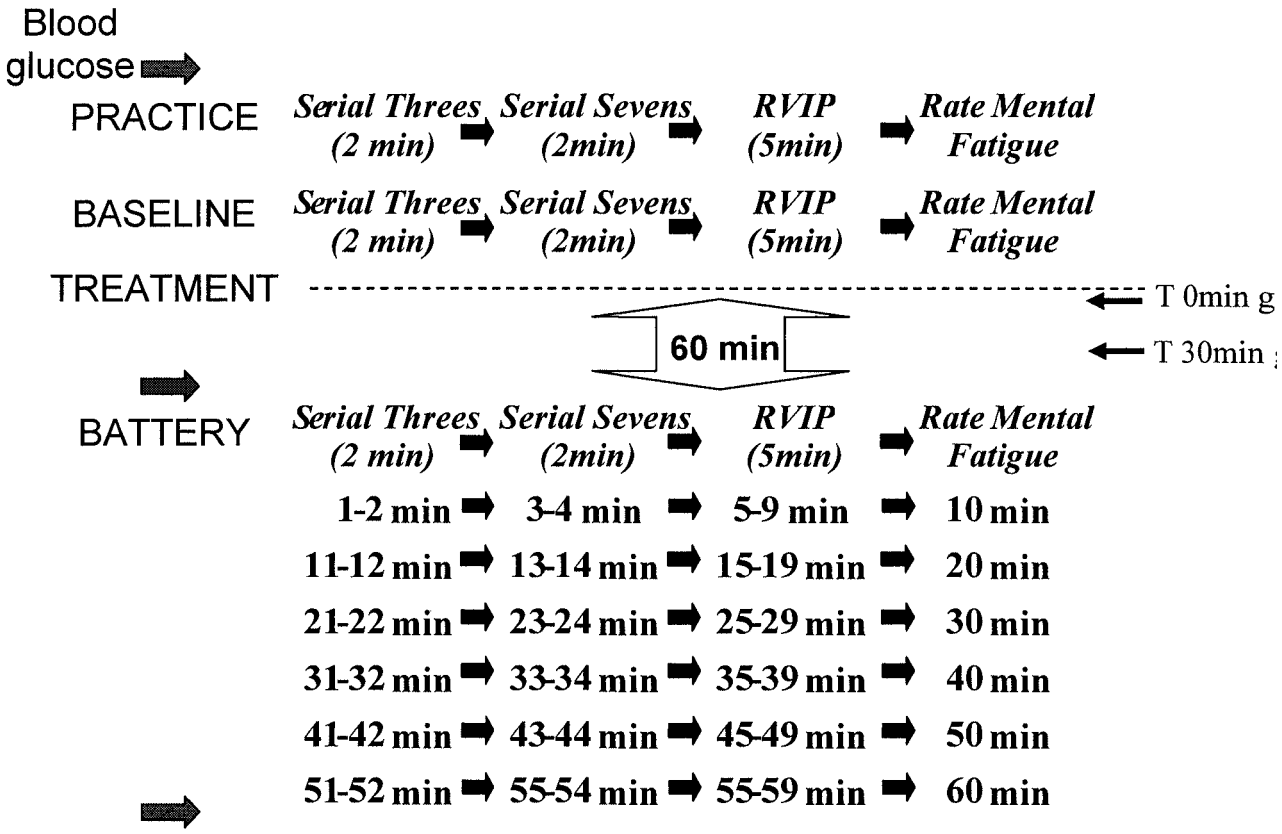

Diagram 2. Timeline and running order for each of the four study days. Participants received one treatment regime: 0mg G115/0 mg glucose (placebo); 200mg G115/0 mg glucose (ginseng); 0mg G115/25g glucose (glucose) or 200mg G115/25g glucose (ginseng/glucose combination) on each study day. Study days were separated by not less than a 7 day washout period.

3.2.3 Cognitive Demand Battery

A 10 minute, computerised 'cognitive demand battery' comprising the Serial Threes subtraction task (2 mins), Serial Sevens subtraction task (2 mins), a Rapid Visual Information Processing task (RVIP – 5 mins), and a 'mental fatigue' visual analogue scale, was utilised. Tasks within this battery have been shown to

be sensitive to the effects of *Ginkgo biloba* and *Panax ginseng* (Scholey and Kennedy, 2002), and a glucose drink (Scholey *et al.*, 2001). The overall experimental paradigm has been used to demonstrate positive effects of a caffeine/glucose energy drink (Kennedy and Scholey, 2004) and *Panax ginseng* (Chapter 2) (see diagram 2).

The individual tasks are described in detail previously in chapter 2 (section 2.2.of this thesis).

3.2.4 Treatments

3.2.4.1 *Ginseng capsule treatment*

Active treatments and placebo capsules (containing Soya-bean oil, Partly hydrogenated Soya-bean oil, Yellow Beeswax, Lecithin,Yellow iron oxide), matched for size, colour, opacity and odour were provided by the manufacturer. The individual capsules contained either an inert placebo, or 100mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland).

3.2.4.2 *Glucose drink treatment*

Active treatments and placebo drinks, matched for sweetness, volume (180ml of tap-water and 20ml of a sugar free fruit cordial drink), odour and colour were mixed in the laboratory on each day of testing. The individual drinks contained either 25g of glucose or 30 mg of saccharin

3.2.4.3 *Treatment preparation and administration*

Prior to the commencement of the study, a disinterested third party, who had no other involvement in the study, prepared the capsule treatments for each of the individual participants (in accordance with the study's Latin Square) (see appendix 9) and sealed them in containers marked only with the participant code and study day number. The same third party prepared the glucose and placebo drinks for each participant (in accordance with the study's Latin Square) on the morning of each study day. Depending on the condition to which the participant was allocated on that particular day, the combination of capsules and drink corresponded to a dose of: 0mg G115/0 mg glucose (placebo); 200mg G115/0 mg glucose (ginseng); 0mg G115/25g glucose (glucose) or 200mg G115/25g glucose (ginseng/glucose combination).

3.2.5 Procedure

Each participant was required to attend a practice day and four active study days that were conducted not less than seven days apart to ensure a sufficient washout period between conditions. Testing took place in a suite of research-dedicated laboratories with participants visually isolated from each other. On arrival on the practice day, participants were randomly allocated to a treatment regimen according to a Latin Square that counterbalanced the order of treatments across the four active days of the study (see appendix 8). The practice day was identical to the four study days with the exception that no treatment was offered, nor analysis of the resulting data undertaken. On the four remaining study days (testing commencing at 9am after an overnight fast), after an initial practice run through the 10 minute 'cognitive demand battery' (Serial Threes – 2 mins, Serial

Sevens- 2 mins, RVIP – 5 mins, Mental fatigue rating scale) on arriving at the laboratory (data not analysed), each participant completed the 10-minute cognitive demand battery pre-dose, followed immediately by ingestion of either 200mg (G115) or placebo capsules. This in turn was followed 30 minutes later by the ingestion of a 200ml drink, which had dissolved within it either 25g of glucose or a saccharine placebo. Thus, the participants received one of the following treatments: 0mg G115/0 mg glucose (placebo); 200mg G115/0 mg glucose (ginseng); 0mg G115/25g glucose (glucose) or 200mg G115/25g glucose (ginseng/glucose combination). Commencing 30 minutes after consuming the day's drink treatment the participants completed the demand battery six times in succession (i.e. a total of 60 minutes continuous task performance). Participant's blood glucose levels were measured pre-dose, one hour post ginseng dose (i.e. before commencing the cognitive tasks), and after the six completions of the 'cognitive demand battery' (see diagram 2).

3.2.6 Statistics

3.2.6.1 Planned Comparisons

'Change from baseline' scores on the serial subtractions, RVIP, subjective mental fatigue and blood glucose levels were analysed using the Minitab statistical package version 13.1. Following an initial repeated measures ANOVA (ginseng x glucose x demand battery completion [or time of blood sample]) conducted to determine main and interaction effects (see appendix 10), planned comparisons were made between placebo (0mg G115/0g glucose) and each of the active treatments at each time point utilising t tests with MSError as an error term (Keppel, 1991) (see appendix 11). To ensure the overall protection level,

comparisons were strictly planned prior to commencement of the study, only probabilities associated with planned comparisons were calculated, and all testing was two-tailed.

3.2.6.2 Post-hoc correlation analysis

Pearson's Product-Moment Correlation Coefficients were carried out to investigate any relationship between cognitive performance and blood glucose levels. 'Change from baseline' blood glucose levels at pre test and the end of testing were correlated with 'change from baseline' task performance at the nearest post-dose completion of the demand battery (i.e. the first and sixth completions respectively). Correlations were conducted separately for each condition (see appendix 12).

3.3 RESULTS

3.3.1 Baseline scores

Prior to analysis of change from baseline data, raw baseline scores for all four conditions (placebo, ginseng, glucose, and ginseng/glucose combination) for each of the primary outcome measures (Blood glucose levels, Mental fatigue, RVIP, Serial Threes, and Serial Sevens) were subject to one-way repeated measures ANOVAs (participant x treatment) (see appendix 13). There were no significant differences in baseline performance on any measure. Mean pre-dose baseline raw scores and change from baseline scores, for each condition at each post-dose time point on blood glucose levels and the individual cognitive tasks, are presented in Table 3.1

Table 3.1. Effects of 200mg (G115), 25g glucose, 200mg /25g ginseng/glucose combination, and placebo, on task performance and blood glucose levels. Mean baseline performance score/glucose level and mean change from baseline performance score/glucose level at each post dose battery completion point/glucose measurement point, with standard errors in italics (se). Significance (planned comparisons – treatment with placebo) is indicated ([A], $p<0.05$; [B], $p<0.01$; [C], $p<0.005$; [D], $p<0.001$; [E], $p<0.0005$).

N=27			post dose change from baseline score											
Measure	**Pre dose baseline score**		**1**	**se**	**2**	**se**	**3**	**se**	**4**	**se**	**5**	**se**	**6**	**se**
Mental fatigue (mm)														
Placebo	26.296	*3.380*	0.296	*1.671*	7.296	*2.663*	13.481	*3.233*	18.481	*4.134*	27.444	*4.683*	33.889	*5.531*
200mg	25.889	*3.687*	0.222	*1.825*	6.296	*2.179*	13.889	*2.883*	18.333	*3.512*	22.519[B]	*3.270*	26.926[D]	*3.737*
Glucose (25g)	27.778	*3.794*	1.333	*1.974*	7.000	*2.473*	14.481	*3.413*	18.630	*3.842*	23.148[A]	*3.921*	28.259[C]	*4.372*
Combination	25.778	*3.237*	2.778	*3.021*	9.593	*2.833*	16.111	*3.099*	21.000	*3.267*	27.852	*4.130*	33.148	*4.099*
RVIP reaction time (sec)														
Placebo	0.533	*0.014*	0.008	*0.008*	0.012	*0.009*	0.006	*0.014*	0.016	*0.018*	0.009	*0.014*	-0.006	*0.011*
200mg	0.530	*0.020*	0.008	*0.015*	0.025	*0.013*	-0.019	*0.010*	0.021[B]	*0.014*	0.005	*0.014*	0.018	*0.015*
Glucose (25g)	0.519	*0.018*	0.014	*0.012*	0.023[A]	*0.010*	0.009	*0.010*	0.018	*0.009*	0.009	*0.012*	0.007	*0.012*
Combination	0.535	*0.016*	0.012	*0.013*	0.017[A]	*0.014*	0.016	*0.017*	0.011	*0.013*	-0.001	*0.009*	-0.009	*0.013*
RVIP (false alarms)														
Placebo	2.407	*0.920*	0.000	*0.358*	1.852	*0.860*	0.926	*0.792*	0.704	*0.762*	0.519	*0.684*	3.556	*2.878*
200mg	2.889	*1.023*	0.148	*0.395*	0.407	*0.382*	1.519	*0.543*	1.148	*0.533*	0.926	*0.503*	0.963[C]	*0.801*
Glucose (25g)	4.593	*1.753*	1.111	*0.783*	0.667[A]	*0.669*	1.185[A]	*0.618*	-0.704	*0.449*	-0.741	*0.584*	-1.259[E]	*1.209*
Combination	3.407	*1.386*	0.222	*0.390*	0.667[A]	*0.381*	-0.074	*0.783*	0.444	*0.892*	0.259	*0.780*	0.222[E]	*0.715*
Serial 3s (total responses)														
Placebo	41.037	*2.070*	0.222	*1.040*	1.704	*1.083*	0.000	*1.345*	-0.704	*1.294*	-0.148	*1.313*	-0.481	*0.992*
200mg	40.074	*2.362*	1.963	*1.205*	3.259	*1.273*	2.704[A]	*1.223*	2.185[A]	*1.402*	2.000	*1.314*	3.407[C]	*2.255*
Glucose (25g)	40.222	*2.629*	0.778	*1.951*	1.000	*1.578*	3.481[B]	*1.502*	3.667[D]	*1.273*	2.259	*2.143*	2.333[A]	*1.844*
Combination	40.259	*2.645*	1.000	*0.887*	0.630	*1.378*	0.889	*1.181*	-0.037	*1.825*	-0.111	*1.889*	-1.556	*1.803*

Measure	Pre dose baseline score		post dose change from baseline score											
			1	se	2	se	3	se	4	se	5	se	6	se
Serial 3s (errors)														
Placebo	1.500	*0.255*	0.111	*0.454*	0.222	*0.566*	0.111	*0.308*	0.259	*0.380*	0.741	*0.485*	0.556	*0.408*
200mg	1.241	*0.210*	0.778	*0.435*	0.148	*0.260*	0.630	*0.457*	0.519	*0.274*	-0.037	*0.340*	0.185	*0.311*
Glucose (25g)	2.304	*0.255*	0.444	*0.351*	0.296	*0.413*	-0.259	*0.511*	0.037	*0.513*	-0.593[A]	*0.553*	-0.185	*0.490*
Combination	1.714	*0.337*	0.148	*0.426*	0.037	*0.442*	-0.296	*0.440*	1.481[A]	*1.436*	0.111	*0.516*	0.926	*0.487*
Serial 7s (total responses)														
Placebo	25.704	*1.630*	1.778	*0.790*	0.000	*0.801*	0.185	*0.967*	1.852	*0.910*	1.630	*0.744*	1.815	*0.974*
200mg	25.963	*2.182*	0.444	*0.752*	1.148	*0.938*	1.519	*0.881*	2.037	*0.914*	1.370	*0.994*	2.259	*0.919*
Glucose (25g)	26.222	*2.091*	0.370[A]	*0.911*	0.074	*0.811*	1.889	*0.725*	0.556	*0.930*	1.407	*1.246*	1.963	*0.944*
Combination	25.444	*2.021*	0.926[B]	*0.792*	0.074	*0.808*	2.000	*0.887*	2.185	*1.041*	2.481	*0.902*	2.778	*0.959*
Serial 7s (errors)														
Placebo	1.704	*0.301*	0.444	*0.431*	0.222	*0.375*	0.296	*0.406*	0.370	*0.404*	0.407	*0.411*	1.000	*0.406*
200mg	1.852	*0.412*	0.222	*0.535*	0.185	*0.437*	0.148	*0.503*	0.111	*0.466*	0.296	*0.562*	0.074[A]	*0.456*
Glucose (25g)	1.593	*0.240*	0.444	*0.411*	0.370	*0.431*	0.667	*0.406*	0.148	*0.409*	0.556	*0.289*	0.000[A]	*0.406*
Combination	1.667	*0.250*	0.000	*0.325*	0.111	*0.355*	0.185	*0.346*	0.593	*0.478*	0.185	*0.396*	0.000[A]	*0.405*
blood glucose levels(mmol/litre)	Pre dose baseline score	se	60 mins post										120 mins post	
Placebo	5.229	*0.125*	0.198	*0.126*									-0.721	*0.114*
200mg	5.462	*0.112*	0.630[A]	*0.096*									-0.708	*0.121*
Glucose (25g)	5.338	*0.153*	1.739[E]	*0.206*									-0.923	*0.156*
Combination	5.077	*0.122*	1.980[E]	*0.268*									-0.603	*0.151*

3.3.2 Blood Glucose levels

The initial repeated measure ANOVA (ginseng x glucose x time of blood sample) revealed a significant interaction between administration of glucose and time of blood sample [F (1,26) = 104.52, P < 0.001]. Planned comparisons, comparing each treatment to placebo at each time point, revealed that the ingestion of a 25g glucose load alone [t (26) = 9.40, P < 0.001; d=3.0] or in combination with 200mg ginseng [t (26) = 10.570, P < 0.001; d=3.3] led to significantly increased blood glucose levels at the one hour post dose measurement point. However, following the ingestion of ginseng alone, blood glucose levels were significantly reduced at the one hour post dose measurement point [t (26) = 2.096, P = 0.046; d=0.6] (see Figure 3.1 and Table 3.1).

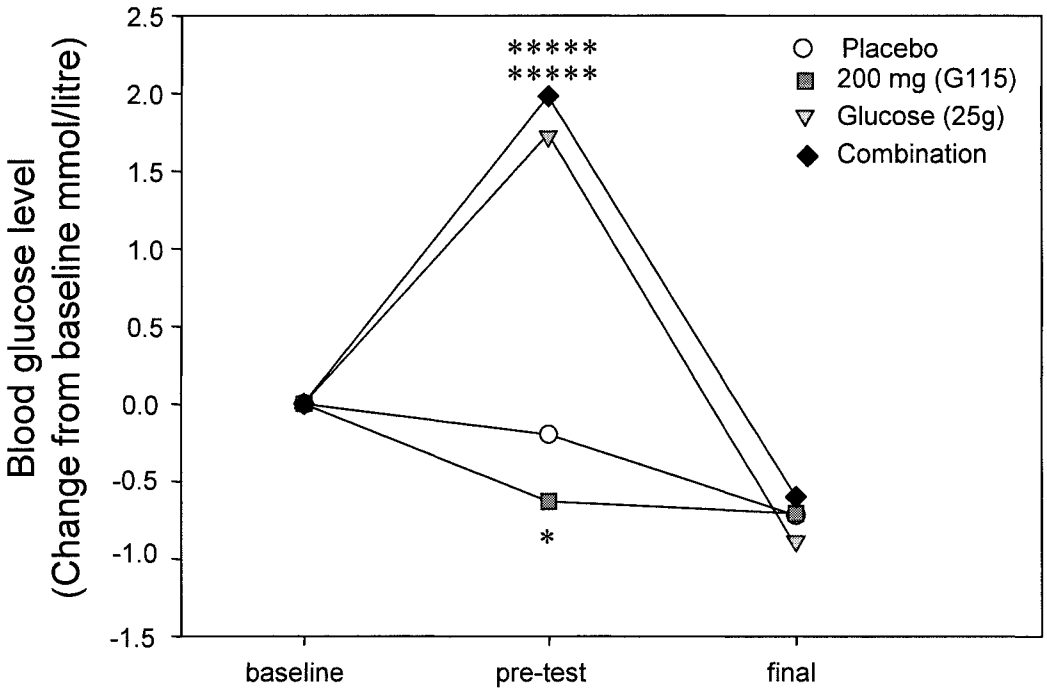

Figure 3.1. Effects of 200mg (G115), 25g glucose, 200mg /25g ginseng/glucose combination, and placebo, on blood glucose levels. Figure depicts mean change from baseline glucose level at one-hour post treatment (pre-test) and after six (final) post-dose completions of the battery. (*, p<0.05; **, p<0.01; ***, p<0.005; ****,p<0.001; *****, p<0.0005). Significance is compared with placebo. N=27.

The ANOVA also revealed a significant interaction between administration of glucose and ginseng [F (1,26) = 5.26, P = 0.03] on blood glucose levels (see Figure 3.2 and Table 3.1). Taken across the two post-dose sessions the pattern of

blood glucose modulation following the administration of ginseng was for an increase in circulating blood glucose levels in the presence of the 25g glucose load but a reduction in blood glucose levels in the absence of the glucose load (although it should be noted that post-hoc comparisons of the effects of ginseng within the glucose/placebo conditions were non-significant in themselves).

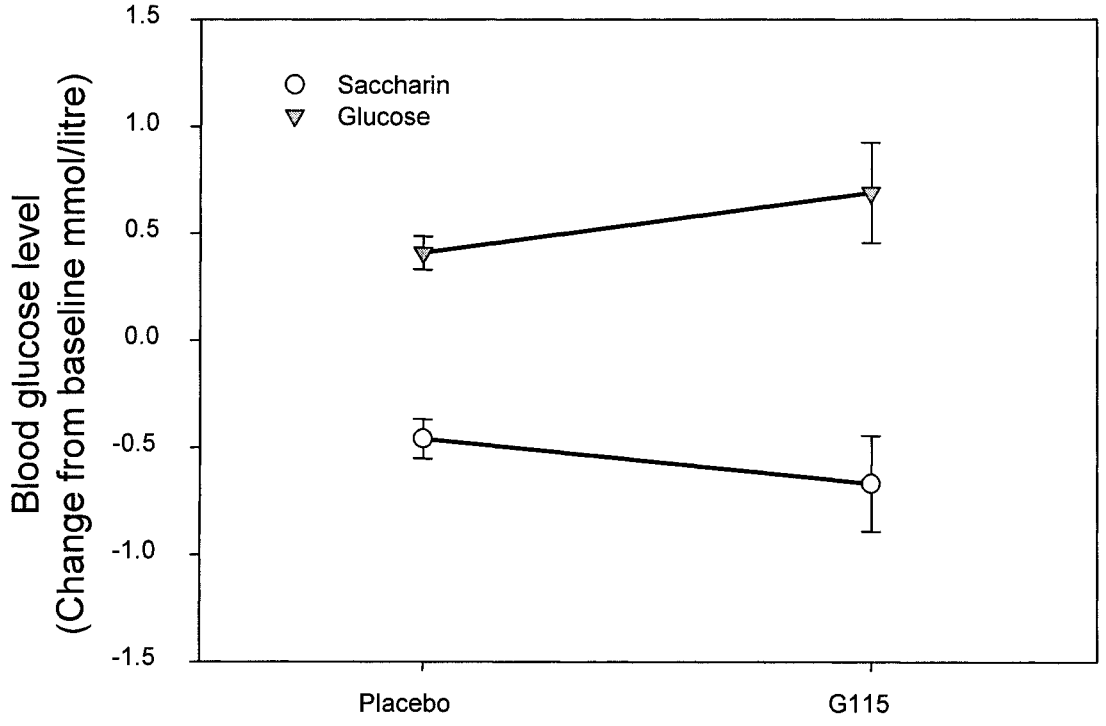

Figure 3.2. Depicts the significant post-dose interaction between ginseng and glucose on blood glucose levels (mean change from baseline values across both post-dose measurements). N=27

3.3.3 Serial Three subtractions

The repeated measure ANOVA (ginseng x glucose x demand battery completion) revealed a significant interaction between the administration of glucose and ginseng on the total number of serial three subtractions made [F (1,130) = 4.52, P = 0.043]. Planned comparisons comparing each treatment to placebo at each demand battery completion revealed significantly more serial three subtractions were performed following 200mg (G115) alone (ginseng condition) at the third [t (130) = 2.088, P = 0.039; d=0.4] fourth [t (130) = 2.231, P = 0.027;d=0.4] and sixth [t (130) = 3.004, P = 0.003; d=0.8] demand battery completions. Similarly, following a 25g glucose load alone (glucose condition) participants produced

significantly more serial three subtractions on the third [t (130) = 2.689, P = 0.008; d=0.5], fourth [t (130) = 3.376, P = 0.001; d=0.7] and sixth [t (130) = 2.174, P = 0.032; d=0.5] demand battery completions. (see Figure 3.3 and Table 3.1)

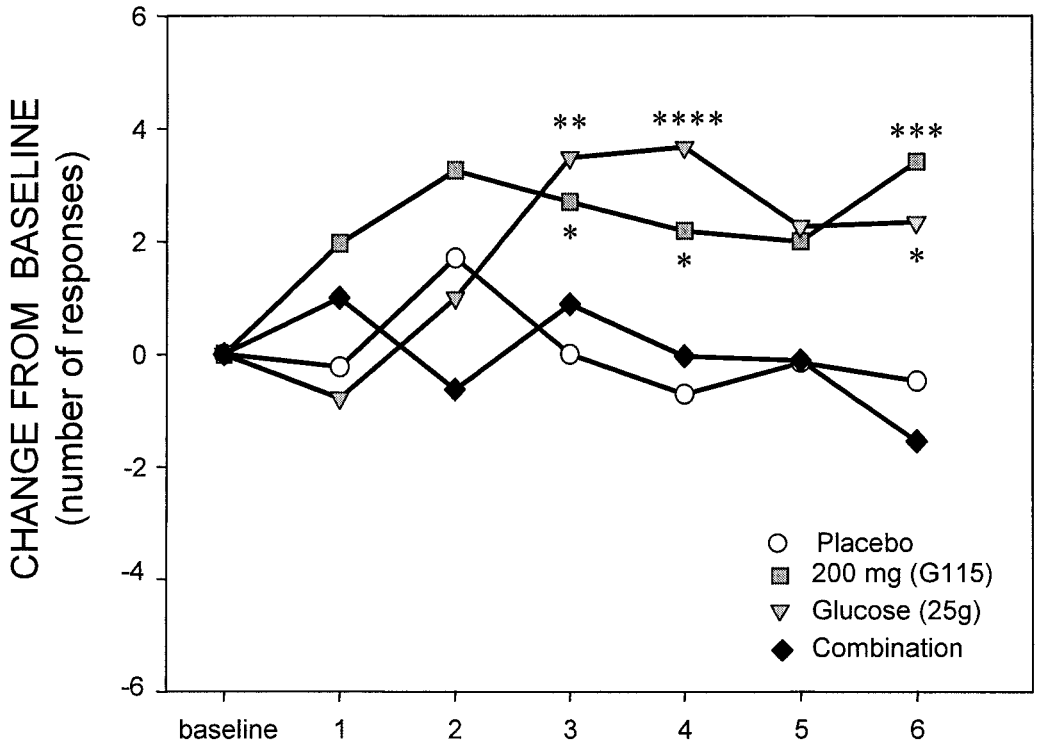

Figure 3.3. Effects of 200mg (G115), 25g glucose, 200mg /25g ginseng/glucose combination, and placebo, on the number of Serial Three subtractions performed. Figure depicts mean change from baseline scores at each post-dose completion (1 to 6) of the battery. (*, p<0.05; **, p<0.01; ***, p<0.005; ****,p<0.001; *****, p<0.0005). Significant is compared with placebo. N=27

3.3.4 Serial Seven subtractions

No significant results were revealed (see Table 3.1).

3.3.5 Rapid Visual Information Processing task (RVIP)

The initial ANOVA (ginseng x glucose x demand battery completion) revealed a significant main effect of glucose administration [F (1,130) = 7.72, P = 0.01] on the number of false alarms participants committed during the RVIP task. There were significantly fewer false alarms committed following a glucose load, irrespective of ginseng administration. Planned comparisons comparing each

treatment to placebo at each demand battery completion revealed a significant reduction in the number of false alarms following a 25g glucose load alone (glucose condition) on the second [t (130) = 2.804, P = 0.006; d=0.6] third [t (130) = 2.35, P = 0.020; d=0.5] and sixth [t (130) = 5.361, P = 0.0000004; d=0.2] demand battery completions. Additionally, a reduction in the number of false alarms was revealed following a glucose load combined with 200mg (G115) on the second [t (130) = 2.804, P = 0.006; d=0.6] and sixth [t (130) = 3.711, P = 0.0003; d=0.3] demand battery completion. A single significant reduction in the number of false alarms was also revealed following 200mg (G115) alone (ginseng condition) on the sixth [t (130) = 2.887, P = 0.005; d=0.2] completion of the demand battery only (Figure 3.4 and Table 3.1).

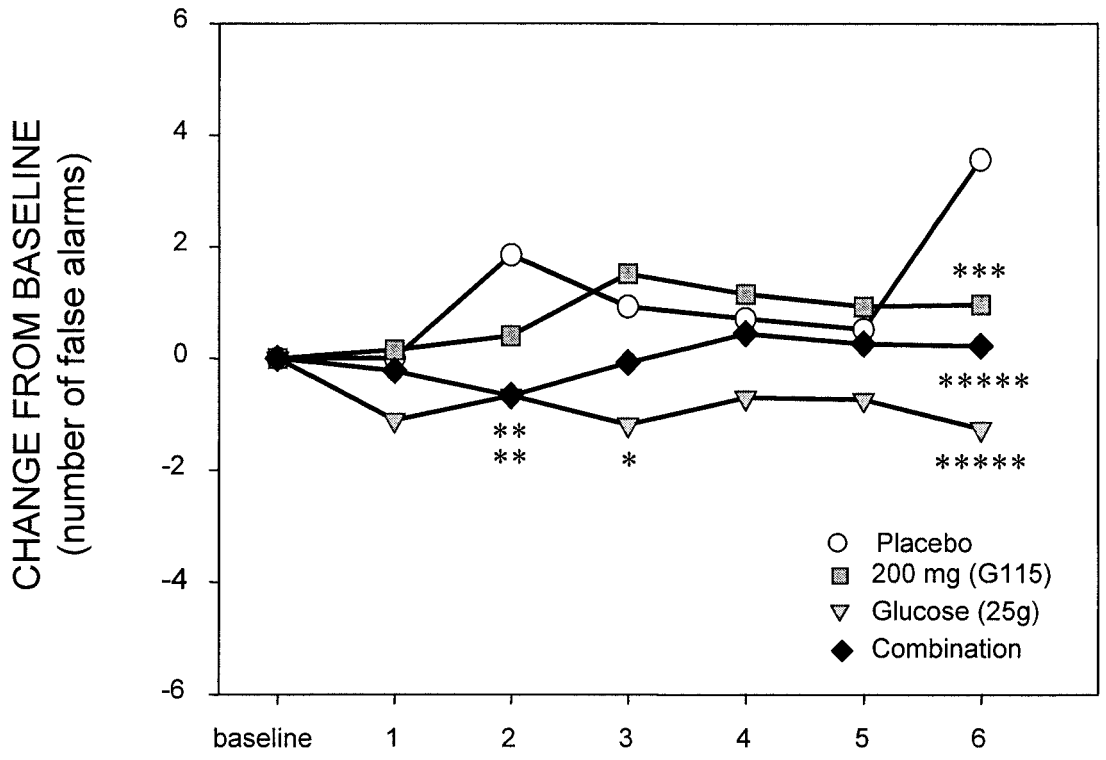

Figure 3.4. Effects of 200mg (G115), 25g glucose, 200mg /25g ginseng/glucose combination, and placebo, on the number of False alarms committed for the RVIP task. Figure depicts mean change from baseline scores at each post-dose completion (1 to 6) of the battery. (*, p<0.05; **, p<0.01; ***, p<0.005; ****, p<0.001; *****, p<0.0005). Significance is compared with placebo. N=27

3.3.6 Mental Fatigue

Planned comparison comparing each treatment to placebo at each demand battery completion revealed a significant amelioration in subjective ratings of mental fatigue following a 25g glucose load alone (glucose condition) on the fifth [t (130) = 2.179, P = 0.031; d=0.2] and sixth [t (130) = 2.855, P = 0.005; d=0.2] completions of the demand battery. Similarly, a significant amelioration in subjective ratings of mental fatigue were revealed following 200mg (G115) alone (ginseng condition) on the fifth [t (130) = 2.498, P = 0.014; d=0.2] and sixth [t (130) = 3.531, P = 0.001; d=0.2] completions of the demand battery (see Figure 3.5 and Table 3.1). There was no effect of the combined treatments.

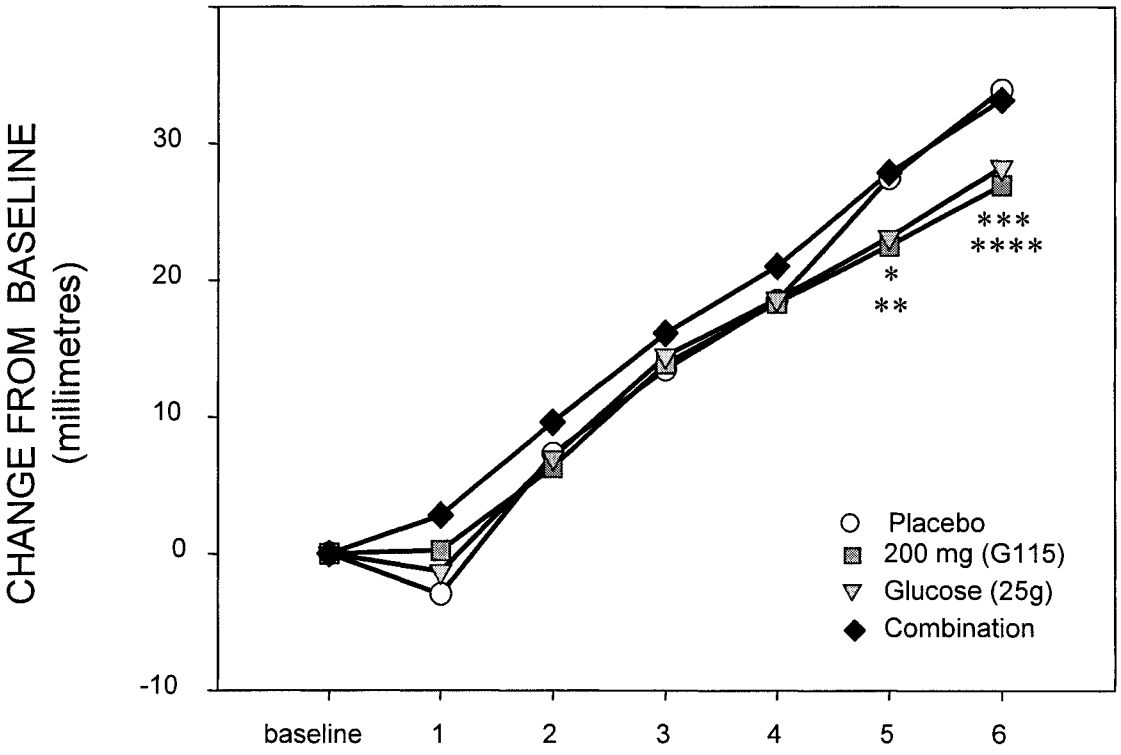

Figure 3.5. Effects of 200mg (G115), 25g glucose, 200mg /25g ginseng/glucose combination, and placebo, on the participants' subjective ratings of mental fatigue. Figure depicts mean change from baseline scores at each post-dose completion (lower scores indicate reduced mental fatigue). (*, p<0.05; **, p<0.01; ***, p<0.005; ****,p<0.001). Significance is compared with placebo. N=27

3.3.7 Post-hoc correlation analysis

The post –hoc correlations revealed no interpretable significant relationships between cognitive performance and blood glucose levels (see appendix 11).

3.4. DISCUSSION

The present study was designed to investigate the effects of *Panax ginseng* on cognitive performance, mood and blood glucose levels, both in the presence and absence of exogenously raised blood glucose levels (via administration of a 25g glucose load 30 minutes post ginseng dose). More specifically, it was intended to address the suggestion that ginseng's acute positive effects on human cognition may be explained, at least in part, through its potential promotion of the cellular uptake of glucose. The methodology employed also allowed an examination of the effect of each individual treatment (200mg G115 and a 25g glucose load) in isolation, in comparison to placebo, on each of the primary outcome measures.

The results revealed no synergistic relationship between *Panax ginseng* (200mg G115) and a 25g glucose load on any of the cognitive outcome measures. The results, however, did reveal that single doses of either *Panax ginseng* (200mg G115) or a 25g glucose load can modulate circulating blood glucose levels, enhance cognitive performance of a mental arithmetic task (Serial Threes), and ameliorate the increase in subjective feelings of mental fatigue experienced by participants during sustained intense cognitive processing. Accuracy of performing the Rapid Visual Information Processing task (RVIP) was also improved following the 25g glucose load.

In relation to blood glucose levels, the results confirmed the absorption of glucose into the blood stream following a 25g glucose load, and also revealed a significant interaction between the administration of ginseng and glucose (Figure 3.2) indicative of *Panax ginseng's* glucose modulating properties. The pattern of results showed that, in the absence of a glucose load (i.e. in overnight fasted participants) a single dose of *Panax ginseng* (200mg G115) resulted in a

significant post-dose fall in circulating blood glucose levels at the assessment one hour following the ingestion of ginseng. There was also a significant interaction between the consumption of ginseng and glucose levels assessed across both post-dose assessments (see Figure. 3.2). This interaction reflected a pattern of increased glucose levels when ginseng was taken with glucose, and reduced glucose levels when ginseng was taken with placebo (i.e. in a fasted state). It should be noted, however, that the individual post-hoc comparison of means for the ginseng and placebo groups while in the glucose drink condition did not show a specific significant increase associated with ginseng per se.

Research has previously addressed the chronic effects of both *Panax ginseng* (Sotaniemi *et al.*, 1995; Vuksan *et al*, 2000) and *Panax quinquefolius* (Tetsutani *et al.*, 2000) and the acute effects of the latter on the glycaemic response to a glucose challenge (Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001; Sievenpiper *et al.*, 2003). Most pertinently the results of the present study are consistent with those of the previous chapter (chapter 2) which demonstrated that the ingestion of either a single dose of 200 mg or 400 mg of *Panax ginseng* (G115) led to a significant reduction in circulating blood glucose levels in a cohort of young, healthy, overnight fasted volunteers. In the case of the current study, planned comparisons confirmed the results of chapter 2, finding that *Panax ginseng* lead to a reduction in blood glucose levels one hour post-dose in the absence of a glucose load.

The pattern of differential modulation of blood glucose levels following *Panax ginseng* with and without a glucose load is not out of line with previous research that demonstrated an increase in blood glucose levels during a 120minute oral glucose tolerance test (75g), using data pooled from 5 different doses of powdered *Panax ginseng* root (Sievenpiper et al., 2003). Interestingly,

Sievenpiper *et al* (2004) reported that an extract of *Panax ginseng,* but not eight other ginseng extracts (*Sanchi, Siberian, American, Asian, Korean red, Japanese, wild American, and Vietnamese)* was associated with an increase in blood glucose levels and a greater insulin response following a glucose load. Taken with the evidence of opposite effects for *Panax quinquefolius* following a glucose load in diabetic and healthy participants (Vuksan *et al.*, 2000a; 2000b; 2001), these results suggest that the differential effects of these extracts and members of the Panax genus require further investigation.

With regards cognitive performance on the serial subtraction tasks, whilst the initial ANOVA of the Serial Sevens data revealed a trend (P = 0.07) towards a treatment effect on the total number of subtractions performed, the only significant results on the ANOVA were obtained for the Serial Threes task. This initial analysis revealed a significant interaction between ginseng and glucose administration for the total number of Serial Three subtractions performed (Figure 3.3). Planned comparisons revealed that 200 mg G115 led to a significantly greater number of Serial Three subtractions being performed at the third, fourth and fifth completions of the demand battery. Similarly a greater number of Serial Three subtractions were performed at the third, fourth and six completions of the demand battery following a 25g glucose load. There was no effect of combining 200 mg with a 25 g glucose load at any time point. This improved speed of performance, for both ginseng and glucose conditions, was not associated with greater production of errors, precluding the possibility of any treatment specific "speed/accuracy trade-off".

The improved Serial Threes performance following both single treatments is generally in line with previous demonstrations of working memory task

enhancement by glucose (e.g. Martin and Benton 1998; Sünram-Lea et al., 2002) and recent findings of faster memory, attention (Kennedy et al., 2004; Sünram-Lea et al., 2006) and Serial Subtraction task performance (Kennedy et al., 2004; chapter 2) and decreased latency of the P300 component of auditory evoked potentials (Kennedy *et al.,* 2003) following *Panax ginseng.* However, they are somewhat at odds with previous reports of the enhancement of Serial Subtraction performance being restricted to the more 'mentally demanding' Serial Sevens task following both 200mg G115 (chapter 2) and a 25g glucose load (Kennedy and Scholey 2000; Scholey et al., 2001). Reference to the planned comparisons for the Serial Sevens task (which are not reported due to a lack of significance on the initial ANOVA) does, however, show that both glucose alone and glucose combined with ginseng led to a significant increase in speed of performance that was restricted to the first completion of the Serial Sevens. This is entirely in keeping with the previous studies (Kennedy and Scholey 2000; Scholey et al., 2001), both of which involved a single completion of these tasks. This also tends to suggest that the pattern of results evinced here might be related to the different demand characteristics of the multiple completions of these tasks, with the benefits of both glucose and ginseng only becoming apparent as fatigue (or another unidentified factor) increased with repeated performance of the Serial Threes task. Again this is in keeping with Fairclough and Houston (2003) who report greater reductions in blood glucose for the more difficult incongruent version of the stroop task, with the reductions becoming more apparent as task duration increased. With regards ginseng the discrepancy in results here in comparison to the results obtained in chapter 2 may well also be due to minor differences in the levels of single ginsenosides, or groups of ginsenosides (e.g. the

ratio of protopanaxadiols to protopanaxatriols) contained in an extract that is standardised to total % content of ginsenosides.

For the RVIP task the initial ANOVA revealed a significant main effect of glucose administration on the number of false alarms committed. There were significantly fewer false alarms committed following the 25 g glucose drink, irrespective of ginseng administration (Figure 3.4). Planned comparisons revealed that significantly fewer false alarms were committed on the second, third and sixth demand battery completions for the glucose condition. Similarly, significantly fewer false alarms were committed on the second and sixth demand battery completions for the ginseng and glucose combination condition (Figure 3.5). Accuracy in performing the RVIP task has previously been shown to be improved following a glucose-caffeine energy drink using the same experimental protocol and demand battery as used in the present study (Kennedy and Scholey 2004).

In relation to the reported subjective feelings of mental fatigue it was found that both 200 mg G115 and a 25g glucose load administered in isolation led to a significant amelioration in the participants' subjective feelings of mental fatigue towards the end of testing. Unfortunately, the current study was not designed to delineate the potential contribution of such mood changes to cognitive performance. Therefore, the behavioural effects observed in both the ginseng and glucose conditions could in all practicality be an artefact of the changes in mood or vice versa. The results further highlight that both glucose and ginseng may only become beneficial for behavioural performance as mental effort increases due to extended processing (indicated through increased self reports of mental fatigue). The result of the present study are consistent with that of chapter 2 that

reported that both 200mg and 400mg of *Panax ginseng* led to a significant amelioration of participants' subjective ratings of the mental fatigue associated with an extended period of intense cognitive processing. The glucose result is consistent with reports of the amelioration of deficits in cognitive performance and subjective fatigue during extended periods of cognitive demand following a glucose caffeine drink (Kennedy and Scholey 2004).

The mechanisms responsible either for ginseng's glycaemic effect or its cognitive effects are not clear at present. With regards the former, Vuksan *et al* (2000a) suggested three possible mechanisms that could account for modulation in blood glucose levels. These include the modulation of glucose disposal, glucose transport or insulin secretion. The latter two may well be mediated by increased nitric oxide (NO) production (Roy *et al.*, 1998; Spinas *et al.*, 1998). The involvement of ginsenosides in this proposed mechanism is supported by the results of a number of studies. For example, 8 weeks administration of American ginseng in type 2 diabetics led to an increase in NO concentration which correlated with improvements observed in HbA_{1c} (Xu *et al.*, 2000).

In the previous chapter 2 it was speculated that *Panax ginseng* may promote the transport of glucose (by an unknown mechanism but it could include the modulation of glucose disposal and / or transport – see introduction, section 1.1.6) into active cells (i.e. leading to reduction in circulating blood glucose levels) and thus facilitating metabolism in task-sensitive structures (i.e. leading to improved behavioural performance) (see: chapter 2). The present study provides no support for such a hypothesis; for example, there were no behavioural improvements or reductions in blood glucose levels revealed following the combination treatment. However, for the second time, *Panax ginseng* did lead to

improved behavioural performance and concomitant reductions in blood glucose levels when ingested by participants in a fasted state. Therefore, it still remains possible that ginseng exerts its beneficial cognition enhancing effects via some unknown gluco-regulatory mechanism. It should be noted that there was no direct correlation between the fall in blood glucose levels and performance.

Whilst the current study utilised participants in an over-night fasting state, and provided increased glucose levels by administering a glucose drink in order to provide adequate experimental control of their gluco-regulatory state, it has to be conceded that this is not necessarily the normal dietary state of the majority of consumers at the time that they consume ginseng. Future research might well be directed towards the effects of ginseng in cohorts in their normal dietary state. This having been said, the current research study does reinforce the potential importance of this line of research with regards treatments for diabetes. Vuksan *et al* (2001) has suggested that *Panax quinquefolius* may be an effective alternative therapy for patients suffering from type 2 diabetes. However, it should be noted that *Panax quinquefolius* is one of the less commonly used members of the Panax genus, whereas *Panax ginseng* is notable for its global ubiquity.

Whilst its potential utility in the treatment of conditions that feature disturbed gluco-regulation is of great interest, the present findings suggest that *Panax ginseng* (G115) may have opposite effects when administered in the absence or presence of glucose. Further research is required to delineate the mechanisms underlying the demonstrated gluco-regulatory effects and whether these effects represent a net benefit or cost to consumers.

In conclusion, one hour following administration of *Panax ginseng* blood glucose levels were reduced in fasted individuals, with an overall pattern of blood glucose

modulation that suggested an opposite effect when administered before exogenous glucose. Both *Panax ginseng* and glucose, when administered in isolation to each other, led to improved task performance, and reduced mental fatigue as a consequence of extended task performance. These latter effects were not directly related to the modulation of blood glucose levels. Given the potential utility of a treatment that beneficially modulates blood glucose levels whilst concomitantly enhancing cognitive performance, the mechanisms underlying these effects require further investigation.

CHAPTER 4. AN INVESTIGATION INTO THE ACUTE, SUB-CHRONIC AND SUPER IMPOSED EFFECTS OF PANAX GINSENG ON SECONDARY MEMORY AND ASPECTS OF WORKING MEMORY

4.1. Introduction

Despite the extensive literature that has documented ginseng's effects on numerous physiological parameters, which may have potential relevance for the modulation of human cognition and mood, there is still a clear lack of empirical human studies investigating the behavioural effect of chronic ginseng ingestion (see Kennedy and Scholey, 2003).

To date only three studies have directly investigated the behavioural effects of daily ginseng ingestion in humans. These studies have revealed improved speed of performing a mental arithmetic task (D' Angelo et al, 1986), faster reaction times on the most rapid auditory reaction time task (Soerensen and Sonne, 1996), and global memory and attention improvements (Labadorf et al, 2004). A further investigation, involving analysis of data from a small cohort of self-reported users of ginseng products drawn from a large prospective study, found no effect on episodic or semantic memory (Persson et al, 2004). However, as this study failed to assess the type of extract, or the dose, regularity or duration of consumption the purported findings are somewhat meaningless. Overall, no conclusive interpretations can be drawn from the above-mentioned studies as they have investigated different cohorts, administered different ginseng extracts and doses, and implemented different assessment tools designed to assess different aspects/domains of cognitive functioning (for further discussion see introduction section 1.2.5.3.3.1 and Scholey et al 2004).

Although the behavioural effects following chronic ginseng ingestion may still be somewhat debateable, there are a recent series of acute trials that have implemented the same placebo controlled, double-blind, balanced crossover design and utilised an identical comprehensive computerised assessment battery. These acute trials have identified both positive, and to a lesser extent, negative cognitive and mood effects of single doses of *Panax ginseng* (standardised extract G115) in healthy young adults. The most consistent finding is of improved secondary memory performance following G115 alone (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004), and in combination with both *Ginkgo biloba* (Kennedy et al., 2001b; Kennedy *et al.*, 2002) and guaraná (*Paullinia cupana*) (Kennedy *et al.*, 2004). Despite the repeated reports of improved secondary memory performance these studies have continually failed to report any effect on an index of working memory. It is possible that this anomaly may simple be due to the insensitivity of the working memory tasks previously employed. This speculation is supported by the fact that previous research has demonstrated that single doses of *Panax ginseng* can elicit improvements in cognitive performance when volunteers performed a mental arithmetic task (e.g. results of chapter 1 and chapter 2). Such a task loads heavily on working memory resources.

Given the clear lack of data available into the cognitive and mood effects following chronic ginseng ingestion and also the possibility that the working memory tasks previously utilised in the acute trials were insensitive to the effects of ginseng, it seems pertinent to address these issues. As chapter 2 of this thesis and previous studies (See Kennedy and Scholey, 2003) have demonstrated the effectiveness of single doses of 200 mg and 400 mg (G115) the present study will

utilise these doses once again. The post dose assessment points utilised in the present study are consistent with those used in previous studies (see Kennedy and Scholey, 2003). The present placebo controlled, double-blind, balanced, crossover study aims to investigate the acute (i.e. following single doses), the sub-chronic (i.e. following 7-days of consecutive ingestion) and the superimposed effects (i.e. the effect of an acute dose following chronic ingestion) of 200 mg and 400 mg (G115) of *Panax ginseng* on mood, secondary memory and aspects of working memory.

4.2. SUBJECTS AND METHODS

4.2.1 Participants

Fifteen male and 15 female undergraduate volunteers (mean age 22.87 years; SD 4.01) participated in the study which was approved by the Northumbria University Division of Psychology Ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation each participant gave informed consent and completed a medical health questionnaire (see appendix 14). All participants reported that they were in good health, and that they were free from heart disorders, high blood pressure, respiratory disorders, epilepsy, panic attacks and diabetes. Additionally, they reported being free from 'over the-counter' treatments, illicit social drugs and prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. Of the 30 participants 4 where light smokers (< 3 per day) and agreed to abstain from smoking on the days of testing. All participants were alcohol and caffeine free for 12 hours prior to baseline measure, and agreed to abstain from products containing caffeine on the days of testing. Volunteers were paid £100 for participation. Participants were randomly allocated a position on a Latin Square counterbalancing the treatment order by the computerised generation of random numbers (see appendix 15).

Due to data capture errors the number of evaluable data sets were reduced to 28 on the corsi block task and the delayed word recognition task. Reduced to 24 on the N-back task and to 23 on the random number generation task.

4.2.2 Cognitive Battery

Each cognitive/mood assessment comprised completion of the following tasks in the order shown.

4.2.2.1 *Bond–Lader visual analogue scales* (Bond and Lader 1974)

Scores from the 16 items, comprising 100 mm visual analogue scales anchored at each end by pairs of by mood antonyms (e.g. calm-tense), were combined as recommended by the authors to form three mood factors: Alert, Calm, and Contented (see appendix 16).

4.2.2.2 *Word presentation*

Twenty words, matched for frequency and concreteness, were presented in sequence on the monitor for the participant to remember. The stimulus duration was 1 s, as was the interstimulus intervals.

4.2.2.3 *Immediate word recall*

The participant was allowed 60s to write down as many of the words as possible. The task was scored as number of correct answers, errors, and intrusions

4.2.2.4 *Corsi Block Tapping task*

The Corsi blocks task was developed as a nonverbal counterpart to the verbal-memory span task (Milner, 1971). It has frequently been used to assess nonverbal short-term memory performance in adults (e.g. Smyth & Scholey, 1992), children (e.g. Orsini, Schiappa, & Grossi, 1981), and patients with neuropsychological deficits (e.g. Vilkki & Holst, 1989). The present study implemented a

computerised version of the task. Nine identical blue squares (each square was 93 x 93 pixels; screen resolution was set at 1024-768) appeared sequentially in random positions on the computer screen. A predetermined number of squares would change colour, from the original blue to red then back to blue, at the rate of one per second, thus identifying the sequence to be remembered. The participant would then repeat the sequence by moving the mouse and clicking each square in the sequence. The sequence span increased until the participant could no longer correctly recall the sequence, resulting in a span measure of nonverbal working memory. Participants were presented with five trials at each span level and were required to correctly recall at least three of the five trials to proceed to the next level. Nonverbal memory span was measured as an average of the last five correct sequences recalled.

4.2.2.5 *N back task*

fMRI studies have demonstrated the involvement of the prefrontal cortex (an area thought to underpin working memory performance) whilst completing the N back task. (Jonides et al 1997; Watter et al 2001). In the present study participants engaged in the N-back task at four levels of difficulty. In the most difficult version (3-back) participants had to indicate whether or not the letter currently on the screen was the same as that which had been presented 3 letters before. In the easier versions volunteers had to engage in '2- back' (2 letters before) and '1-back' (the letter immediately before) matching. In the least demanding version (0-back) participants had to match each letter in a series against a constant target that was specified prior to the beginning of the series. For each level of difficulty there were 14 targets and 41 non-targets. Each letter had an interstimulus interval

(ISI) of 2.5 seconds. Responses were made via the 'M' key (match) and 'Z' key (no match). Responses were marked for correct, error and reaction time for target and novel stimuli. A Sensitivity index (ranging from -1 to +1) was also calculated.

4.2.2.6 *Random Number Generation Task*

Successful random number generation is considered an indication of executive function and draws heavily on processes responsible for "inhibition" thought to be located in the left dorsolateral pre-frontal cortex (Jahanshahi *et al.*, 2000). In this task participants were instructed to produce a string of random numbers, using the linear number keys (0 to 9). Participants were instructed to keep pace with an asterisk that appeared on the computer screen, once per second for 100 seconds. Participant's responses were scored for "randomness" using RG calc (Towse and Neil, 1998), which is a computerised algorithm generating scores representing indicators of "randomness".

4.2.2.7 *Delayed word recall*

The participant was allowed 60s to write down as many of the words presented at the beginning of the battery as possible. The task was scored for number of correct answers, errors, and intrusions

4.2.2.8 Delayed word recognition

The original words presented at the beginning of the battery plus 20 distractor words were presented one at a time in a randomized order. For each word, the participant indicated whether or not they recognised it as being included in the

original list of words by pressing the 'M' key to indicate yes or 'Z' key to indicate no as appropriate and as quickly as possible. Accuracy and reaction time to each stimulus was recorded.

4.2.3 Treatments

Active treatments and placebo capsules, matched for size, colour, opacity and odour were provided by the manufacturer. Prior to the commencement of the study, a disinterested third party, who had no other involvement in the study, prepared the capsule treatments for each of the individual participants (in accordance with the study's Latin Square) (see appendix 15) and sealed them in containers marked only with the participant code and study day number. On each study day, participants received four capsules. The individual capsules contained either an inert placebo, or 100 mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland). Depending on the condition to which the participant was allocated on that particular day, the combination of capsules treatments corresponded to a dose of 0 mg (placebo), 200 mg (G115), or 400 mg (G115).

4.2.4 Procedure

The study commenced with a practice day that was identical to subsequent study days with the exception that no treatment was offered and the data acquired was not entered into any analysis. Participants received each treatment (placebo, 200 mg and 400 mg) for 8 days in total, with a wash-out period of 6 days between treatments. The order of presentation of the treatments was counterbalanced by random allocation to a Latin Square. Participants were assessed on the first (day 1) and last (day 8) day of each treatment period (i.e. 6 assessment days in total across the three treatments) (see diagram 3). On each of these days the

cognitive/mood testing regime comprised assessments (see above for tasks and running order) pre dose (followed immediately by the ingestion of that day's treatment) and thereafter at 1hr, 2.5hrs, and 4hrs post-dose. Testing took place in a suite of laboratories with participants visually isolated from each other. Upon completion of the last post-dose testing session on day 1 for each treatment period participants were provided with six identical containers, containing treatments for each day (days 2 to 8) until the next laboratory visit. Treatments on day 1 and day 8 of each period were consumed in the laboratory (see diagram 4).

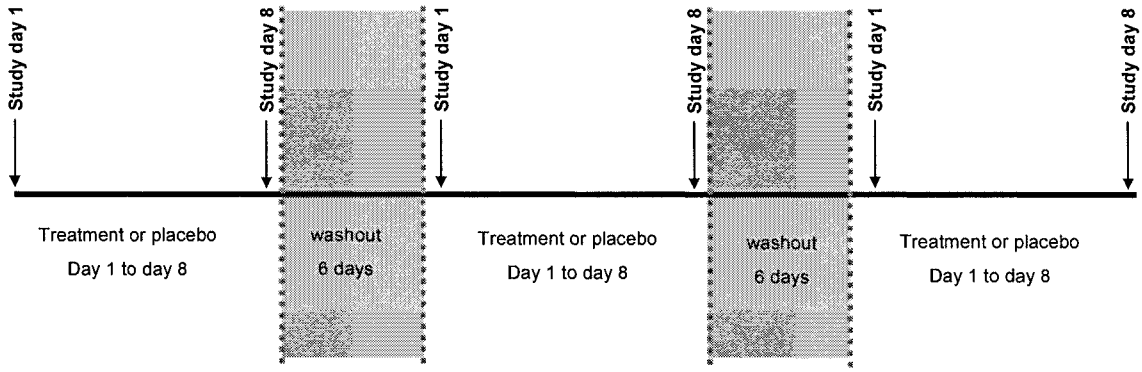

Diagram 3. Timelines of the study. Participants received either placebo or ginseng (200 or 400 mg G115) during each arm of the study. All participants received placebo during the washout period (beginning of week 9 to end of week 12).

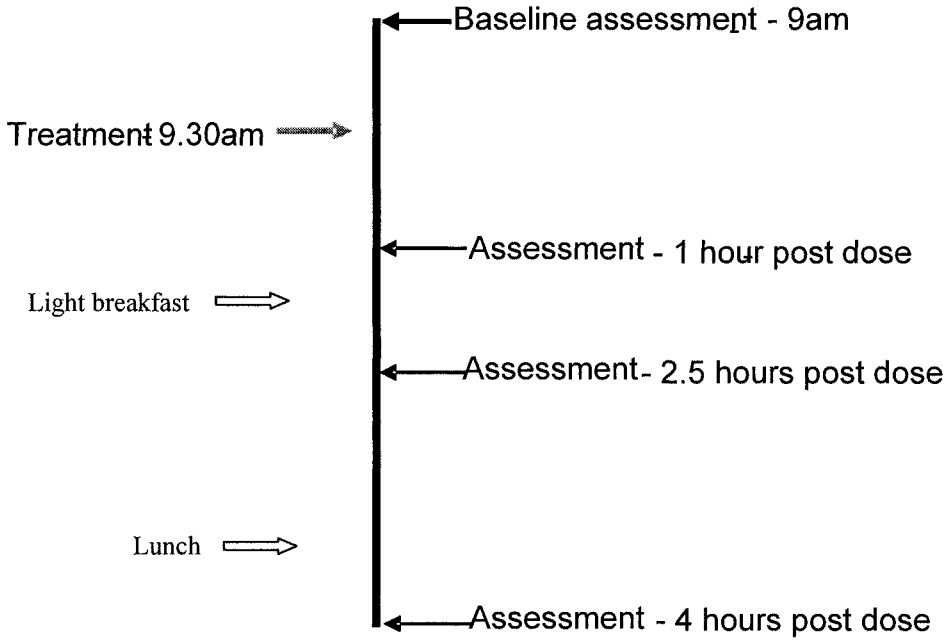

Diagram 4. Running order of each study day (day 1, 8) of both the 1st, 2nd and 3rdarms.

4.2.5 Statistics

Data was analysed using the Minitab statistical package version 13.1. All data, for each outcome measures: Bond Lader mood scale; Immediate Word Recall; Delayed Word Recall; Delayed Word Recognition; N Back tasks; Random Number Generation task and the Corsi-Block tapping test, was analysed as "change from pre-dose day 1 baseline score".

4.2.5.1 *Pure Sub-chronic effects*

In order to assess the effects of sub-chronic ginseng use, in the absence of any acute effects or any superimposed effects, pre-dose data on day 8 (scored as 'change from day 1 baseline') was analysed by one-way repeated measures ANOVA (see appendix 17).

4.2.5.2 *Acute, sub-chronic and superimposed effects*

In order to assess the acute, sub-chronic and superimposed effects of ginseng administration a three way repeated measures ANOVA (treatment [0, 200, 400mg] x session [1 hr, 2.5, 4hr post-dose] x day [1,8]) was conducted on data from the 1hr, 2.5hr and 4hr post-dose assessments on day 1 and day 8 of each treatment regime. All data were scored as 'change from day 1 pre-dose baseline' (see appendix 18).

4.2.5.3 *Using this analysis:*

A main effect of 'treatment' or a significant 'treatment' x 'session' interaction would indicate an acute effect of ginseng across both assessment days.

A treatment x day interaction would indicate a differential effect of sub-chronic and acute dosing (superimposed with one another).

4.2.5.4 *Planned comparisons*

On those measures that yielded a significant main effect or interaction effect with treatment, planned comparisons were made between placebo and each of the active treatments utilising t tests with MSError as an error term (Keppel, 1991). To ensure the overall protection level, comparisons were strictly planned prior to commencement of the study and only conducted on those outcome measures that reached statistical significance on the initial ANOVA. Additionally, only probabilities associated with planned comparisons were calculated, and all testing was two-tailed (see appendix 19).

4.3. RESULTS

4.3.1 Baseline scores

Prior to analysis of 'change from baseline' data, raw baseline scores for all three conditions (placebo, 200 mg, 400 mg) for each of the primary outcome measures (Bond Lader visual analogue scale, Immediate and Delayed word recall, Delayed word recognition, computerised Corsi Block tapping task, Random Number Generation task, N Back tasks) were subject to one-way, repeated-measures ANOVAs. There were no significant differences in baseline performance on any measure. Mean pre-dose baseline raw scores and change from baseline scores, for each treatment, at each post-dose time point, on each individual cognitive task, are represented in Table 4.1.

Table 4.1. Effects of 200mg (G115), 400 mg (G115) and placebo on task performance. Values represent mean baseline performance score and mean change from baseline performance score at each post-dose testing session on day 1; mean change from baseline performance score at pre-dose on day 8 and at each post-dose testing session on day 8. Standard errors in italics.

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
Bond-lader mood scale (N=30)									
Alert	Placebo	55.76 *4.45*	4.98 3.60	4.56 3.39	2.00 3.82	2.72 3.29	4.86 3.96	5.43 3.79	6.23 4.90
	200mg	59.66 *2.95*	0.29 1.95	2.52 2.35	-0.81 2.99	0.78 2.67	1.63 2.78	-0.22 3.00	1.18 2.57
	400mg	61.79 *3.04*	3.29 1.66	1.74 2.14	-2.65 2.89	-2.94 2.90	0.23 2.36	-0.22 3.25	-1.37 3.22
Calm	Placebo	65.69 *2.25*	-2.63 2.29	-5.93 2.29	-14.74 2.56	-1.98 1.63	-3.56 1.65	-3.85 2.41	-4.15 2.49
	200mg	60.69 *2.53*	0.92 2.51	0.43 2.53	-2.33 2.70	1.40 2.29	1.93 2.54	-1.43 2.72	1.99 2.38
	400mg	62.88 *2.92*	1.99 1.35	0.87 2.08	-3.38 2.72	1.98 2.51	1.02 2.68	0.89 2.21	0.44 3.24
Content	placebo	67.37 *2.97*	-10.03 3.02	-3.58 3.32	-8.85 3.46	-2.57 2.93	-5.83 2.94	-8.07 2.98	-9.32 6.23
	200mg	61.92 *2.81*	1.47 3.14	-1.28 3.15	-1.83 3.88	0.77 3.20	-3.02 3.75	-3.87 3.50	-1.42 3.42
	400mg	65.60 *3.65*	-2.75 2.59	-5.33 2.14	-3.93 3.35	-0.85 3.11	-6.85 2.55	-3.88 2.56	-5.05 3.47
Corsi block task (N=28)									
Span	placebo	6.04 *0.19*	0.26 0.18	0.11 0.25	0.07 0.22	0.23 0.21	0.01 0.26	0.12 0.20	0.33 0.23
	200mg	6.10 *0.22*	-0.15 0.19	0.09 0.18	-0.01 0.22	0.21 0.23	0.09 0.22	-0.01 0.23	-0.01 0.20
	400mg	6.31 *0.21*	-0.38 0.24	-0.24 0.16	-0.24 0.19	-0.22 0.19	-0.28 0.20	-0.02 0.16	-0.26 0.21
Immediate word recall (N=30)									
Correct	placebo	9.47 *0.65*	-0.10 0.64	-0.83 0.42	-0.63 0.53	0.40 0.46	0.00 0.48	0.80 0.54	-0.30 0.44
	200mg	9.80 *0.72*	-0.27 0.53	-0.63 0.60	-1.27 0.45	0.20 0.48	-0.60 0.46	-0.50 0.69	-0.17 0.49
	400mg	9.33 *0.55*	-0.83 0.47	-0.30 0.42	-0.33 0.46	0.07 0.45	-0.83 0.48	-0.70 0.43	0.77 0.51
Errors	placebo	0.47 *0.12*	-0.20 0.14	-0.20 0.15	-0.13 0.11	-0.20 0.13	-0.23 0.14	-0.20 0.18	0.03 0.17
	200mg	0.53 *0.16*	-0.20 0.15	-0.03 0.21	-0.10 0.16	-0.27 0.13	-0.30 0.16	-0.23 0.13	-0.07 0.19
	400mg	0.20 *0.07*	0.03 0.11	0.20 0.14	0.10 0.11	-0.10 0.07	0.27 0.19	0.03 0.11	0.30 0.15
Intrusions	placebo	0.13 *0.10*	-0.07 0.13	-0.07 0.12	-0.13 0.10	-0.13 0.10	-0.10 0.11	-0.13 0.10	-0.13 0.10
	200mg	0.17 *0.11*	-0.10 0.12	-0.13 0.11	-0.13 0.11	0.03 0.13	-0.07 0.13	-0.17 0.11	-0.13 0.11
	400mg	0.07 *0.05*	-0.03 0.06	0.00 0.07	0.00 0.07	-0.07 0.05	0.03 0.06	-0.07 0.05	0.03 0.06

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
Delayed word recall (N=30)									
Correct	placebo	7.10 *0.61*	-0.47 0.45	-0.27 0.48	-1.37 0.49	0.63 0.39	-1.67 0.49	-0.70 0.52	-1.30 0.51
	200mg	7.23 *0.65*	-1.17 0.48	-0.63 0.41	-1.23 0.43	0.10 0.53	-0.63 0.62	-1.93 0.54	-0.53 0.48
	400mg	7.27 *0.59*	-1.03 0.38	0.07 0.49	-1.13 0.55	0.20 0.44	-1.33 0.38	-1.13 0.55	-1.70 0.58
Errors	placebo	0.60 *0.31*	-0.43 0.32	-0.27 0.32	-0.07 0.11	-0.13 0.32	0.27 0.17	-0.17 0.21	-0.23 0.27
	200mg	0.53 *0.19*	-0.33 0.29	-0.20 0.32	0.03 0.14	-0.30 0.20	0.10 0.13	-0.30 0.20	-0.27 0.18
	400mg	0.20 *0.07*	-0.33 0.33	-0.30 0.34	0.23 0.18	0.00 0.08	-0.07 0.12	-0.17 0.19	-0.13 0.20
intrusions	placebo	0.07 *0.05*	0.10 0.13	0.17 0.14	0.10 0.06	-0.03 0.03	0.10 0.09	0.03 0.08	0.07 0.10
	200mg	0.07 *0.05*	0.17 0.15	0.10 0.13	0.13 0.14	0.07 0.07	0.07 0.11	0.17 0.13	-0.03 0.06
	400mg	0.03 *0.03*	0.10 0.10	0.07 0.10	0.10 0.09	0.00 0.05	0.07 0.08	-0.03 0.06	0.10 0.12
Delayed word recognition (N=30)									
targets	placebo	15.04 *0.68*	0.39 0.55	-0.82 0.45	-1.11 0.47	-0.07 0.55	-0.11 0.53	-0.43 0.55	-0.36 0.50
	200mg	15.39 *0.57*	-0.89 0.49	-1.36 0.70	-1.14 0.57	-1.04 0.59	-1.04 0.68	-1.46 0.88	-1.04 0.62
	400mg	15.18 *0.60*	-0.54 0.61	-0.64 0.65	-0.79 0.66	-0.50 0.52	-0.86 0.54	-0.07 0.69	-0.93 0.63
errors	placebo	4.96 *0.68*	-0.07 0.72	1.57 0.72	2.86 0.71	0.07 0.55	0.57 0.73	1.68 0.74	1.57 0.79
	200mg	4.61 *0.57*	2.32 0.64	2.79 0.97	2.57 0.72	1.04 0.59	2.25 0.86	3.00 1.03	3.11 0.97
	400mg	4.82 *0.60*	1.64 0.78	1.18 0.66	1.71 0.93	0.50 0.52	1.29 0.65	0.96 0.79	1.75 1.02
distractors	placebo	17.96 *0.48*	-0.32 0.42	-0.75 0.47	-1.75 0.49	0.86 0.50	-0.46 0.47	-1.25 0.56	-1.21 0.56
	200mg	18.50 *0.32*	-1.11 0.54	-0.54 0.46	-0.93 0.62	-0.29 0.39	-0.43 0.50	-0.89 0.67	-0.82 0.73
	400mg	17.46 *0.49*	-1.43 0.42	-1.43 0.62	-1.43 0.45	0.43 0.49	-1.21 0.50	-1.54 0.48	-2.07 0.67
false alarms	placebo	2.04 *0.48*	0.32 0.42	0.75 0.47	1.75 0.49	-0.86 0.50	0.46 0.47	1.25 0.56	1.21 0.56
	200mg	1.50 *0.32*	1.11 0.54	0.54 0.46	0.93 0.62	0.29 0.39	0.43 0.50	0.89 0.67	0.82 0.73
	400mg	2.54 *0.49*	1.43 0.42	1.43 0.62	1.43 0.45	-0.43 0.49	1.21 0.50	1.54 0.48	2.07 0.67
S I	placebo	0.70 *0.03*	-0.01 0.03	-0.09 0.04	-0.15 0.04	0.03 0.04	-0.04 0.04	-0.10 0.04	-0.09 0.04
	200mg	0.72 *0.03*	-0.08 0.04	-0.05 0.03	-0.08 0.04	-0.05 0.03	-0.06 0.03	-0.03 0.04	-0.08 0.05
	400mg	0.67 *0.04*	-0.12 0.03	-0.13 0.05	-0.13 0.03	0.01 0.03	-0.10 0.04	-0.15 0.05	-0.15 0.05

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
reaction time (ms)	placebo	0.97 *0.04*	0.11 0.06	-0.01 0.04	-0.05 0.05	0.01 0.03	0.03 0.06	0.06 0.09	0.03 0.05
	200mg	0.97 *0.03*	0.03 0.04	0.05 0.06	0.04 0.06	0.00 0.06	0.03 0.04	0.11 0.09	-0.04 0.06
	400mg	0.98 *0.05*	-0.10 0.05	-0.15 0.07	-0.03 0.12	-0.05 0.05	-0.08 0.06	-0.12 0.08	-0.08 0.11
Zero back (N=24)									
Target %	placebo	97.57 *0.79*	-0.60 1.32	-2.11 1.29	-1.49 0.88	-0.02 1.08	-7.44 4.05	-0.05 0.62	-4.24 1.98
	200mg	95.44 *2.18*	-0.39 2.29	0.96 2.66	2.15 2.30	0.92 2.50	1.24 3.04	0.00 2.41	-0.27 2.83
	400mg	97.23 *1.34*	-0.92 1.06	-0.92 1.06	-1.17 0.82	-0.82 0.95	-1.81 1.04	-1.95 0.96	-0.55 1.24
errors	placebo	0.29 *0.10*	0.08 0.18	0.33 0.18	0.21 0.13	0.00 0.14	1.04 0.53	0.04 0.11	0.58 0.28
	200mg	0.63 *0.30*	-0.04 0.30	-0.21 0.35	-0.38 0.32	-0.13 0.35	-0.17 0.42	0.00 0.34	0.00 0.40
	400mg	0.29 *0.16*	0.21 0.12	0.21 0.12	0.17 0.12	0.17 0.09	0.29 0.13	0.21 0.08	0.13 0.16
distractors %	placebo	98.12 *0.76*	0.68 0.87	1.09 0.84	0.82 0.78	0.56 0.89	0.14 1.16	1.22 0.97	0.54 1.24
	200mg	97.19 *1.15*	1.08 1.28	1.60 1.44	1.61 1.37	1.35 1.25	1.21 1.38	2.14 1.30	2.15 1.27
	400mg	98.68 *0.39*	-1.45 1.16	0.92 0.40	0.51 0.37	-1.10 1.46	-2.30 3.43	-2.04 3.04	-3.25 3.17
false alarms	placebo	0.33 *0.16*	-0.17 0.16	-0.25 0.19	0.00 0.17	-0.08 0.18	0.04 0.28	-0.29 0.19	-0.08 0.23
	200mg	0.75 *0.36*	-0.42 0.40	-0.54 0.42	-0.38 0.42	-0.63 0.36	-0.33 0.42	-0.58 0.39	-0.63 0.39
	400mg	0.21 *0.08*	0.13 0.16	-0.21 0.08	-0.13 0.09	0.00 0.11	-0.21 0.08	-0.17 0.10	0.08 0.20
targets RT	placebo	0.61 *0.03*	-0.01 0.03	-0.04 0.03	0.01 0.04	0.00 0.03	0.05 0.05	-0.02 0.03	0.01 0.05
	200mg	0.65 *0.04*	-0.04 0.03	0.02 0.03	0.00 0.03	-0.01 0.02	0.05 0.06	-0.04 0.02	-0.02 0.03
	400mg	0.66 *0.04*	-0.05 0.03	-0.09 0.04	-0.07 0.03	-0.05 0.03	-0.07 0.04	-0.06 0.03	-0.05 0.04
distractor RT	placebo	0.58 *0.03*	-0.01 0.02	-0.05 0.02	-0.03 0.02	0.01 0.03	-0.03 0.03	-0.03 0.03	-0.04 0.04
	200mg	0.62 *0.04*	-0.02 0.03	0.00 0.03	-0.06 0.02	0.00 0.03	-0.04 0.03	-0.05 0.03	-0.07 0.03
	400mg	0.60 *0.04*	-0.03 0.03	-0.06 0.03	-0.04 0.03	-0.02 0.03	-0.06 0.03	-0.08 0.02	-0.04 0.03
average RT	placebo	0.59 *0.03*	-0.01 0.02	-0.05 0.02	-0.02 0.03	0.01 0.03	-0.02 0.03	-0.03 0.03	-0.03 0.04
	200mg	0.63 *0.04*	-0.03 0.03	0.01 0.03	-0.04 0.02	0.00 0.02	-0.01 0.04	-0.04 0.02	-0.06 0.03
	400mg	0.61 *0.03*	-0.04 0.03	-0.07 0.03	-0.05 0.03	-0.03 0.02	-0.06 0.03	-0.07 0.02	-0.04 0.03
S I	placebo	0.97 *0.01*	0.00 0.01	-0.01 0.01	-0.01 0.01	0.00 0.01	-0.06 0.04	0.01 0.01	-0.03 0.02
	200mg	0.94 *0.03*	0.01 0.02	0.02 0.03	0.03 0.02	0.03 0.03	0.02 0.03	0.02 0.03	0.02 0.03
	400mg	0.97 *0.01*	-0.02 0.01	0.00 0.01	-0.01 0.01	-0.01 0.01	-0.01 0.01	-0.01 0.01	-0.01 0.01

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
One back (N=24)									
target %	placebo	85.51 *3.62*	1.08 2.97	-6.07 4.38	-5.15 4.95	-1.17 3.11	-4.26 4.44	-4.74 4.28	-0.76 4.43
	200mg	87.66 *2.81*	0.05 2.52	-8.77 4.33	-7.97 3.50	1.88 1.91	-5.40 2.65	-5.56 2.53	-3.94 2.77
	400mg	85.35 *4.13*	-4.33 4.89	-5.36 4.29	-0.87 3.91	-1.44 3.22	-1.10 5.27	1.95 4.32	-5.52 4.87
errors	placebo	2.00 *0.50*	-0.21 0.41	0.83 0.61	0.67 0.69	-0.13 0.45	0.46 0.59	0.58 0.58	-0.04 0.53
	200mg	1.71 *0.39*	-0.08 0.34	0.71 0.47	1.04 0.47	-0.29 0.29	0.63 0.34	0.75 0.35	0.42 0.38
	400mg	1.92 *0.53*	0.63 0.65	0.79 0.57	0.21 0.50	0.17 0.42	0.13 0.68	-0.33 0.57	0.50 0.60
distractors %	placebo	95.78 *2.31*	2.71 2.46	2.30 2.56	2.43 2.52	2.31 2.40	2.43 2.30	2.03 2.72	2.16 2.55
	200mg	98.76 *0.55*	-1.39 0.75	-3.81 1.91	-0.82 0.41	-0.14 0.43	-0.81 0.75	-0.13 0.58	-1.07 0.82
	400mg	96.98 *0.74*	-0.86 1.86	-0.82 1.52	0.83 0.72	1.36 1.15	0.59 1.77	-1.39 3.13	-3.04 3.57
false alarms	placebo	0.92 *0.69*	-0.50 0.74	-0.33 0.79	-0.58 0.77	-0.67 0.71	-0.75 0.76	-0.58 0.78	-0.50 0.77
	200mg	0.33 *0.13*	0.17 0.16	0.42 0.21	0.00 0.16	0.04 0.13	0.13 0.20	0.04 0.14	0.08 0.22
	400mg	0.63 *0.13*	-0.13 0.20	-0.04 0.15	-0.13 0.19	-0.42 0.12	-0.42 0.15	-0.33 0.28	-0.21 0.29
targets RT	placebo	0.65 *0.04*	0.02 0.04	-0.04 0.04	-0.02 0.03	-0.01 0.03	-0.04 0.03	0.02 0.05	-0.01 0.03
	200mg	0.70 *0.04*	0.02 0.03	0.07 0.05	-0.02 0.03	-0.05 0.03	-0.05 0.03	-0.02 0.03	-0.04 0.03
	400mg	0.67 *0.03*	-0.01 0.02	0.05 0.04	-0.01 0.04	-0.04 0.02	-0.04 0.03	-0.02 0.04	-0.02 0.04
distractor RT	placebo	0.64 *0.04*	-0.03 0.03	-0.05 0.04	-0.04 0.04	-0.04 0.03	-0.03 0.04	-0.04 0.04	-0.03 0.04
	200mg	0.66 *0.04*	-0.01 0.02	0.06 0.04	-0.05 0.03	-0.02 0.04	-0.05 0.03	-0.02 0.04	-0.04 0.03
	400mg	0.65 *0.03*	-0.02 0.02	-0.04 0.03	-0.05 0.03	-0.04 0.03	-0.08 0.03	-0.06 0.03	-0.05 0.05
average RT	placebo	0.64 *0.04*	-0.02 0.03	-0.05 0.03	-0.03 0.03	-0.03 0.03	-0.03 0.04	-0.03 0.04	-0.03 0.04
	200mg	0.67 *0.04*	-0.01 0.02	0.06 0.04	-0.04 0.03	-0.03 0.03	-0.05 0.03	-0.03 0.03	-0.05 0.03
	400mg	0.66 *0.03*	-0.03 0.02	-0.03 0.03	-0.04 0.03	-0.04 0.03	-0.08 0.03	-0.06 0.04	-0.03 0.05
S I	placebo	0.85 *0.04*	0.02 0.04	-0.04 0.03	-0.02 0.05	0.02 0.03	0.00 0.03	-0.02 0.03	0.01 0.04
	200mg	0.88 *0.02*	-0.01 0.02	-0.09 0.03	-0.05 0.03	0.01 0.02	-0.05 0.02	-0.04 0.02	-0.03 0.02
	400mg	0.85 *0.03*	-0.03 0.04	-0.04 0.03	-0.01 0.03	0.01 0.02	0.02 0.04	0.03 0.04	-0.02 0.04

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
Two back (N=24)									
target %	placebo	81.94 *4.55*	-3.75 3.30	0.00 3.56	-6.00 4.66	0.71 3.12	-0.11 4.76	-6.36 5.77	-3.78 5.27
	200mg	84.00 *3.59*	3.14 2.81	-4.49 2.19	-8.49 3.37	0.41 3.29	-4.06 4.18	-1.81 3.05	-1.56 3.58
	400mg	83.40 *4.17*	1.74 3.47	-0.30 3.95	-1.63 4.32	-1.03 3.70	-5.22 5.12	1.95 2.86	-2.04 3.63
errors	placebo	2.33 *0.58*	0.58 0.43	0.08 0.46	0.79 0.63	-0.17 0.42	-0.13 0.68	0.79 0.80	0.63 0.74
	200mg	2.00 *0.41*	-0.38 0.31	0.46 0.25	1.21 0.43	0.00 0.36	0.42 0.48	0.33 0.30	0.29 0.41
	400mg	2.08 *0.55*	-0.21 0.50	0.13 0.55	0.25 0.58	0.33 0.55	0.63 0.70	-0.21 0.39	0.21 0.46
distractors %	placebo	95.88 *1.08*	1.69 0.97	0.88 0.98	0.70 1.03	-0.47 1.44	0.68 1.17	-0.94 2.01	0.81 1.22
	200mg	97.43 *0.75*	-1.67 0.86	-1.99 1.82	0.30 0.64	0.57 1.06	-1.35 1.26	0.28 1.03	-1.52 1.13
	400mg	92.45 *3.85*	4.26 3.91	4.14 4.20	5.40 4.20	2.83 4.27	4.75 4.54	2.12 4.93	1.83 5.51
false alarms	placebo	0.96 *0.26*	-0.50 0.26	-0.50 0.25	-0.25 0.23	-0.33 0.25	-0.54 0.26	-0.08 0.49	-0.67 0.30
	200mg	0.67 *0.21*	0.08 0.23	0.17 0.36	-0.13 0.19	-0.38 0.23	0.08 0.27	-0.25 0.21	0.29 0.27
	400mg	1.63 *1.07*	-1.04 1.14	-1.13 1.18	-1.21 1.18	-1.04 1.06	-1.46 1.17	-1.17 1.05	-0.92 1.22
targets RT	placebo	0.75 *0.05*	-0.01 0.05	0.01 0.05	0.01 0.06	0.02 0.04	0.01 0.04	0.03 0.06	0.06 0.05
	200mg	0.85 *0.05*	-0.07 0.04	-0.06 0.03	-0.06 0.04	-0.07 0.03	-0.10 0.04	-0.03 0.04	-0.08 0.04
	400mg	0.80 *0.04*	-0.01 0.05	-0.03 0.06	-0.08 0.05	-0.04 0.05	-0.04 0.06	-0.05 0.05	-0.06 0.05
distractor RT	placebo	0.73 *0.05*	-0.01 0.02	-0.01 0.03	-0.01 0.03	-0.03 0.02	-0.01 0.03	-0.02 0.03	-0.07 0.03
	200mg	0.78 *0.05*	-0.02 0.04	-0.07 0.03	-0.02 0.05	-0.04 0.03	-0.06 0.04	-0.01 0.04	-0.07 0.04
	400mg	0.80 *0.05*	-0.04 0.04	-0.10 0.04	-0.10 0.05	-0.08 0.04	-0.10 0.05	-0.12 0.05	-0.13 0.05
average RT	placebo	0.74 *0.04*	-0.01 0.02	-0.01 0.03	-0.01 0.04	-0.02 0.02	0.00 0.03	-0.01 0.03	-0.04 0.03
	200mg	0.80 *0.05*	-0.04 0.03	-0.07 0.03	-0.04 0.04	-0.05 0.03	-0.07 0.04	-0.01 0.04	-0.08 0.03
	400mg	0.80 *0.05*	-0.03 0.04	-0.08 0.04	-0.09 0.05	-0.07 0.04	-0.09 0.05	-0.10 0.05	-0.11 0.05
S I	placebo	0.81 *0.05*	0.01 0.03	0.04 0.03	-0.02 0.03	0.04 0.03	0.04 0.05	-0.02 0.05	0.02 0.05
	200mg	0.85 *0.03*	0.02 0.02	-0.05 0.02	-0.06 0.03	0.02 0.03	-0.04 0.03	0.00 0.02	-0.03 0.03
	400mg	0.80 *0.07*	0.06 0.07	0.05 0.08	0.04 0.08	0.04 0.07	0.03 0.08	0.06 0.06	0.02 0.07

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
Three back (N=24)									
target %	placebo	68.13 *4.96*	-4.08 4.54	-3.64 4.41	-1.81 4.12	1.99 4.66	-0.25 5.64	1.21 4.92	1.97 4.86
	200mg	70.60 *4.72*	-1.92 4.86	-2.31 2.85	-1.17 3.39	-0.32 2.90	-8.74 4.04	-1.26 4.31	-4.42 3.54
	400mg	63.69 *4.66*	6.41 3.48	1.74 3.40	6.25 3.89	7.05 3.87	8.36 3.14	8.93 3.11	4.60 3.45
errors	placebo	4.25 *0.67*	0.38 0.66	0.42 0.57	0.04 0.55	-0.46 0.61	-0.25 0.73	-0.46 0.63	-0.38 0.65
	200mg	3.67 *0.55*	0.25 0.53	0.25 0.34	0.46 0.39	0.08 0.37	1.21 0.49	0.29 0.51	0.83 0.41
	400mg	4.42 *0.55*	-0.54 0.53	-0.13 0.48	-0.63 0.53	-0.67 0.54	-0.83 0.50	-0.92 0.44	-0.21 0.53
distractors %	placebo	92.73 *1.59*	-0.22 1.34	-2.14 1.67	-1.05 1.51	1.64 1.71	0.30 2.15	0.68 1.64	0.90 1.55
	200mg	93.55 *1.54*	-2.74 1.90	-3.10 2.16	-1.27 1.07	-0.37 1.56	-1.16 1.48	-0.18 1.63	-1.80 1.41
	400mg	84.62 *4.42*	6.00 4.37	7.82 3.87	5.95 4.49	6.69 5.25	7.09 5.30	7.09 5.23	6.80 4.42
false alarms	placebo	1.58 *0.31*	-0.04 0.34	0.58 0.34	0.17 0.37	-0.29 0.33	-0.50 0.39	-0.58 0.39	-0.42 0.28
	200mg	1.71 *0.38*	0.58 0.38	0.21 0.39	0.00 0.26	-0.63 0.21	0.00 0.31	-0.13 0.42	0.33 0.30
	400mg	3.67 *1.22*	-1.50 1.29	-2.08 1.17	-1.75 1.19	-2.21 1.28	-2.42 1.27	-2.25 1.27	-2.08 1.20
targets RT	placebo	0.93 *0.06*	-0.15 0.04	-0.10 0.06	-0.14 0.04	-0.02 0.05	-0.06 0.05	-0.09 0.05	-0.04 0.05
	200mg	0.89 *0.07*	0.02 0.05	-0.10 0.04	-0.06 0.04	-0.02 0.05	-0.04 0.05	-0.03 0.05	-0.12 0.04
	400mg	0.99 *0.08*	-0.18 0.06	-0.20 0.07	-0.17 0.06	-0.13 0.05	-0.15 0.06	-0.19 0.06	-0.12 0.10
distractor RT	placebo	0.88 *0.06*	-0.08 0.04	-0.09 0.04	-0.12 0.04	-0.07 0.04	-0.10 0.04	-0.08 0.04	-0.14 0.03
	200mg	0.78 *0.04*	-0.01 0.03	-0.03 0.03	-0.05 0.03	0.03 0.03	0.01 0.03	0.04 0.04	-0.08 0.03
	400mg	0.89 *0.06*	-0.09 0.02	-0.14 0.03	-0.12 0.03	-0.06 0.04	-0.12 0.04	-0.16 0.04	-0.11 0.06
average RT	placebo	0.88 *0.06*	-0.10 0.03	-0.10 0.04	-0.13 0.04	-0.06 0.04	-0.08 0.04	-0.08 0.03	-0.11 0.03
	200mg	0.81 *0.05*	0.00 0.03	-0.04 0.03	-0.05 0.03	0.01 0.04	-0.01 0.03	0.02 0.04	-0.09 0.03
	400mg	0.92 *0.06*	-0.11 0.03	-0.16 0.04	-0.14 0.03	-0.08 0.03	-0.13 0.04	-0.17 0.05	-0.12 0.06
S I	placebo	0.69 *0.04*	-0.05 0.04	-0.09 0.04	-0.05 0.04	0.00 0.04	0.02 0.04	0.02 0.05	0.02 0.03
	200mg	0.69 *0.05*	-0.05 0.06	-0.04 0.04	-0.01 0.03	0.03 0.03	-0.05 0.04	-0.01 0.05	-0.06 0.04
	400mg	0.55 *0.08*	0.11 0.07	0.10 0.06	0.13 0.06	0.16 0.07	0.17 0.05	0.18 0.05	0.12 0.06

			POST-DOSE DAY 1				POST-DOSE DAY 8			
		Baseline day 1 *se*	1hour se	2.5hour se	4hour se	Pre-dose day 8 se	1hour se	2.5hour se	4hour Se
Random number generation (N=23)									
R	placebo	4.09 *0.79*	0.56 0.44	0.14 0.49	-0.76 0.61	0.07 0.35	0.50 0.44	0.30 0.61	0.95 0.64
	200mg	3.56 *0.51*	0.61 0.44	1.05 0.40	1.16 0.54	0.52 0.37	1.87 1.20	1.09 1.14	2.46 1.32
	400mg	3.69 *0.48*	-0.02 0.62	0.98 0.61	-0.11 0.52	1.16 0.74	1.04 0.81	1.24 0.82	-0.60 0.32
RNG	placebo	0.30 *0.02*	0.01 0.01	-0.01 0.02	-0.01 0.01	0.01 0.01	0.00 0.02	-0.01 0.02	-0.01 0.01
	200mg	0.31 *0.01*	-0.01 0.01	0.00 0.02	0.00 0.01	0.01 0.01	0.00 0.02	-0.01 0.01	0.02 0.02
	400mg	0.31 *0.01*	-0.01 0.02	0.00 0.02	-0.02 0.01	-0.01 0.02	0.01 0.02	0.00 0.02	-0.01 0.01
NSQ	placebo	53.20 *1.37*	0.75 1.16	1.19 1.93	-0.83 1.28	-0.83 1.16	2.46 1.24	0.48 1.32	1.49 1.62
	200mg	54.00 *1.17*	-0.35 1.26	0.26 1.49	1.67 1.26	0.28 1.54	0.70 1.62	0.04 1.73	1.62 1.83
	400mg	52.48 *1.10*	0.48 1.32	1.67 1.29	-0.66 1.18	0.76 1.20	2.99 1.25	1.71 1.70	0.13 0.98
RNG2	placebo	0.25 *0.01*	0.01 0.01	-0.01 0.02	-0.01 0.01	0.01 0.01	0.00 0.02	0.01 0.01	0.01 0.01
	200mg	0.26 *0.01*	-0.01 0.01	0.00 0.01	0.00 0.01	-0.02 0.01	0.01 0.02	-0.01 0.01	0.02 0.02
	400mg	0.26 *0.01*	-0.02 0.01	0.00 0.02	-0.01 0.01	-0.02 0.01	0.01 0.01	-0.02 0.02	-0.02 0.01
TPI	placebo	0.72 *0.07*	-0.03 0.07	0.15 0.09	-0.01 0.11	0.25 0.07	0.19 0.11	0.07 0.10	0.05 0.09
	200mg	0.79 *0.10*	0.00 0.11	0.02 0.11	0.06 0.12	-0.06 0.07	0.04 0.11	-0.04 0.11	0.10 0.18
	400mg	0.75 *0.07*	-0.01 0.09	0.06 0.06	0.06 0.06	0.05 0.08	0.03 0.11	0.07 0.08	0.10 0.07
RUNS	placebo	19.07 *1.94*	1.16 2.15	1.36 2.65	2.06 2.06	5.18 3.72	3.56 2.90	4.37 2.90	2.60 3.18
	200mg	22.92 *2.84*	-0.37 2.82	-2.89 3.47	-1.42 2.77	-2.07 3.13	4.54 4.54	-1.10 2.90	-2.07 3.37
	400mg	20.90 *2.46*	4.33 4.95	0.35 3.17	-0.90 2.96	-3.30 2.39	5.04 3.68	0.81 1.63	3.72 3.56

4.3.2 Sub-chronic effects

There were no significant treatment effects revealed for any outcome measure. Mean pre-dose data for day 8 (change from day 1 baseline), for each treatment, for each outcome measure are represented in Table 4.1.

4.3.3 Acute effects

4.3.3.1 Bond Lader visual analogue scale:

The initial ANOVA revealed a significant main effect of treatment [F (2,116) = 4.64, P = 0.014] on subjective self-report ratings of calmness, as assessed by the Bond Lader visual analogue scales (see Figure 4.1, 1a and Table 4.1). Planned comparisons (Figure 4.1, 1b and Table 4.1) comparing each treatment to placebo, at each time point, on day 1 and day 8 revealed that 200 mg significantly improved calmness at 2.5hours [t (116) = 2.56, P = 0.012; d=0.5], and 4 hours [t (116) = 4.99, P = 0.000002; d=0.9] post treatment on day 1. 200 mg also led to significant improved calmness ratings at 1hour [t (116) = 2.21, P = 0.029; d=0.6], and 4 hours [t (116) = 2.46, P=0.015; d=0.5] post treatment on day 8. 400 mg improved calmness at 2.5hours [t (116) = 2.74, P = 0.007; d=0.5], and 4hours [t (116) = 4.57, P = 0.000012; d=0.8] post treatment on day 1 only.

4.3.3.2 3 Back reaction time

There was a significant main effect of treatment on average reaction time [F(2,92) = 5.64,P = 0.006] (Figure 4.1, 2a and table 4.1). Planned comparisons revealed (Figure 4.1, 2b) that, compared to placebo, 400 mg led to significantly faster response times at 2.5 hours post dose on day 1 [t (92) = 2.30, P = 0.023; d=0.3] and day 8 [t (92) = 3.37, P = 0.001; d=0.5]. However, 200mg led to significantly

slower response times on day 1 at 1hour [t (92) = 3.664, P = 0.0004; d=0.6], 2.5 hours [t (92) = 2.021, P=0.046; d=0.3], 4hours [t (92) = 3.046, P = 0.003; d=0.5]. Similarly, on day 7, 200 mg led to significantly slower response times at 1hour [t (92) = 3.938, P = 0.004; d=0.4] and 2.5 hours [t (92) = 4.017, P = 0.00012; d=0.6] post dose.

4.3.3.3 3 Back sensitivity index

There was a significant main effect of treatment on sensitivity index [F (2,92) = 6.57, P= 0.003] (Figure 4.1,3a and Table 4.1). Planned comparisons (Figure 4.1,3b and Table 4.1) revealed that, compared to placebo, the 400 mg dose led to significantly improved sensitivity on day 1 at 1 hour [t (92) = 4.229, P = 0.00006; d=0.7], 2.5 hours [t (92) = 5.15, P = 0.0000015; d=0.9], and 4 hours [t (92) = 4.866, P = 0.000005; d=0.8]. Similarly, 400 mg led to significantly improved sensitivity on day 8 at 1 hour [t (92) = 4.384, P = 0.00003; d=0.9], 2.5 hours [t (92) = 4.38, P = 0.00003; d=0.6] and 4 hours [t (92) = 2.91, P = 0.004; d=0.6] post treatment. However, 200 mg led to significant impairment in sensitivity on day 8 at 4 hours [t (92) = 2.25, P = 0.02; d=0.5] post treatment.

4.3.3.4 Immediate word recall

The initial ANOVA revealed a significant treatment x session interaction [F (4,116) = 2.47, P = 0.048]. The interaction and planned comparisons were uninterruptible and render any further discussion of these results open to type 1 error. Planned comparisons revealed one single time point improvement for 200 mg and one single time point decrement following 400 mg.

4.3.4 Superimposed effects

4.3.4.1 1 Back Reaction time

The initial ANOVA revealed a significant treatment x day interaction on reaction time for the 1 back task [F (92) = 3.35, P = 0.044]. The pattern of results would indicate that repeated dosing ameliorates an initial slowing in performance following 200 mg and further speeds performance following 400 mg. However, planned comparisons comparing each treatment to placebo at each time point revealed only one single significant time point slowing following 200 mg on day 1 and no significant differences between treatments and placebo on day 8. Therefore, to control the risk of type one error, further discussion of this result will be limited.

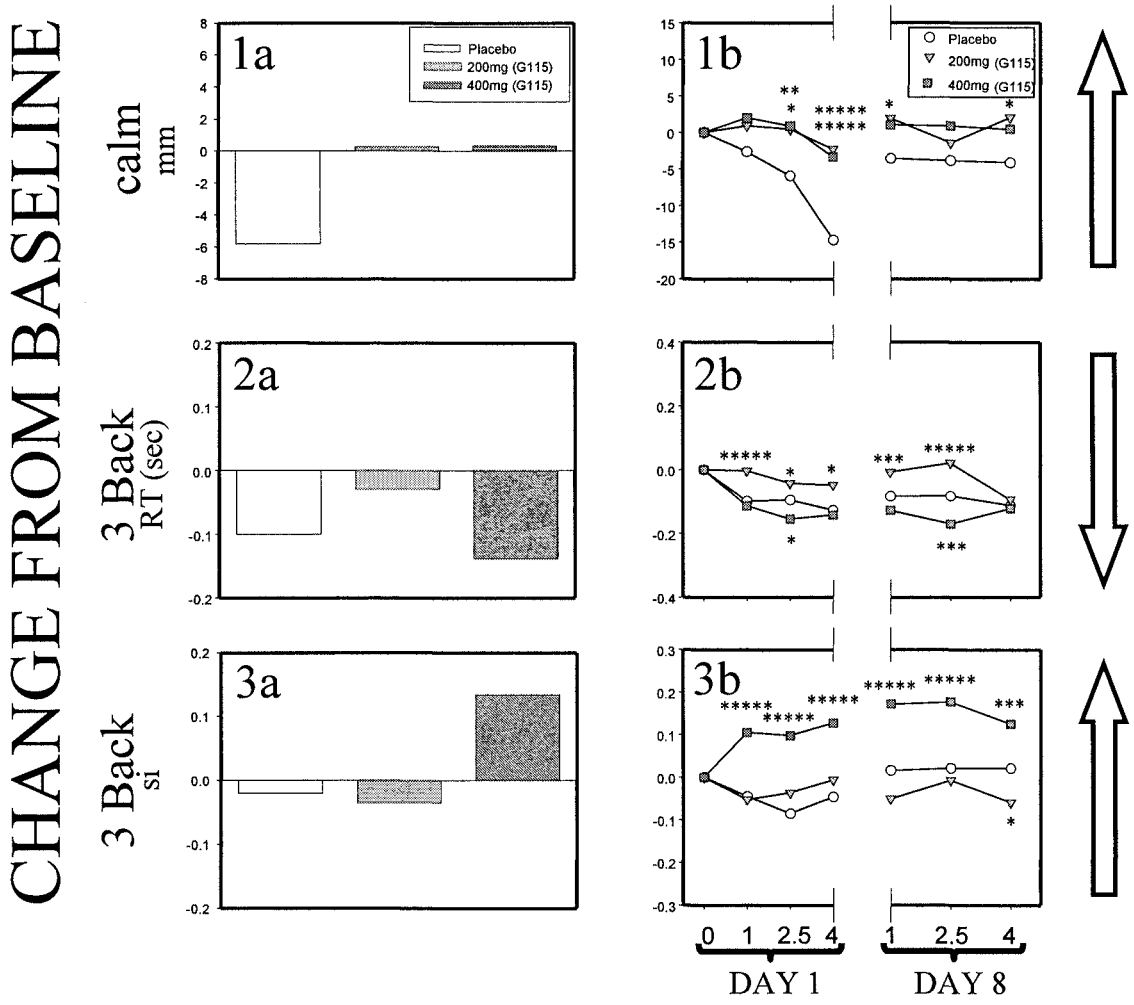

Figure 4: Panel 1a, 2a, and 3a depicts a significant main effect of ginseng treatment on self-reported ratings of calmness, average reaction time for the 3 back task and sensitivity index for the 3 back task respectively, in young healthy volunteers. Values represent "change from baseline" group means summed across day (post dose day 1 and post dose day 8) and session (1, 2.5, 4hours post dose). Panel 1b, 2b and 3b depicts the pattern of results broken down over day (day 1 and day 8) and session (1, 2.5 and 4 hours post dose) for placebo, 200 mg and 400 mg of *P. ginseng* (G115). Asterisks represent significance of planned comparison as compared to placebo. Values represent "change from day 1 pre-dose baseline". Directional arrows indicate improvements on those measures. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$ *****, $p<0.0005$). Significance is compared with placebo.

4.4. DISCUSSION

The results of the present study demonstrate that acute single doses of *Panax ginseng* (both on day 1 and on day 8) can improve participants' subjective ratings of calmness and improve and impair working memory performance (depending on dose) in healthy young volunteers. The study also revealed that there were no sub-chronic effects, although, there was a single outcome measure suggesting the superimposed behavioural effects of *Panax ginseng.* However, due to the exploratory nature of this study and for caution and risk of type 1 error there will be no further discussion of this pattern of results.

With regards to mood; the initial ANOVA revealed a significant main effect (means summed across session and day) of *Panax ginseng* treatment on subjective self-reports of calmness. This main effect indicates a 'simple' acute treatment effect on day 1 and day 8 with no effect/interaction of/with sub-chronic ingestion. Planned comparisons revealed that both 200 mg and 400 mg G115 led to significant ameliorations in subjective self-reports of calmness at the latter 2 testing session on day 1. Additionally, 200 mg significantly improved subjective calmness ratings at the first and last testing sessions on day 8, as compared to placebo. The present result may represent ginseng's 'anti-stress' adaptogenic properties as inspection of the pattern of results suggests that the volunteers are becoming less calm as the day progresses (i.e. in the placebo group). This may be related to the battery itself or to unidentified external influences (i.e. the working day). However, unfortunately there was no objective or subjective measure of stress levels taken in the present study.

Mood, which may fall under the umbrella term of 'quality of life' and 'well-being' has been investigated in numerous studies. Ginseng or its active components have been associated with improvements in such measures in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations [although findings of this nature are by no means unequivocal (Kennedy and Scholey, 2003)] as well as attenuating the effects of fatigue in night nurses (Wesnes *et al.*, 2003) and that associated with sustained mental performance (chapter 2 and chapter 3).

With regards to cognitive performance the initial analyses revealed a significant main effect of treatment on the speed of performing the most demanding version of the N back task (3 back). Again this result indicates a simple acute effect of ginseng treatment. Planned comparisons revealed that 200 mg led to a significant slowing in performing of the 3 back task at all but the very last testing session on day 8, as compared with placebo. Conversely, 400 mg was associated with a significant speeding in performance of the same task at the mid-testing session on day 1 and day 8. Interestingly, this mid-session testing commenced between 12.15 and 12.30 when hunger and tiredness may have been highest following a full morning's works (this session took place before participants had eaten lunch). This may be further evidence of ginseng's ability to alleviate a fall in performance that we might expect when fatigued or stressed (see chapters 2 and 3).

The ANOVA also revealed a significant main effect of treatment on sensitivity index of performing the 3 back task. Once again this is indicative of a simple acute effect. Planned comparisons revealed that 400 mg led to significant

improvements in the accuracy of performing the 3 back task at all post-dose testing sessions, as compared to placebo. Conversely, 200 mg led to a significant decrement in performance at the last testing session on day 8.

The behavioural effects of *Panax ginseng* on working memory performance, revealed in the present study, are in direct contrast to repeated failures to elicit any behavioural effect, of any dose, on any direct parameter/measure of working memory performance previously utilised (see Kennedy at al 2003). However, acute improvements and decrements have been reported in the performance of mental arithmetic tasks, which will load heavily on working memory resources (Scholey and Kennedy, 2002; Chapter 1 and Chapter 2).

The significant slowing in working memory performing revealed in the present study, following 200 mg, is consistent with the observation of slower performance in other cognitive domains, previously reported in a different sample drawn from the same population (i.e. young healthy adults), following the same 200 mg dose. For instance, significant slowing in the performance of attentional tasks (Kennedy *et al.*, 2001) and a mental arithmetic task (Scholey and Kennedy, 2002). However, the reported slowing in performance is not consistent with the recent findings of faster memory, attention, and serial subtraction task performance (Kennedy et al., 2004; chapter 2 and chapter 3), and decreased latency of the P300 component of auditory evoked potentials following the same 200 mg dose (Kennedy *et al.,* 2003). This pattern of results may suggest that 200 mg will affect the speed of performing cognitive tasks.

However, the improved accuracy in working memory performance following 400 mg dose is somewhat consistent with the improved accuracy of performing a mental arithmetic task (Scholey and Kennedy, 2002) and faster responses on an

attentional task (Sünram-Lea et al., 2004). However, is inconsistent with the repeated failure to find any effect on previous measures of working memory performance (see Kennedy et al 2003). The pattern of results may suggest that 400 mg has an effect on the accuracy of performing cognitive tasks.

The present study was specifically designed to investigate ginseng's effects on working memory performance. The observed improvements in working memory are the first to be reported. To date, the most consistent findings, following an acute dose of 400 mg *Panax ginseng* was that of improved secondary memory performance, but not working memory performance. This effect has been reported following G115 alone (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004), and in combination with both *Ginkgo biloba* (Kennedy et al., 2001b; Kennedy *et al.*, 2002) and guaraná (*Paullinia cupana*) (Kennedy *et al.*, 2004). The previous failure to educe an affect on working memory performance may have been due to the 'under loaded' nature of the working memory tasks utilised in the previous studies. Indeed, this suggestion is supported by the observation that, in the present study, ginseng only exerted its effects on the most cognitively demanding level on the N Back task (3 back) and the more demanding version of the serial subtraction task (Chapter 2), or on an easier version of the subtraction task but only when concomitant subjective self reported mental fatigue was at its greatest (Chapter 3).

It is difficult, if not impossible, with the present knowledge of the mechanism responsible for ginseng effects, to explain the disparity in results obtained from study to study following the 200 mg dose of *Panax ginseng* or even the non-linear dose response relationship. However, in the present study the slowing in performance following the 200 mg dose was associated with a concomitant

increased in subjective reports of calmness. This improved state of calmness may be accountable for the slowed speed of performance (although this pattern was not evident for the 400 mg dose). Unfortunately, the study was not designed to delineate any cause/effect relationship between mood and cognitive performance. Therefore the most parsimonious explanation is that of a simple cohort effect (all studies report data from the same healthy young population). Alternatively, whilst the extract used is standardised to total ginsenoside content, it is possible that even minor differences in the levels of single ginsenosides, or groups of ginsenosides (e.g. the ratio of protopanaxadiols to protopanaxatriols), may have exerted an effect. Additionally, while the effect following 200mg may appear curious, previous ginseng research, both in humans and animals, is replete with dose-specific effects and non-linear dose response profiles (for review see: Kennedy and Scholey, 2003).

As mentioned earlier the mechanisms underlying ginsengs cognitive effects are, as yet, not well understood. Potential candidate mechanisms include effects on the cardiovascular and HPA systems, acceleration of platelet aggregation, cardio- and neuro protective effects, modulation of neurotransmission, promotion of nitric oxide synthesis and gluco-regulatory effects (Kennedy and Scholey 2003). With reference to the latter, in chapter 2 and chapter 3 an attempt was made to relate ginseng's acute gluco-regulatory effects to its cognitive efficacy, reporting cognitive enhancement in the presence of concomitant reduction in circulating blood glucose levels in a healthy young cohort (Chapter 1 and Chapter 2). However, there was a clear lack of any relationship between the modulation of blood glucose levels and cognitive performance.

In conclusion, the present study has revealed that 7 consecutive days of ginseng ingestion has no affect on self-reported mood, secondary memory performance or working memory performance. However, results did reveal that single doses (administered on day 1 and day 8) of *Panax ginseng* can modulate working memory performance and improve participants' subjective self-reports of calmness. Given that ginseng is typically self-medicated for longer periods of time further research is needed.

CHAPTER 5. AN INVESTIGATION INTO THE ACUTE, CHRONIC AND SUPERIMPOSED EFFECTS OF PANAX GINSENG ON COGNITIVE PERFORMANCE AND MOOD AND INDICES OF GLUCOSE REGULATION: A 20WEEK TRIAL IN HEALTHY VOLUNTEERS.

5.1. Introduction

Despite the fact that ginseng products are often self-administered globally by millions of people for weeks at a time, there is a clear lack of empirical studies to support the purported chronic behavioural effects in humans. However, there is a growing support for its acute efficacy (Kennedy and Scholey, 2003; and also Chapters 2 and 3).

To date only three studies have directly investigated the behavioural effects of daily ginseng ingestion in humans. These studies have revealed improved speed of performing a mental arithmetic task (D' Angelo et al, 1986), faster reaction times on the most rapid auditory reaction time task (Soerensen and Sonne, 1996), and global memory and attention improvements (Labadorf et al, 2004). A further investigation, involving analysis of data from a small cohort of self-reported users of ginseng products drawn from a large prospective study, found no effect on episodic or semantic memory (Persson et al, 2004). However, as this study failed to assess the type of extract, or the dose, regularity or duration of consumption the purported negative findings are somewhat meaningless (Scholey et al., 2005). Overall, no conclusive interpretations can be drawn from the above-mentioned studies because of methodological differences with regards to cohorts, ginseng extracts and doses, and the measurement tools utilised (which may assess different aspects/domains of cognitive function). For a full discussion of these studies see the introduction section 1.2.5.3.3. Additionally, the lack of behavioural effect reported in these studies may be accounted for by the

methodological shortcomings of the studies (Scholey et al., 2004; Kennedy and Scholey, 2003; Bahrke and Morgan, 1994; 2000; Vogler *et al.*, 1999;).

In line with the methodological issues raised (Scholey et al., 2004; Kennedy and Scholey, 2003; Bahrke and Morgan, 1994; 2000; Vogler *et al.*, 1999;) and integrating some of the suggestions made by these authors, a further study (Chapter 4) aimed to investigate the acute, sub-chronic and superimposed behavioural working memory effects of *Panax ginseng* for the first time. The study utilised the same standardised extract, doses and methodology previously used in the assessment of *Panax ginseng's* acute effects (e.g. Kennedy *et al.*, 2001a; 2001b; 2002; 2004; Sünram-Lea., 2004). Results found no behavioural effect of 7 consecutive days of ginseng ingestion. However, results did reveal that the acute ingestion of both 200 mg and 400 mg of *Panax ginseng* caused a significant amelioration in subjective self-report ratings of calmness and also dose-specific improvements and decrements on aspects of working memory performance, in healthy young volunteers. The amelioration of subjective mood are in keeping with similar improvements reported on measures pertaining to 'quality of life' or 'well being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations [although findings of this nature are by no means unequivocal (Kennedy and Scholey, 2003)] as well as attenuating the effects of fatigue in night nurses (Wesnes *et al.*, 2003) and fatigue associated with sustained mental performance (Chapter 2 and Chapter 3).

Conversely, the present study was the first human study to report the modulation of working memory performance following an acute dose of *Panax ginseng*. Specifically, 200 mg led to significant slowing in working memory performance

whereas 400 mg led to significant improvements in the accuracy of working memory performance and also significant speeded performance. The behavioural effects of *Panax ginseng* on working memory performance, revealed in chapter 4 are in direct contrast to repeated failures to elicit any behavioural effect on any direct parameter/measure of working memory performance previously utilised (see: Kennedy at al 2003). However, acute improvements and decrements have been reported in the performance of mental arithmetic tasks, which will load heavily on working memory resources (Scholey and Kennedy, 2002; Chapter 1 and Chapter 2). The significantly improved accuracy in working memory performance following 400 mg of *Panax ginseng* reported in Chapter 4 is somewhat consistent with the reports of improved accuracy, following the same dose, in the performance on a mental arithmetic task (Scholey and Kennedy, 2002) and faster responses on an attentional task (Sünram-Lea et al., 2004). The significant slowing, following 200 mg *Panax ginseng*, in working memory performing reported in chapter 4 is also somewhat consistent with the reports of slower performance in other cognitive domains. For example, 200 mg of *Panax Ginseng* has previously led to significant slowing in the performance of attentional tasks (Kennedy *et al.*, 2001) and a mental arithmetic task (Scholey and Kennedy, 2002). However, this reported slowing in performance contrasts with the recent findings of faster memory, attention, and serial subtraction task performance (Kennedy et al., 2004; chapter 2 and chapter 3), and decreased latency of the P300 component of auditory evoked potentials following the same 200 mg dose (Kennedy *et al.*, 2003).

The mechanisms by which ginseng might modulate human cognitive performance are not yet well understood, but they may involve several central and peripheral

physiological effects that have potential relevance to human cognitive performance (see: Kennedy and Scholey, 2003). One such physiological effect, on which this thesis has focused, is *Panax ginseng's* ability to acutely modulate circulating blood glucose levels. In Chapter 2 and Chapter 3 it was reported, for the first time, that single doses of *Panax ginseng* caused significant reductions in blood glucose levels whilst also improving aspects of cognitive function in a group of overnight fasted healthy volunteers. This pattern of results was unexpected given the relationship that has been previously identified between peripheral blood glucose levels (varying from normoglycaemic levels) and cognitive performance that has been evoked in recent years (Messier, 2004) and therefore deserves further investigation.

Given the recent findings with regards to ginseng's glucose modulating properties (e.g. Chapter 2 and Chapter 3), the lack of controlled behavioural studies into the chronic effect of ginseng, and the findings of chapter 4 (i.e. 7 consecutive days of ginseng ingestion is not sufficient to bring about behavioural change) the present placebo-controlled, double-blind, balanced, crossover study aimed to further investigate ginseng's potential for behavioural/physiological modulation following longer periods of ginseng ingestion.

The current study aimed to assess the acute (following a single dose), chronic (following 28 and 56 consecutive days of *Panax ginseng* ingestion) and superimposed effects of *Panax ginseng* ingestion on cognitive performance, mood and indices of glucose regulation in healthy volunteers. The study utilises the same standardised, computerised assessment battery used in the series of acute studies (Kennedy *et al.*, 2001a; 2001b; 2002; 2004; Sünram-Lea., 2004). Additionally, those tasks that proved sensitive to ginseng ingestion in Chapter 4

will also be incorporated into the present assessment. Post – dose testing was limited to one session timed at three hours post ginseng ingestion. This time point was chosen for two reasons. Firstly, previous studies had tested at 1, 1.5, 2.5, 4 and 6 hours post dose; therefore testing at three hours post dose would add more insight into the acute effects following *Panax ginseng* ingestion. Additionally, the present study moved away from using the student population as there sample group. Therefore, to aid recruitment and completion rates the post-dose testing session was designed to fall into the time frame of a normal working lunch hour – volunteers ingested ginseng at 9am (before work) they could return at 12 noon (start of lunch hour) to complete their 3 hour post-dose testing session.

5.2 Subjects and methods

5.2.1 Participants

Thirteen female and 12 male volunteers (mean age = 35.28, SD =10.50), recruited through local media advertisements, participated in the study. One participant failed to complete all study sessions of the study, stating time constraints as the reason. The study was approved by the Northumbria University Division of Psychology Ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation each participant gave informed consent and completed a medical health questionnaire (see appendix 20). All participants reported that they were in good health and were not taking any illicit social drugs. Additionally, they were free from 'over the-counter' or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. All participants fasted overnight and were alcohol and caffeine free for 12 hours prior to all assessment days, and abstained from products containing these substances on the days of testing. Volunteers were paid £150 for participation. Treatment order was counterbalanced and participants were randomly allocated to a treatment regimen (see appendix 21).

5.2.1.1 Treatment compliance

Participants were asked to record any days that they forgot to take their dose. Two participants reported forgetting to take their dose when in the placebo condition. Only two further participants reported that they forgot to take their dose when in the active treatment condition. One participant forgot to take day

14's treatment dose. A second participant forgot to take day 6's treatment dose. All participants were blind to the treatment condition throughout the study.

5.2.2 Cognitive and mood assessment

5.2.2.1 Subjective self-report mood measure

World health organisation quality of life questionnaire – bref:

The World health organisation quality of life questionnaire – bref (WHOQOL – BREF) is an abbreviated version of the WHOQOL –100 (WHOQOL group 1994a; 1994b; 1995). The WHOQOL –BREF contains a total of 26 questions, one item from each of the 24 facets contained in the WHOQOL-100 and two items from the overall quality of life and general health facet. The 26 questions are factored down into one of four domain factors; physical health, psychological health, social relationships, environment (WHOQOL group, 1996) (see appendix 22).

5.2.2.2 *Brunel scale*

The Brunel scale assesses anger, confusion, depression, fatigue, tension, and vigour. Items are rated on a 5-point scale anchored by "not at all" (0) and "extremely" (4) (Terry et al., 1999). The scale was completed at every testing session (see appendix 23).

5.2.2.3 Objective behavioural measures

5.2.2.4 *CDR computerised assessment battery*

The CDR computerised assessment battery (Wesnes et al., 1987) has been shown to be a sensitive tool in exploring the acute cognitive effects of mild cognition enhancers (Moss et al., 1998, Scholey et al., 1999) as well as wide variety of other substances (Ebert et al., 1998, O'Neill et al 1995). In the present study a tailored version of the CDR battery was utilised. This tailored version has previously been shown to capture the acute modulation of cognitive performance following the ingestion of *G. biloba* (Kennedy et al., 2000) and *Panax ginseng* (Kennedy et al., 2001; Sünram-Lea et al., 2004; Labadorf et al., 2004), and a G. biloba/ *Panax ginseng* combination (Wesnes et al., 1997; 2000, Kennedy et al., 2001). The selection of computer-controlled tasks from the system was administered with parallel forms of the tests being presented at each testing session. Presentation was via laptop with high-resolution TFT colour monitors and, with the exception of written word recall tests, all responses were recorded via two-button (yes/no) response boxes. The entire selection of tasks took approximately 20 min. Tests were administered in the following order

5.2.2.5 W*ord presentation*

Fifteen words, matched for frequency and concreteness, were presented in sequence on the monitor for the participant to remember. The stimulus duration was 1 s, as was the interstimulus interval.

5.2.2.6 *Immediate word recall*

The participant was allowed 60 s to write down as many of the words as possible. The task was scored as number of correct answers, errors, and intrusions and the resulting score was converted into a percentage.

5.2.2.7 *Picture presentation*

A series of 20 photographic images was presented on the monitor at the rate of 1 every 3 s, with a stimulus duration of 1 s, for the participant to remember.

Simple reaction time:

The participant was instructed to press the 'yes' response button as quickly as possible every time the word 'yes' was presented on the monitor. Fifty stimuli were presented with an interstimulus interval that varied randomly between 1 and 3.5 s. Reaction times were recorded in milliseconds.

5.2.2.8 *Digit vigilance task*

A target digit was randomly selected and constantly displayed to the right of the monitor screen. A series of digits was presented in the center of the screen at the rate of 80/min and the participant was required to press the 'yes' button as quickly as possible every time the digit in the series matched the target digit. The task lasted 3 minutes and there were 45 stimulus–target matches. Task measures were accuracy (%), reaction time (milliseconds), and number of false alarms.

5.2.2.9 *Choice reaction time*

Either the word 'no' or the word 'yes' was presented on the monitor and the participant was required to press the corresponding button as quickly as possible. There were 50 trials, of which the stimulus word was chosen randomly with equal probability, with a randomly varying interstimulus interval of between 1 and 3.5 s. Reaction times (millisecond) and accuracy (%) were recorded.

5.2.2.10 Spatial working memory:

A pictorial representation of a house was presented on the screen with four of its nine windows lit. The participant was instructed to memorise the position of the illuminated windows. In 36 subsequent presentations of the house, one of the windows was illuminated and the participant decided whether or not this matched one of the lighted windows in the original presentation. The participant made their response by pressing the 'yes' or 'no' response button as quickly as possible. Mean reaction times were measured in milliseconds and the accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages that were used to derive a 'percentage greater than chance performance' score.

5.2.2.11 *Numeric working memory*

Five digits were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the 'yes' or 'no' response button as appropriate, as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were

measured in milliseconds and the accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages that were used to derive a 'percentage greater than chance performance' score.

5.2.2.12 *Delayed word recall*

The participant was again given 60 s to write down as many of the words as possible. The task was scored as number of correct answers, errors, and intrusions and the resulting score was converted into a percentage.

5.2.2.13 Delayed word recognition

The original words plus 15 distractor words were presented one at a time in a randomised order. For each word, the participant indicated whether or not he/she recognised it as being included in the original list of words by pressing the 'yes' or 'no' button as appropriate and as quickly as possible. Mean reaction times were measured in milliseconds and the accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages that were used to derive a 'percentage greater than chance performance' score.

5.2.2.14 *Delayed picture recognition*

The original pictures plus 20 distractor pictures were presented one at a time in a randomized order. For each picture, participants indicated whether or not it was recognised as being from the original series by pressing the 'yes' or 'no' button as appropriate and as quickly as possible. Mean reaction times were measured in

milliseconds and the accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages that were used to derive a 'percentage greater than chance performance score.

5.2.2.15 *The Bond–Lader visual analogue scales (Bond and Lader, 1974)*

The 16 visual analogue scales of Bond–Lader were combined as recommended by the authors to form three mood factors: Alert, Calm, and Contented (see appendix 24.

5.2.2.16 Primary cognitive outcome measures

The above measures were collapsed into the cognitive outcome factors (Secondary Memory, Working Memory, Speed of Memory, Accuracy of Attention, and Speed of Attention) derived from the battery by factor analysis (see Wesnes et al., 2000 for details). These primary cognitive outcome measures have previously be shown to be a valuable tool in reporting cognitive effects of herbal supplements (Wesnes et al., 2000; 1997; Kennedy et al., 2000; 2001a; 2001b). The original factor analysis was performed on the data from a cohort of 272 healthy middle-aged individuals, and there is no reason to assume that scores from such a population should differ from those in the current sample. The names used to describe the factors are those previously used in a series of acute studies (Kennedy et al., 2000; 2001a; 2001b). Two factors, Speed of Attention and Quality of Attention, were described as Power of Attention and Continuity of Attention, respectively, in the original factor analysis (Wesnes et al., 2000).

5.2.2.17 *Quality of Memory factor*

This is derived by combining the scores obtained on the Secondary and Working Memory factors. One hundred percent accuracy on this measure would generate a maximum score of 600.

5.2.2.18 *Secondary Memory factor*

This is derived by combining the percentage accuracy scores (adjusted for proportions of novel and original stimuli where appropriate) from all of the secondary memory tests—word recognition, picture recognition, immediate word recall, and delayed word recall (with adjustments to the total percent correct for errors and intrusions on the latter two tasks). One hundred percent accuracy across the four tasks would generate a maximum score of 400 on this index.

5.2.2.19 *Working Memory factor*

This is derived by combining the percentage accuracy scores from the two working memory tests—spatial working memory and numeric working memory. One hundred percent accuracy across the two tasks would generate a maximum score of 200 on this index.

5.2.2.20 *Speed of Memory*

This is derived by combining the reaction times of the four computerised memory tasks—numeric working memory, spatial memory, delayed word recognition, and delayed picture recognition (units are in milliseconds for the four tasks).

5.2.2.21 *Speed of Attention*

This is derived by combining the reaction times of the three attentional tasks—simple reaction time, choice reaction time, and digit vigilance (units are in milliseconds for the three tasks).

5.2.2.22 *Accuracy of Attention*

This is derived by calculating the combined percentage accuracy across the choice reaction time and digit vigilance tasks with adjustment for false alarms from the latter test. One hundred percent accuracy across the two tasks would generate a maximum score of 100.

5.2.2.23 *N back task*

fMRI has confirmed the working memory demands made by the N back task (Jonides et al 1997; Watter et al 2001). In the present study participants engaged in the N back task at the most difficult level only (3 back). In this version participants had to match each letter to the one that had appeared three items back in the series. Each letter had an ISI of 2.5 seconds. Responses were made via the 'M' key (match) and 'Z' key (no match). The tasks were scored for accuracy and reaction time to each stimulus.

5.2.2.24 *Corsi Block Tapping task:*

The Corsi blocks task was developed in the early 1970s as a nonverbal counterpart to the verbal-memory span task (Milner, 1971). Over the years, it has frequently been used to assess nonverbal short-term memory performance in adults (e.g. Smyth & Scholey, 1992), children (e.g. Orsini, Schiappa, & Grossi, 1981), and patients with neuropsychological deficits (e.g. Vilkki & Holst, 1989).

The present study implemented a computerised version of the task. Nine identical blue squares appeared in a random position on the computer screen. A predetermined number of squares would change colour, from the original blue to red then back to blue, at the rate of one per second, thus identifying a sequence. The participant would then repeat the sequence by clicking each square with the mouse. The sequence span increases until the participant can no longer correctly recall the sequence, resulting in a span measure of nonverbal working memory. Participants were presented with five trials at each span level. Participants were required to correctly recall at least three of the five to proceed to the next level. Nonverbal memory span was measured as an average of the last five correct sequences recalled.

5.2.2.25 *Alphabetic working memory*

The task was implemented as it was thought that participants would find it more difficult to use mnemonic strategies (e.g. chunking or forming a representation of "non-target" items) to aid in the storage of the target letters than NWM. Thus the demands of the Alphabetic working memory task are higher than that of the Numeric working memory task despite recruiting the same underlying cognitive domains. Five letters were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe letters for each of which the participant decided whether or not it had been in the original series and pressed the 'yes' ('M' key) or 'no' ('Z' key) response button as appropriate, as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were measured in milliseconds and the accuracy of responses to both original and novel (distractor) stimuli were recorded as

percentages that were used to derive a 'percentage greater than chance performance' score.

5.2.3 Physiological measures

5.2.3.1 *Finger prick blood samples*

Blood glucose levels were measured using a Reflotron Plus diagnostic machine and Reflotron test sticks (Roche Diagnostics, Germany). The reliability of the test has previously been confirmed (Price and Koller, 1988). On each of the six active study days, blood glucose levels were measured at pre-dose and at 3 hours post dose (before commencement of the post-dose behavioural; assessment).

5.2.3.2 *Insulin*

A 2.5ml blood sample was taken from the arm of each participant at the beginning of each testing day. Blood samples were collected in a serum gel monovette and immediately centrifuged for 10 mins at 3000rpm temp 7oC using an Allegra X-22R centrifuge supplied by Beckman Coulter. The resulting supernatant was poured off into an eppendorf tube and kept at -20oC prior to analysis. Blood insulin levels were established using a solid phase enzyme amplified sensitivity immunoassay (BioSource INS-EASIA) performed on microtiter plates. The assay uses monoclonal antibodies directed against distinct epitopes of insulin

5.2.3.3 *HbA1c*

A 2.5ml blood sample was taken from the arm of each participant at the beginning of each testing day. Bloods were collected in an edta monovette and

whole blood was stored at -28C prior to analysis. HbA1c levels were established using a Tosoh G7 HbA1c analyser. This system uses a cation exchange column to separate haemoglobin components by different ionic charge. The various components of haemoglobin, including A1c, are separated into 6 fractions and assayed.

5.2.4 Treatments

Active treatments and placebo capsules, matched for size, colour, opacity and odour were provided by the manufacturer Prior to the commencement of the study, a disinterested third party, who had no other involvement in the study prepared the treatments for each individual participant (treatment order was counter-balanced and participants were randomly allocated to a treatment regime) (see appendix 21) and sealed them in containers marked only with the participant code and study day number. On each study day, participants received two capsules. The individual capsules contained either an inert placebo, or 100 mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland). Depending on the condition to which the participant was allocated on that particular day, the combination of capsules treatments corresponded to a dose of 0 mg (placebo) or 200 mg (G115).

5.2.5 Procedure

The study commenced with a practice day that was identical to subsequent study days with the exception that no treatment was offered, no blood samples were taken and the behavioural data acquired was not entered into any analysis (except to check that it fell within established norms). Participants received each

treatment (placebo, and 200 mg) for 57 days in total, with a wash-out period of 27 days between treatments. The order of presentation of the treatments was counterbalanced by random allocation. Participants were assessed on the first day (day 1), the halfway point (day 29) and last day (day 57) of each treatment period (i.e. 6 assessment days in total across the two treatments (see Diagram 5) On each of these days the cognitive/mood testing regimen comprised assessments (see above for tasks and running order) pre-dose, followed immediately by ingestion of the day's treatment, and thereafter at 3hrs post-dose. Testing took place in a suite of laboratories with participants visually isolated from each other. Upon completion of the 3hr post-dose testing session for each treatment period participants were provided with a container containing treatments for each day (days 2 to 28; days 30 to 56) until the next laboratory visit. Treatments on day 1, day 29 and day 57 of each period were consumed in the laboratory. Capillary finger prick blood samples and whole blood (2 x 2.5 ml) was collected on each study day, in both treatment regimes, immediately prior to the commencement of the pre-dose and post-dose (finger prick sample only) behavioural assessments (see Diagram 6). All participants were in an overnight fasting state during the pre-dose data collection and consumed the same (i.e. across all 6 testing days) self-selected breakfast immediately after treatment ingestion.

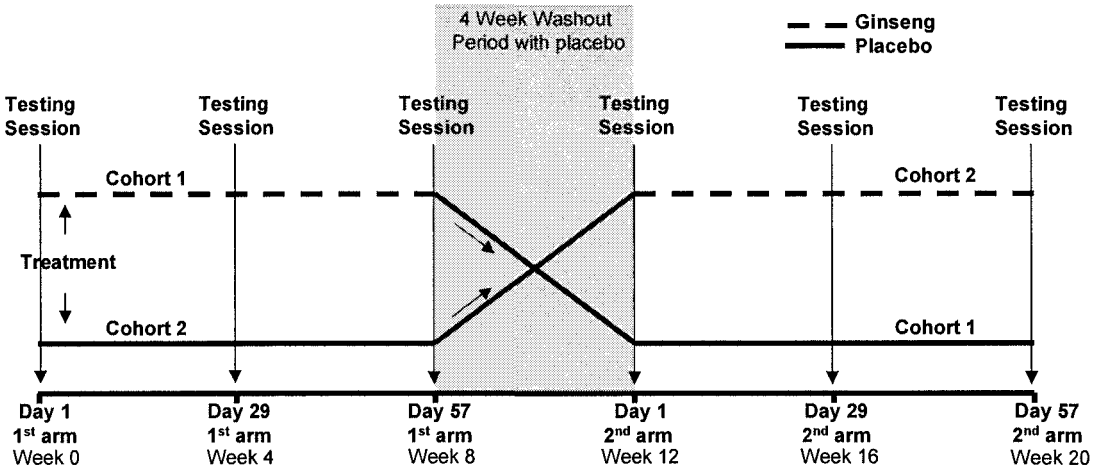

Diagram 5. Timelines of the study. Participants received either placebo or ginseng (200 mg G115) during the first arm of the study (weeks 0 to 8), and the opposite treatment in the second arm (weeks 12 to 20). All participants received placebo during the washout period (beginning of week 9 to end of week 12). Testing days took place on days 1, 29, and 57 of each arm.

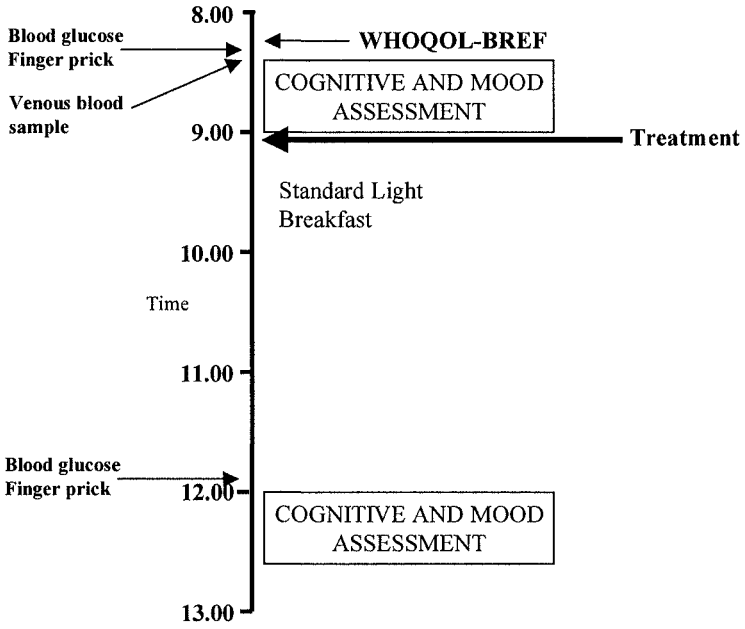

Diagram 6. Running order of each study day (day 1, 29 and 57) of both the 1st and 2nd arms.

5.2.6 Statistics

All data were analysed using the Minitab statistical package version 13.1. An initial one-way repeated measures ANOVA was conducted on day 1 baseline raw score data, for each outcome measure to check for baseline differences (see appendix 25). For all subsequent analysis, for all outcome measures, 'Change from pre-dose day 1 baseline' scores were used.

5.2.6.1 *Acute effects*

Data obtained from the 3hr post-dose testing session on day 1 (change from day 1 pre-dose baseline) was analysed by one-way repeated measures ANOVA to reveal any acute effects (see appendix 26).

5.2.6.2 *Chronic effects*

When only pre-dose data were obtained (HbA1c; insulin; WHOQOL) on day 29 and 57 (all data was change from day 1 pre-dose baseline), analysis was by two way repeated measures ANOVA (treatment x day) to reveal any chronic effects (see appendix 27).

5.2.6.3 *Using a 2-way ANOVA with treatment and day as the I.Vs*

Using this analyse a significant main effect of treatment would indicate a simple chronic effect summed across both days. A significant treatment x day interaction would also indicate a chronic effect that is complicated by a differential influence of 29 and 57 consecutive days of treatment.

5.2.6.4 *Chronic and superimposed effects*

Pre and post-dose data obtained from day 29 and 57 (all data was change from day 1 pre-dose baseline) was analysed by three way repeated measures ANOVA (treatment x day x pre/post) to reveal any chronic and super-imposed chronic/acute effects (see appendix 28).

5.2.6.5 *Using a 3-way ANOVA with treatment, day and pre/post as the I.Vs*

Using this analysis a significant main effect of treatment would indicate a simple chronic effect summed across both days and all four testing sessions. A significant treatment x day interaction would also indicate a chronic effect that is complicated by a differential influence of 29 and 57 consecutive days of treatment. A significant treatment x pre/post interaction would indicate both chronic (i.e. pre-dose data) and the superimposed (i.e. post-dose data).

5.2.6.6 *Planned comparisons*

On those measures that yielded a significant main effect or interaction with treatment, planned comparisons were made between placebo and the active treatment utilising t tests with MSError as an error term (Keppel, 1991). To ensure the overall protection level, comparisons were strictly planned prior to commencement of the study and only conducted on those outcome measures that reached statistical significance on the initial ANOVA. Additionally, only probabilities associated with planned comparisons were calculated, and all testing was two-tailed (see appendix 29).

5.3. Results

5.3.1 Baseline scores

Prior to analysis of change from baseline data, raw baseline scores for both treatment conditions (placebo, 200mg) for each of the primary outcome measures were subject to one-way repeated-measures ANOVAs. There was a significant baseline difference revealed for the social relations factor of the quality of life questionnaire. Participants reported significantly higher baseline social relation scores when in the ginseng conditions (mean = 15.00) as compared to the placebo condition (mean = 13.83) [F (1,22) = 4.67, P = 0.042]. There was a significant baseline difference revealed for the number of errors committed on the delayed word recall task. When in the ginseng condition participants committed significantly more errors (mean = 0.76) than the placebo condition (mean = 0.28) [F (1,24) = 4.9, P = 0.037]. Mean pre-dose baseline raw scores and change from day 1 baseline scores, for each condition, at each post-dose time point on each primary outcome measure and for blood glucose levels, are represented in Table 5.1.

Table 5.1. Effects of 200mg (G115) and placebo on task performance, blood glucose levels and indices of glucose regulation. Values represent mean baseline performance/glucose score/levels and mean change from baseline performance score at post-dose on day 1; mean change from baseline performance score at pre-dose and post-dose on day 29; mean change from baseline performance score at pre-dose and post-dose on day 57. Standard errors in italics.

N=25			Acute effects	Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Primary Outcome Factors							
Quality of memory (% X 6)	placebo	405.878 *11.458*	-24.816 *7.416*	8.690 *8.647*	-21.927 *8.854*	11.451 *8.080*	-13.893 *9.149*
	200mg	397.190 *11.604*	-30.323 *11.636*	11.262 *10.344*	-21.376 *14.048*	14.444 *12.802*	-6.301 *13.043*
Secondary memory (% X 4)	placebo	221.932 *11.047*	-18.865 *6.667*	12.934 *9.168*	-21.133 *8.778*	12.534 *7.319*	-10.798 *7.946*
	200mg	217.800 *9.501*	-32.667 *10.032*	7.000 *9.765*	-18.867 *12.804*	7.733 *10.825*	-6.402 *10.748*
Working memory (% X 2)	placebo	183.946 *2.635*	-5.950 *4.691*	-4.244 *3.547*	-0.794 *2.637*	-1.083 *2.509*	-3.094 *3.056*
	200mg	179.390 *4.313*	2.344 *3.025*	4.262 *3.401*	-2.509 *5.543*	6.711 *3.530*	0.101 *4.199*
Speed of attention (summed ms)	placebo	1084.623 *20.128*	-726.089 *19.340*	-720.676 *20.030*	-720.742 *20.029*	-722.142 *19.684*	-724.596 *19.940*
	200mg	1074.000 *19.435*	-713.546 *18.927*	-710.973 *19.366*	-711.559 *19.013*	-707.879 *19.490*	-712.359 *19.347*
Speed of memory (summed ms)	placebo	2727.419 *96.528*	-751.212 *45.051*	-701.587 *47.821*	-733.071 *46.972*	-803.309 *56.567*	-802.977 *51.005*
	200mg	2711.540 *93.166*	-766.116 *45.365*	-683.584 *41.058*	-756.517 *58.390*	-725.572 *57.644*	-785.188 *56.852*
Accuracy of attention (%)	placebo	361.694 *1.771*	-271.533 *1.509*	-270.653 *1.781*	-270.493 *1.733*	-270.533 *1.827*	-271.173 *1.653*
	200mg	364.840 *2.321*	-274.120 *2.031*	-274.040 *2.214*	-274.400 *2.061*	-273.440 *2.336*	-274.520 *2.169*
Individual CDR tasks							
Simple reaction time (ms)	placebo	266.444 *4.950*	6.110 *4.580*	0.165 *3.022*	5.169 *5.295*	-1.387 *4.682*	3.738 *4.646*
	200mg	264.949 *5.008*	6.570 *2.654*	2.938 *2.955*	10.537 *4.188*	9.026 *3.425*	15.921 *3.972*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Digit Vigilance							
Accuracy (%)	placebo	96.978 *0.872*	-0.089 *0.859*	0.090 *0.792*	-0.089 *0.782*	0.800 *0.786*	0.178 *0.701*
	200mg	97.068 *0.753*	-0.178 *0.712*	0.177 *0.796*	-0.800 *0.853*	0.533 *0.553*	-1.156 *1.190*
Reaction time (ms)	placebo	405.323 *7.411*	8.642 *6.160*	1.409 *4.675*	9.849 *4.972*	7.707 *5.620*	11.799 *5.664*
	200mg	404.585 *7.681*	6.594 *4.306*	9.844 *5.006*	10.197 *5.108*	10.064 *5.205*	15.896 *6.727*
False alarms	placebo	0.880 *0.229*	-0.080 *0.277*	-0.080 *0.266*	-0.360 *0.226*	-0.160 *0.258*	-0.120 *0.312*
	200mg	0.440 *0.100*	0.040 *0.144*	0.560 *0.227*	0.440 *0.220*	0.560 *0.447*	0.280 *0.308*
Choice reaction time (ms)	placebo	412.856 *11.248*	-8.406 *5.049*	-0.530 *7.721*	0.022 *6.589*	1.218 *6.396*	-6.746 *6.540*
	200mg	404.465 *10.234*	1.187 *4.660*	9.060 *5.538*	13.084 *6.738*	19.743 *6.200*	17.974 *7.781*
Choice RT accuracy (%)	placebo	95.680 *0.503*	-0.960 *0.395*	0.640 *0.541*	0.560 *0.562*	0.080 *0.605*	-0.560 *0.798*
	200mg	96.480 *0.693*	-1.280 *0.599*	-0.400 *0.744*	-0.480 *0.559*	0.480 *0.664*	-0.720 *0.650*
Spatial Memory							
Targets (%)	placebo	94.500 *1.475*	-0.750 *2.612*	-1.000 *1.963*	1.250 *1.807*	-0.750 *1.886*	-0.250 *2.081*
	200mg	92.500 *2.242*	3.500 *1.775*	1.750 *1.791*	-1.000 *3.001*	4.000 *2.182*	1.500 *2.706*
Distractors (%)	placebo	97.800 *0.854*	-3.600 *1.827*	-1.200 *1.245*	-0.800 *1.192*	-1.400 *1.253*	-1.600 *1.381*
	200mg	95.600 *1.801*	1.600 *2.000*	1.000 *1.859*	-0.800 *2.499*	1.200 *1.636*	1.000 *1.793*
Sensitivity index	placebo	0.932 *0.013*	-0.048 *0.039*	-0.026 *0.027*	0.002 *0.018*	-0.028 *0.023*	-0.023 *0.028*
	200mg	0.883 *0.039*	0.053 *0.034*	0.028 *0.034*	-0.015 *0.052*	0.052 *0.035*	0.026 *0.042*
Targets RT (ms)	placebo	624.678 *48.904*	-77.186 *24.724*	16.951 *24.924*	-41.454 *28.947*	-42.762 *35.094*	-82.808 *38.415*
	200mg	612.925 *41.660*	-60.502 *29.597*	-12.132 *23.232*	-47.930 *42.000*	-11.426 *33.138*	-45.134 *32.054*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se		Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Distractors RT (ms)	placebo	619.026 *36.416*	-21.773 *18.889*		69.754 *24.219*	5.421 *26.641*	15.730 *25.422*	-15.442 *27.754*
	200mg	618.028 *30.402*	-14.034 *24.014*		62.612 *15.149*	-28.123 *21.342*	23.528 *15.646*	10.466 *26.100*
Average RT (ms)	placebo	621.634 *40.542*	-47.261 *18.354*		47.147 *22.632*	-15.720 *25.716*	-9.176 *27.007*	-46.053 *29.658*
	200mg	615.183 *33.907*	-34.261 *24.347*		30.517 *14.899*	-36.609 *28.526*	8.912 *21.356*	-13.160 *27.343*
Numeric Working Memory								
Targets (%)	placebo	94.223 *1.039*	-0.623 *1.166*		-2.044 *1.273*	-0.089 *0.868*	0.356 *0.998*	-0.533 *1.262*
	200mg	93.956 *0.896*	-1.422 *0.983*		1.779 *1.077*	-0.176 *1.279*	1.245 *0.919*	-0.888 *0.976*
Distractors (%)	placebo	97.423 *0.870*	-0.977 *0.961*		0.000 *0.846*	-1.155 *0.865*	0.712 *0.941*	-0.711 *1.240*
	200mg	97.334 *0.655*	-1.334 *0.900*		-0.266 *0.634*	-0.533 *0.646*	0.266 *0.568*	-1.512 *0.764*
Sensitivity index	placebo	0.920 *0.016*	-0.017 *0.019*		-0.019 *0.016*	-0.013 *0.013*	0.010 *0.013*	-0.013 *0.021*
	200mg	0.916 *0.012*	-0.026 *0.016*		0.013 *0.014*	-0.008 *0.017*	0.014 *0.010*	-0.025 *0.015*
Targets RT (ms)	placebo	572.440 *21.829*	-35.880 *9.961*		-24.128 *11.793*	-38.119 *12.559*	-41.108 *10.419*	-45.433 *12.545*
	200mg	568.974 *21.976*	-33.259 *8.998*		-12.575 *9.297*	-32.990 *10.415*	-31.013 *10.018*	-34.945 *10.818*
Distractors RT (ms)	placebo	621.632 *22.164*	-10.579 *9.723*		-3.865 *11.102*	-28.334 *13.314*	-36.317 *10.988*	-36.158 *14.160*
	200mg	627.896 *23.967*	-39.527 *10.909*		-4.765 *12.203*	-29.061 *13.115*	-18.337 *12.812*	-22.144 *13.852*
Average RT (ms)	placebo	597.266 *21.314*	-23.294 *8.473*		-13.513 *9.421*	-33.567 *11.081*	-38.237 *9.008*	-40.751 *12.155*
	200mg	598.505 *22.561*	-36.760 *9.235*		-8.688 *9.756*	-30.964 *10.904*	-24.807 *10.508*	-28.376 *11.162*
Word Recognition								
Targets (%)	placebo	75.200 *3.884*	-2.133 *2.897*		0.000 *2.421*	1.066 *3.659*	2.666 *3.163*	-1.334 *1.928*
	200mg	77.867 *2.922*	-5.600 *2.555*		-5.067 *2.544*	-4.000 *3.230*	-1.867 *2.089*	-4.801 *2.941*
Distractors (%)	placebo	90.666 *2.205*	-6.399 *2.375*		-0.266 *2.765*	-5.066 *2.308*	2.668 *2.071*	-3.465 *2.303*
	200mg	89.333 *2.804*	-6.134 *2.534*		-2.666 *2.564*	-8.267 *3.345*	0.000 *1.648*	-3.468 *2.272*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se		Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Sensitivity index	placebo	0.714 *0.025*	-0.092 *0.037*		-0.019 *0.038*	-0.047 *0.033*	0.041 *0.035*	-0.069 *0.032*
	200mg	0.706 *0.036*	-0.101 *0.036*		-0.056 *0.041*	-0.125 *0.044*	0.005 *0.028*	-0.062 *0.035*
Targets RT (ms)	placebo	675.120 *22.263*	34.038 *21.493*		56.006 *27.442*	26.722 *17.210*	-14.821 *20.356*	24.884 *25.083*
	200mg	685.480 *21.174*	17.350 *18.916*		-12.273 *13.657*	-20.969 *14.158*	-28.159 *16.884*	-17.815 *14.514*
Distractors RT (ms)	placebo	729.624 *27.923*	37.971 *34.404*		0.647 *20.880*	19.644 *19.557*	-17.614 *14.865*	-1.359 *22.418*
	200mg	742.059 *35.317*	4.677 *17.426*		6.547 *19.218*	25.716 *25.468*	-7.824 *22.882*	-23.680 *23.690*
Average RT (ms)	placebo	698.960 *24.690*	33.245 *24.819*		27.266 *22.490*	20.220 *15.777*	-14.588 *14.062*	9.139 *20.044*
	200mg	708.999 *27.244*	9.902 *16.355*		-2.418 *14.472*	3.336 *18.464*	-12.120 *15.711*	-19.610 *18.177*
Picture Recognition								
Targets (%)	placebo	82.200 *2.879*	2.400 *2.795*		6.600 *2.210*	1.800 *2.862*	2.800 *2.522*	2.200 *2.879*
	200mg	82.800 *3.774*	-1.800 *2.101*		4.600 *1.814*	2.000 *2.304*	0.400 *2.337*	0.400 *2.320*
Distractors (%)	placebo	90.400 *2.082*	-0.200 *1.848*		2.600 *1.987*	0.000 *2.215*	2.800 *2.161*	0.600 *2.477*
	200mg	89.400 *2.071*	-1.800 *1.576*		4.800 *1.804*	-1.400 *1.827*	2.800 *1.773*	2.800 *1.555*
Sensitivity index	placebo	0.741 *0.040*	0.023 *0.037*		0.085 *0.036*	0.017 *0.042*	0.054 *0.041*	0.022 *0.049*
	200mg	0.733 *0.051*	-0.034 *0.028*		0.098 *0.028*	0.003 *0.033*	0.026 *0.036*	0.038 *0.034*
Targets RT (ms)	placebo	781.079 *31.295*	28.053 *22.504*		-15.276 *20.734*	31.656 *24.271*	-42.326 *20.680*	3.599 *19.612*
	200mg	771.872 *28.649*	41.261 *28.529*		-0.992 *13.712*	22.152 *25.449*	12.836 *22.925*	-29.016 *24.801*
Distractors RT (ms)	placebo	836.999 *38.350*	14.600 *23.667*		-52.869 *29.731*	3.385 *15.713*	-70.159 *30.679*	-31.082 *29.055*
	200mg	807.477 *27.921*	-3.638 *23.466*		6.117 *22.044*	19.217 *22.581*	-8.967 *22.503*	-37.852 *19.765*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Average RT (ms)	placebo	809.559 *33.612*	18.302 *21.086*	-36.261 *23.326*	15.176 *13.626*	-56.936 *24.008*	-17.213 *20.216*
	200mg	788.853 *26.223*	13.904 *20.971*	3.586 *14.672*	20.054 *22.245*	-0.678 *17.915*	-34.654 *17.658*
Bond-lader							
Alert	placebo	54.480 *2.900*	1.320 *2.131*	8.192 *2.633*	4.420 *3.004*	6.288 *2.472*	2.260 *2.872*
	200mg	59.944 *2.309*	-3.160 *2.154*	0.020 *2.524*	-2.140 *2.370*	3.172 *2.930*	-3.076 *3.524*
Content	placebo	62.416 *2.464*	1.480 *2.166*	6.984 *2.940*	2.776 *2.464*	1.248 *2.926*	2.696 *3.011*
	200mg	66.464 *2.334*	-2.160 *1.374*	-2.880 *2.663*	-2.936 *2.176*	3.912 *2.010*	2.040 *1.918*
Calm	placebo	56.940 *3.106*	1.720 *2.417*	3.420 *2.474*	0.000 *2.967*	2.760 *2.486*	2.400 *2.411*
	200mg	56.920 *3.169*	-1.560 *2.493*	0.440 *2.114*	-0.260 *2.130*	3.920 *2.616*	3.720 *1.640*
Immediate Word Recall							
No of words	placebo	7.440 *0.488*	-0.560 *0.355*	0.280 *0.399*	-0.480 *0.397*	0.320 *0.438*	-0.280 *0.434*
	200mg	7.480 *0.364*	-0.880 *0.660*	0.440 *0.532*	-0.360 *0.735*	0.120 *0.698*	0.000 *0.724*
Errors	placebo	0.240 *0.086*	-0.040 *0.089*	0.000 *0.113*	0.080 *0.126*	-0.040 *0.120*	0.040 *0.106*
	200mg	0.280 *0.090*	0.040 *0.133*	-0.040 *0.133*	0.080 *0.169*	0.040 *0.165*	-0.120 *0.103*
Intrusions	placebo	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*
	200mg	0.000 *0.000*	0.040 *0.039*	0.160 *0.157*	0.280 *0.275*	0.160 *0.157*	0.000 *0.000*
*D*elayed word recall							
No of words	placebo	5.600 *0.581*	-1.200 *0.523*	0.400 *0.368*	-1.840 *0.531*	0.160 *0.298*	-0.600 *0.266*
	200mg	5.320 *0.412*	-1.520 *0.724*	0.360 *0.631*	-0.720 *0.745*	0.840 *0.685*	-0.360 *0.710*
Errors	placebo	0.280 *0.106*	0.040 *0.106*	0.040 *0.120*	0.360 *0.178*	0.280 *0.184*	0.360 *0.195*
	200mg	0.760 *0.182*	-0.040 *0.275*	-0.120 *0.256*	-0.400 *0.227*	-0.200 *0.266*	-0.080 *0.349*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Intrusions	placebo	0.000 *0.000*	0.120 *0.065*	0.040 *0.039*	0.080 *0.054*	0.000 *0.000*	0.040 *0.039*
	200mg	0.000 *0.000*	0.160 *0.093*	0.000 *0.000*	0.040 *0.039*	0.000 *0.000*	0.040 *0.039*
Brunnel scale							
Tension	placebo	1.240 *0.337*	-0.400 *0.170*	-0.600 *0.311*	-0.600 *0.311*	-0.560 *0.260*	-0.560 *0.260*
	200mg	0.720 *0.228*	-0.280 *0.216*	0.320 *0.344*	-0.080 *0.240*	0.080 *0.305*	-0.440 *0.213*
Depression	placebo	0.960 *0.370*	-0.440 *0.272*	-0.560 *0.389*	-0.560 *0.389*	-0.080 *0.420*	-0.080 *0.420*
	200mg	0.320 *0.221*	0.040 *0.165*	0.880 *0.458*	0.520 *0.532*	0.480 *0.228*	-0.080 *0.187*
Aggression	placebo	0.240 *0.130*	0.000 *0.204*	-0.040 *0.184*	-0.040 *0.184*	0.040 *0.184*	0.040 *0.184*
	200mg	0.040 *0.051*	0.120 *0.131*	0.120 *0.086*	0.080 *0.097*	0.560 *0.368*	0.080 *0.097*
Vigour	placebo	5.280 *0.754*	1.200 *0.514*	0.200 *0.491*	0.200 *0.491*	0.080 *0.478*	0.080 *0.478*
	200mg	6.160 *0.523*	0.480 *0.432*	0.400 *0.634*	0.200 *0.669*	-0.480 *0.812*	-0.480 *0.674*
Fatigue	placebo	3.440 *0.792*	-1.080 *0.555*	-1.120 *0.632*	-1.120 *0.632*	-0.600 *0.676*	-0.600 *0.676*
	200mg	2.680 *0.379*	-1.360 *0.420*	0.040 *0.645*	-0.920 *0.605*	-0.520 *0.690*	-0.720 *0.612*
Confusion	placebo	0.880 *0.337*	-0.440 *0.171*	-0.760 *0.342*	-0.760 *0.342*	-0.400 *0.336*	-0.400 *0.336*
	200mg	0.440 *0.126*	-0.320 *0.136*	0.120 *0.199*	-0.280 *0.121*	0.280 *0.287*	-0.280 *0.145*
Corsi Block							
Span	placebo	4.928 *0.337*	0.384 *0.279*	0.312 *0.256*	-0.248 *0.337*	-0.160 *0.310*	0.120 *0.259*
	200mg	4.776 *0.361*	0.376 *0.298*	0.928 *0.317*	0.303 *0.368*	0.704 *0.343*	0.496 *0.264*
Alphabetic Working Memory							
Targets	placebo	13.387 *0.245*	0.227 *0.218*	0.240 *0.178*	0.240 *0.203*	0.040 *0.182*	-0.067 *0.199*
	200mg	13.587 *0.269*	-0.240 *0.263*	-0.133 *0.196*	0.080 *0.251*	-0.133 *0.204*	-0.133 *0.224*

				Acute effects		Chronic / superimposed effects					
		Baseline	se	Day 1 post-dose	se	Day 29 pre-dose	se	Day 29 post-dose	se	Day 57 pre-dose	se	Day 57 post-dose	se
Distractors	placebo	14.307	*0.155*	0.227	*0.122*	0.200	*0.104*	-2.177	*0.463*	0.093	*0.138*	0.173	*0.201*
	200mg	14.440	*0.201*	0.133	*0.143*	0.080	*0.102*	-2.241	*0.500*	-0.080	*0.162*	0.040	*0.208*
Targets RT (ms)	placebo	0.656	*0.027*	-0.017	*0.008*	-0.013	*0.014*	-0.014	*0.013*	-0.031	*0.019*	-0.022	*0.015*
	200mg	0.653	*0.027*	-0.014	*0.014*	-0.024	*0.011*	0.012	*0.015*	-0.016	*0.013*	-0.026	*0.014*
Distractors RT (ms)	placebo	0.691	*0.028*	-0.001	*0.014*	0.108	*0.077*	-0.004	*0.057*	-0.010	*0.016*	0.000	*0.014*
	200mg	0.697	*0.030*	0.007	*0.015*	-0.020	*0.010*	0.207	*0.107*	-0.014	*0.017*	-0.001	*0.018*
Error	placebo	1.600	*0.249*	-0.160	*0.212*	-0.227	*0.192*	-0.200	*0.206*	-0.027	*0.180*	0.000	*0.187*
	200mg	1.387	*0.257*	0.267	*0.262*	0.147	*0.202*	-0.027	*0.256*	0.160	*0.215*	0.093	*0.202*
False alarms	placebo	0.707	*0.155*	-0.240	*0.120*	-0.200	*0.102*	2.013	*0.438*	-0.107	*0.141*	-0.320	*0.142*
	200mg	0.573	*0.198*	-0.147	*0.143*	-0.093	*0.103*	2.000	*0.463*	0.053	*0.160*	-0.187	*0.128*
N Back													
Targets	placebo	10.280	*0.466*	-0.480	*0.461*	0.320	*0.431*	0.120	*0.322*	0.360	*0.310*	0.280	*0.423*
	200mg	9.640	*0.457*	0.840	*0.477*	0.520	*0.405*	0.680	*0.480*	0.920	*0.608*	0.880	*0.369*
Error	placebo	3.680	*0.474*	0.400	*0.481*	-0.280	*0.445*	-0.200	*0.340*	-0.440	*0.306*	-0.320	*0.416*
	200mg	4.320	*0.446*	-0.880	*0.465*	-0.520	*0.397*	-0.640	*0.484*	-0.920	*0.621*	-0.880	*0.369*
Distractors	placebo	25.040	*0.462*	0.000	*0.405*	0.120	*0.495*	0.080	*0.446*	0.040	*0.378*	0.800	*0.413*
	200mg	24.440	*0.592*	0.360	*0.584*	0.400	*0.385*	0.680	*0.531*	0.720	*0.434*	1.320	*0.431*
False alarms	placebo	5.880	*0.458*	-0.040	*0.352*	-0.240	*0.462*	-0.080	*0.432*	-0.160	*0.383*	-0.800	*0.421*
	200mg	6.360	*0.570*	-0.360	*0.575*	-0.200	*0.350*	-0.680	*0.543*	-0.840	*0.453*	-1.240	*0.394*

			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 pre-dose se	Day 57 post-dose se
Targets RT (ms)	placebo	0.753 *0.040*	0.039 *0.031*	-0.010 *0.020*	-0.057 *0.030*	-0.049 *0.031*	-0.098 *0.035*
	200mg	0.769 *0.042*	-0.018 *0.026*	-0.017 *0.040*	-0.020 *0.053*	-0.040 *0.037*	-0.037 *0.039*
Distractors RT (ms)	placebo	0.774 *0.040*	0.015 *0.025*	-0.005 *0.023*	-0.016 *0.029*	-0.066 *0.028*	-0.061 *0.028*
	200mg	0.774 *0.046*	-0.016 *0.022*	-0.012 *0.029*	-0.043 *0.029*	-0.028 *0.029*	-0.049 *0.036*
Average RT (ms)	placebo	0.767 *0.038*	0.022 *0.024*	-0.006 *0.019*	-0.027 *0.025*	-0.064 *0.025*	-0.070 *0.026*
	200mg	0.772 *0.043*	-0.016 *0.020*	-0.013 *0.030*	-0.042 *0.031*	-0.031 *0.029*	-0.044 *0.035*
Sensitivity index	placebo	0.560 *0.036*	-0.036 *0.036*	0.027 *0.032*	0.008 *0.027*	0.028 *0.025*	0.044 *0.031*
	200mg	0.495 *0.042*	0.070 *0.046*	0.046 *0.031*	0.069 *0.051*	0.094 *0.051*	0.106 *0.031*
Finger prick blood glucose levels	placebo	4.90 *0.18*	0.04 0.22	0.143 *0.158*	0.243 *0.233*	0.094 *0.186*	0.520 *0.218*
	200mg	5.04 *0.15*	0.03 0.17	0.143 *0.136*	0.187 *0.238*	0.253 *0.133*	0.172 *0.170*
Quality of life							
Physical Health	placebo	16.57 *0.35*		0.043 *0.248*		0.217 *0.302*	
	200mg	16.74 *0.31*		-0.174 *0.233*		0.043 *0.298*	
Psychological Health	placebo	15.00 *0.34*		0.000 *0.252*		0.000 *0.218*	
	200mg	15.00 *0.30*		0.043 *0.160*		0.522 *0.226*	
Social relations	placebo	13.83 *0.76*		0.783 *0.388*		0.826 *0.525*	
	200mg	15.00 *0.45*		-0.522 *0.366*		-0.174 *0.482*	
Environmental	placebo	15.35 *0.45*		0.000 *0.260*		0.435 *0.320*	
	200mg	15.61 *0.29*		-0.130 *0.212*		0.130 *0.246*	

			Acute effects		Chronic / superimposed effects					
		Baseline	se	Day 1 post-dose	se	Day 29 pre-dose	se	Day 29 post-dose	se	Day 57 pre-dose	se	Day 57 post-dose	se
Insulin	placebo	10.51	*0.84*			3.347	*1.436*			3.155	*1.216*		
	200mg	11.03	*0.62*			1.895	*1.615*			3.098	*2.365*		
HbA1c	placebo	5.12	*0.28*			-0.141	*0.294*			0.012	*0.013*		
	200mg	5.34	*0.07*			0.024	*0.058*			-0.053	*0.046*		

5.3.2 Acute effects

5.3.2.1 CDR Tasks

There were no significant results revealed on any of the CDR primary outcome measures or the individual tasks (see Table 5.1).

5.3.2.2 Non – CDR Tasks

The only significant result was revealed on the 3-back task (see Table 5.1).

5.3.2.3 3-back

The ANOVA revealed a significant main effect of treatment on the correct number of 3-back identifications. A greater number of correct 3-back identifications were made following 200 mg (G115) (mean 0.84) as compared to placebo (mean -0.48) [F (1,24) = 4.37, P = 0.047; d = 0.2] (Figure 5.1 and Table 5.1).

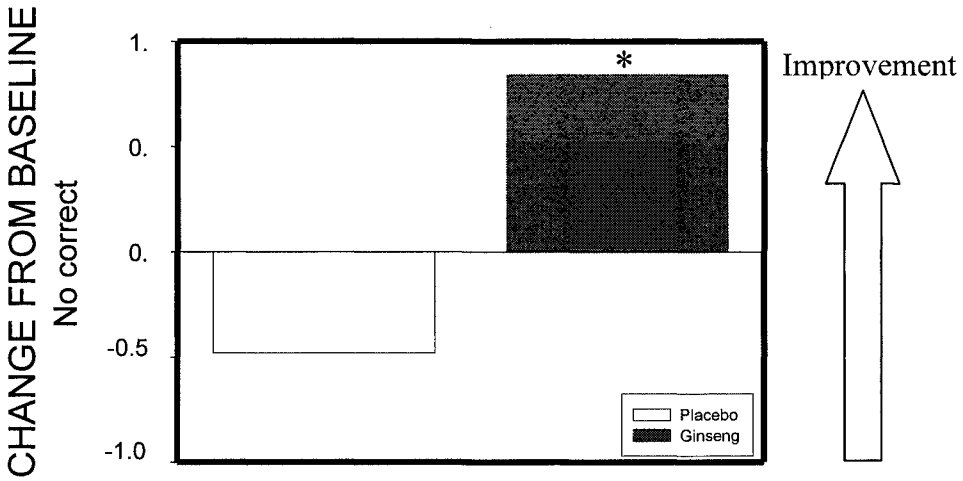

Figure 5.1. The acute effect of 200 mg *Panax ginseng* (G115) on working memory performance (3 back task) 3 hours post-dose in healthy volunteers (*, P< 0.05). N=25.

5.3.3 Chronic and superimposed effects

5.3.3.1 CDR Primary factor results

5.3.3.2 Working memory:

The ANOVA revealed a significant treatment X pre/post interaction [F (1,24) = 4.34, P = 0.048] on an index of working memory, indicative of a superimposed effect (Figure 5.2a and Table 5.1). The pattern of results suggests that the improvements in working memory performance following chronic ingestion of *Panax ginseng* (means summed across day 29 and 57 pre-dose testing sessions) are some what attenuated by an acute dose (means summed across day 27 and day 57 post dose testing sessions). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly improved working memory performance at the pre-dose testing session on day 29 (mean = 4.26) compared with placebo (mean –4.24) [t (24) = 3.140, P = 0.0044; d=0.5] and day 56 (mean = 6.71) as compared with placebo (mean = -1.10), [t (24) = 2.877, P = 0.0083; d=0.6]. There was no significant difference between *Panax ginseng* and placebo at either post-dose testing session (Figure 5.2d and Table 5.1).

5.3.3.3 Speed of attention

The ANOVA revealed a significant treatment x week interaction [F (1,24) = 4.44, P = 0.046] on speed of attention (Figure 5.3a) indicative of a chronic effect. The pattern of results suggests that *Panax ginseng* led to slower performance of attentional tasks on day 27, which was slowed further by day 57 (means summed across pre and post-dose testing sessions). Planned comparisons comparing

treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly slower speed of attention at pre-dose on day 29 (mean = -710.97) as compared with placebo (mean = -720.66) [t(24) = 4.043, P = 0.0005; d= 0.1] and post-dose (mean = -711.56) as compared with placebo (mean = -720.74) [t (24) = 3.826, P = 0.0008; d= 0.1]. Additionally, *Panax ginseng* led to significantly slower speed of attention at pre-dose on day 57 (mean = - 707.88) as compared with placebo (mean = – 722.14) [t (24) = 5.94, P = 0.000004; d= 0.1] and post-dose (mean = -712.36) as compared with placebo (mean = –724.60) [t (24) = 5.098, P = 0.00003; d= 0.1] (Figure 5.3c and Table 5.1).

5.3.3.4 CDR individual tasks

5.3.3.5 Numeric working memory task

The ANOVA revealed a significant treatment x pre/post interaction the number of correct identification [F (1,24) = 4.86, P = 0.037], indicative of a superimposed effect (Figure 5.2b and Table 5.1). The pattern of results suggests that the improvements in numeric working memory performance of chronic ingestion of *Panax ginseng* (means summed across day 29 and 57 pre-dose testing sessions) are some what attenuated by an acute dose (means summed across day 29 and day 57 post dose testing sessions) Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to a significantly greater number of correct identifications being made pre-dose (mean = 1.78) on day 29 as compared with placebo (mean = -2.04) [t (24) = 4.428, P = 0.0002; d=0.6]. There were no other significant differences (Figure 5.2e and Table 5.1).

5.3.3.6 Choice reaction time

The ANOVA revealed a significant main effect of treatment on CRT indicative of a simple chronic effect. Ginseng led to a significant slowing (mean 14.966) as compared with the placebo (mean -1.509) [F (1,24) = 7.81, P = 0.010] (Figure 5.3b and Table 5.1). Planned comparisons comparing treatment with placebo at each testing session (Figure 5.3d and Table 5.1) revealed that *Panax ginseng* led to significantly slower choice reaction time at the post-dose testing sessions (mean = 13.08) on day 29 as compared with placebo (mean = – 0.53) [t (24) = 2.35, P = 0.027; d=0.4]. Additionally, *Panax ginseng* led to significantly slower choice reaction time on day 57 at pre-dose (mean = 19.74) as compared with placebo (mean = 0.02) [t (24) = 3.33, P = 0.0028; d=0.6] and post-dose (mean = 17.97) as compared with placebo (mean = – 6.75) [t (24) = 4.445 , P = 0.0002; d=0.7].

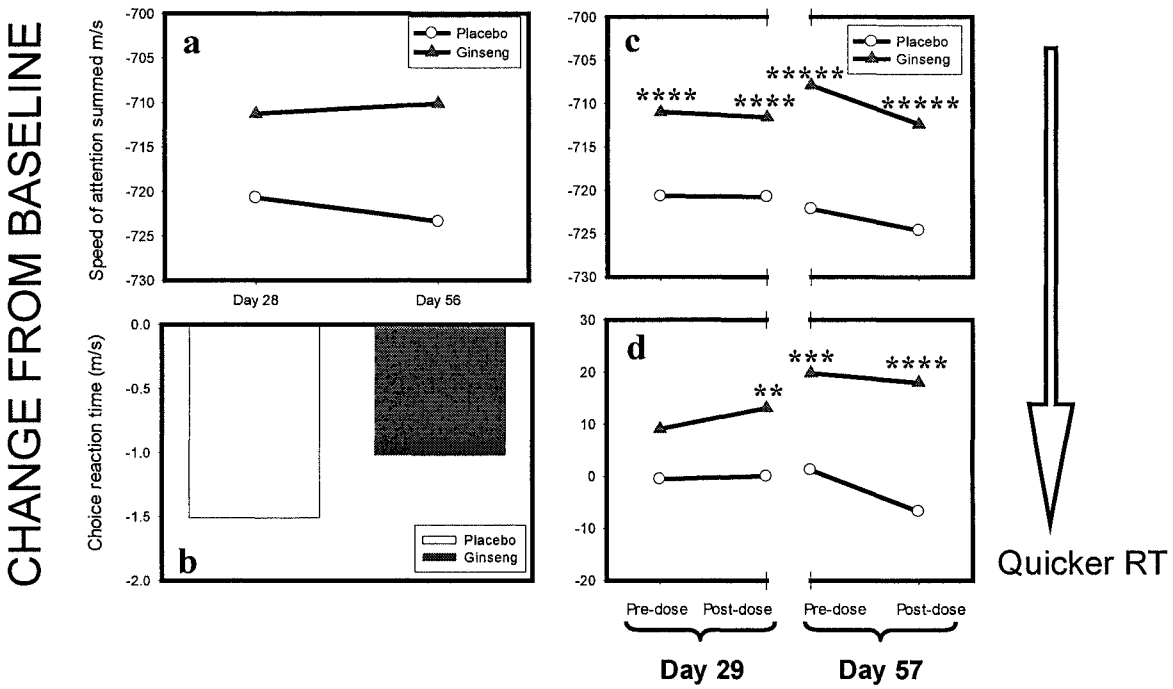

Figure 5.3 depicts the chronic effect of *Panax ginseng* G115 (200 mg/d) on speed of attention (a) and choice reaction time (b). Figure also depicts planned comparisons between treatment and placebo at pre and post-dose testing sessions on day 29 and day 57 (c and d). **, P <0.01; ***, P < 0.005; ****, P < 0.001; *****, P < 0.0005. Significance is compared with placebo. N=25.

5.3.3.7 Delayed picture recognition (RT)

The ANOVA revealed a significant treatment X pre/post interaction [F (1, 24) = 5.24, P = 0.031], indicative of a superimposed effect (Figure 5.4a and Table 5.1). The pattern of results suggests a post-dose slowing in performance for the placebo group (in comparison to their own pre-dose performance – means summed across day 29 and day 57). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly slower performance at the pre-dose session on day 29 (mean = 3.59) as compared with placebo (mean = – 36.26) [t (24) = 2.214, P = 0.037; d=0.3]. Additionally, *Panax ginseng* led to significantly slower performance at pre-dose on day 57 (mean = – 0.68) as compared with placebo (mean = - 56.94) [t (24) = 3.126, P = 0.0046; d=0.5] (Figure 5.4b and Table 5.1).

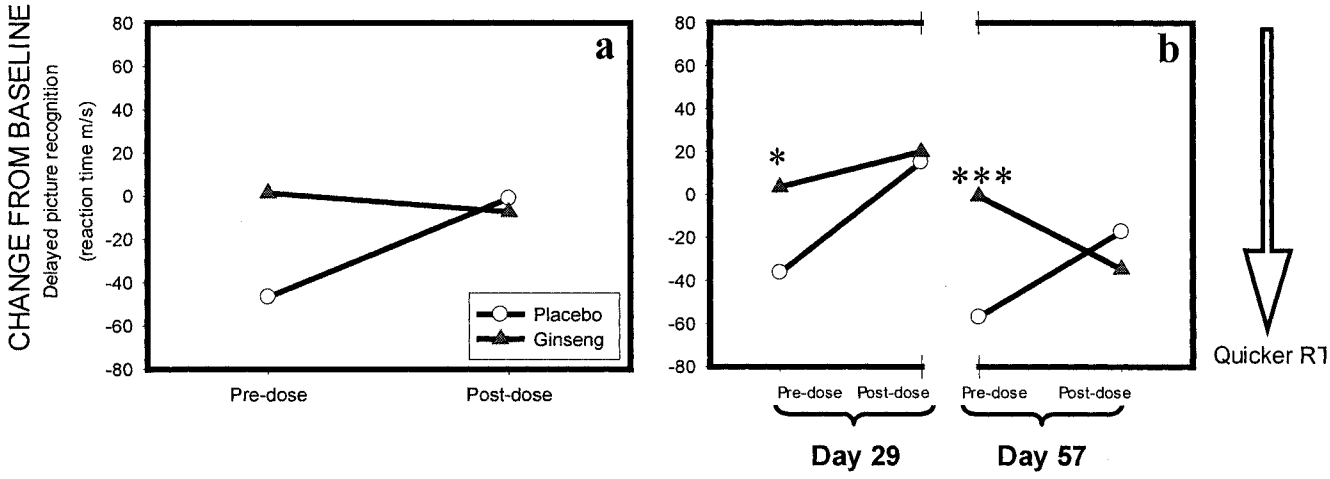

Figure 5.4 depicts the superimposed effect of *Panax ginseng* G115 (200 mg/d) on delayed picture recognition reaction time (a). Figure also depicts planned comparisons between treatment and placebo at pre and post-dose testing sessions on day 29 and day 57 (b). *, P <0.05; ***, P < 0.005. Significance is compared with placebo. N=25.

5.3.3.8 Subjective mood

5.3.3.9 Content (Bond Lader 1974)

The ANOVA revealed a significant treatment x week interaction [F (1,24) = 8.18, P = 0.009] indicative of a chronic effect (Figure 5.5a and Table 5.1). The pattern of results suggests that an initial elevation in content ratings in the placebo group on day 27 (means summed across pre and post dose testing sessions) was attenuated by day 57 (means summed across pre and post dose testing sessions). Conversely, the initial fall in content ratings in the ginseng group, on day 27 (means summed across pre and post dose testing), were reversed by day 57 (means summed across pre and post dose testing sessions). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly worse ratings of contentedness on day 29 at pre-dose (mean = - 2.88) as compared with placebo (mean = 6.984) [t (24) = 5.734, P = 0.00001; d=0.7] and post-dose (mean = - 2.93) as compared with placebo (mean = 2.776) [t (24) = 3.320, P = 0.0029; d=0.5]. There were no significant differences between treatment and placebo on day 57 (Figure 5.5b and Table 5.1).

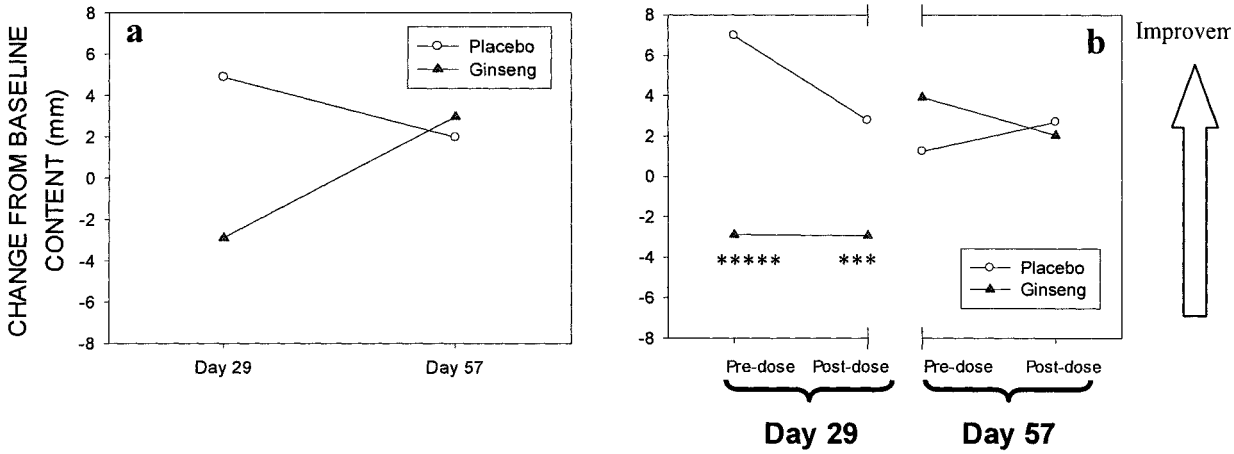

Figure 5.5 depicts the chronic effect of *Panax ginseng* G115 (200 mg/d) on self-report ratings of contentedness following 29 and 57 days of treatment (means summed across pre and post dose testing sessions)(a). Figure also depicts planned comparisons between treatment and placebo at pre and post-dose testing sessions on day 29 and day 57 (b). ***, P < 0.005; ****, P < 0.001. Significance is compared with placebo. N=25.

5.3.3.10 Confusion

The ANOVA revealed a significant treatment x pre/post interaction [F (1,24) = 7.79, P = 0.010) on subjective self-report ratings of confusion, indicative of a superimposed effect (Figure 5.6a and Table 5.1). The pattern of results suggests that the initial worse ratings of confusion following chronic ingestion of ginseng (means summed across day 29 and 57 pre-dose testing sessions) are some what attenuated by an acute dose (means summed across day 29 and day 57 post dose testing sessions). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly worse ratings of confusion on day 29 at pre-dose (mean = 0.12) as compared to placebo (mean = –0.76) [t (24) = 4.369, P = 0.0001; d=0.5] and post-dose (mean = –0.28) as compared with placebo (mean = -0.76) [t (24) = 2.38, P = 0.021; d=0.3]. Additionally, *Panax ginseng* led to significantly worse ratings of confusion on day 57 at pre-dose (mean = 0.28) as compared with placebo (mean = –0.40) [t (24) = 3.376, P = 0.001; d=0.4] (Figure 5.6d and Table 5.1).

5.3.3.11 Tension

The ANOVA revealed a significant treatment X pre/post interaction on subjective self-report ratings of tension {F (1,24) = 6.21, P = 0.020) indicative of a superimposed effect (Figure 5.6b and Table 5.1). The pattern of results suggests that the initial worse ratings of tension following chronic ingestion of ginseng (means summed across day 29 and 57 pre-dose testing sessions) are some what attenuated by an acute dose (means summed across day 29 and day 57 post dose testing sessions). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly worse ratings of

tension on day 29 at pre-dose (mean = 0.32) as compared with placebo (mean = – 0.60) [t (24) = 5.36 , P =0.00002; d=0.6] and post-dose (mean = – 0.08) as compared with placebo (mean = - 0.60) t (24) = 3.031, P = 0.006; d=0.3]. Additionally, *Panax ginseng* led to significant increased ratings of tension on day 57 at pre-dose (mean = 0.08) as compared with placebo (mean = –0.56) [t (24) = 3.731, P = 0.001; d=0.5] (Figure 5.6e and Table 5.1).

5.3.3.12 Depression

The ANOVA revealed a significant treatment X pre/post interaction on subjective self-report ratings of depression [F (1, 24) = 6.88, P = 0.015] indicative of a superimposed effect (Figure 5.6c and Table 5.1). The pattern of results suggests that the initial worse ratings of depression following chronic ingestion of ginseng (means summed across day 29 and 57 pre-dose testing sessions) are then being attenuated by that day's acute dose (means summed across day 29 and day 57 post dose testing sessions). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly worse ratings of depression on day 29 at pre-dose (mean = 0.88) as compared with placebo (mean = –0.56) [t (24) = 9.599, P = 0.000000000001; d=0.7] and post-dose (mean = 0.52) as compared with placebo (mean = - 0.56) [t (24) = 7.20, P = 0.000000004; d=0.5]. Additionally, *Panax ginseng* led to significant worse ratings of depression on day 57 at pre-dose (mean = 0.48) as compared with placebo (mean = – 0.08) [t (24) = 3.73, P = 0.001; d=0.3] (Figure 5.6f and Table 5.1).

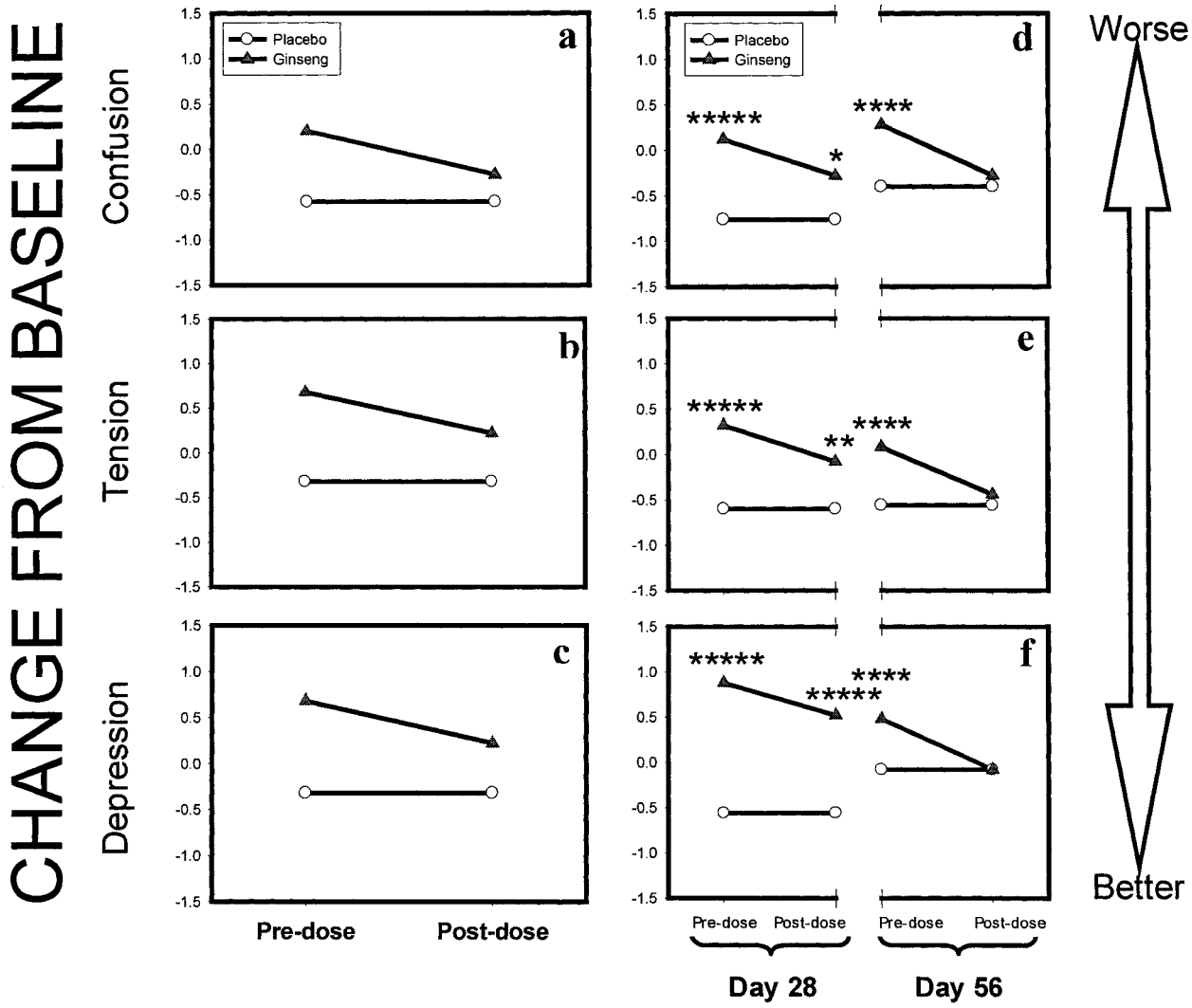

Figure 5.6 depicts the superimposed effect of *Panax ginseng* G115 (200 mg/d) on indices of subjective mood measured by the Brunnel scale: confusion (a); tension (b); depression (c). Figure also depicts planned comparisons between treatment and placebo at pre and post-dose testing sessions on day 29 and day 57 for each mood dimension (d, e and f respectively). *, P <0.05; **, P < 0.01;****, P < 0.001;*****, P < 0.0005. Significance is compared with placebo. N=25.

5.3.3.13 Non-CDR tasks

Computerised Corsi-block task

The ANOVA revealed a significant main effect of treatment on the computerised Corsi-block tapping task, indicative of a simple chronic effect. Ginseng led to a significant improvement in Visuo-spatial span (mean = 0.61) as compared with placebo (mean 0.006) [F (1, 24) = 4.75, P = 0.039] (Figure 5.2c and Table 5.1). Planned comparisons comparing treatment to placebo at each testing session revealed that *Panax ginseng* led to a significantly greater Visuo-Spatial span on day 29 at pre-dose (mean = 0.93) as compared with placebo (mean = 0.312) [t (24) = 2.75, P = 0.011; d=0.5], and post-dose (mean = 0.32) as compared with placebo (mean = –0.25) [t (24) = 2.46, P = 0.022; d=0.3]. Additionally, *Panax ginseng* led to significantly greater Visuo-Spatial span on day 57 at pre-dose (mean = 0.70;) as compared with placebo (mean = –0.16) [t(24) = 3.85, P = 0.0008; d=0.5] (Figure 5.2f and Table 5.1).

5.3.3.14 Physiological measures.

There were no significant differences revealed on finger prick blood glucose levels, HbA1c or insulin levels (see Table 5.1).

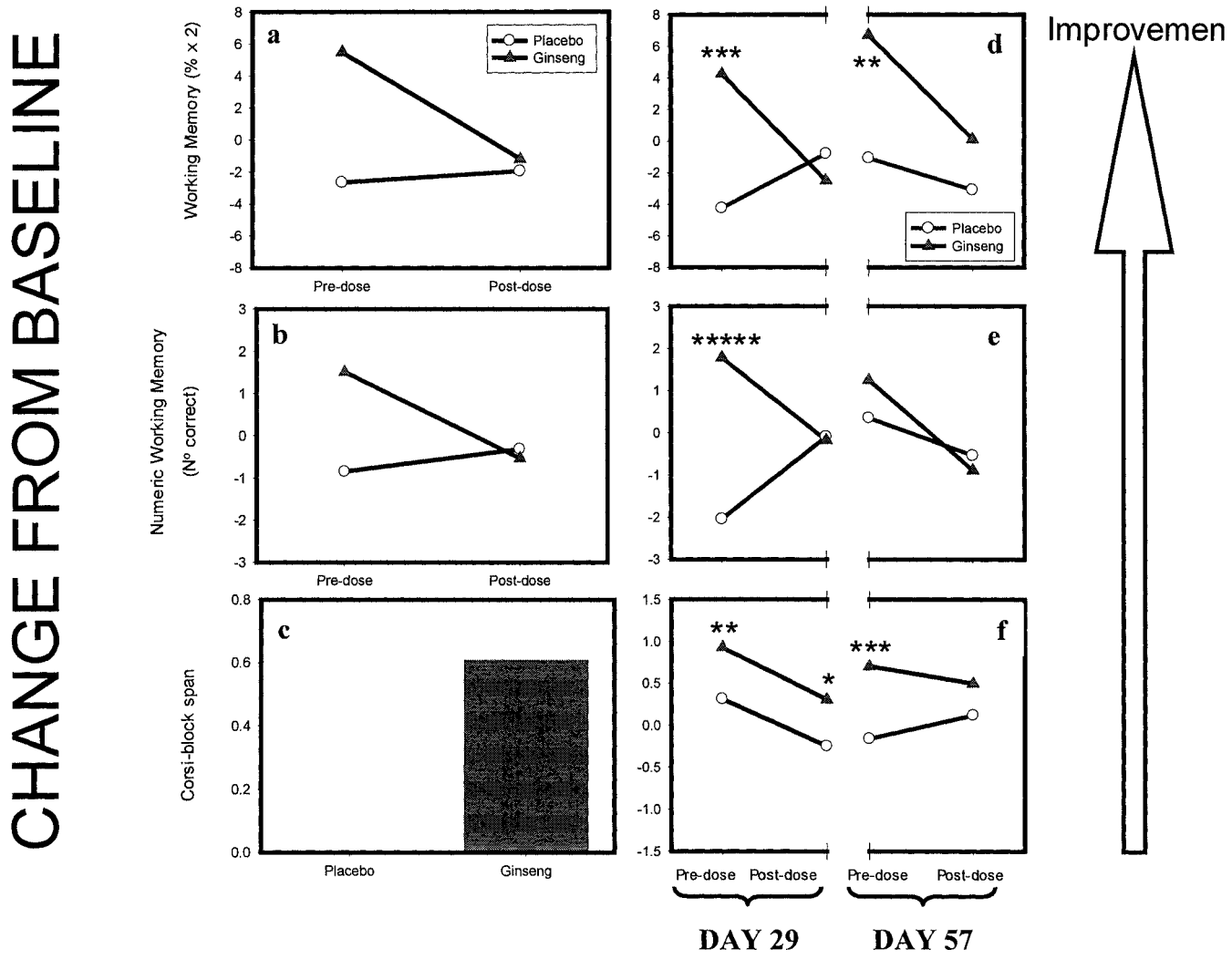

Figure 5.2 depicts the chronic and superimposed effect of *Panax ginseng* G115 (200 mg/d) on indices of working memory performance (a); numeric working memory performance (b); and spatial working memory performance (c). Figure also depicts planned comparisons between treatment and placebo at pre and post-dose testing sessions on day 29 and day 57 for each outcome measure (d, e and f respectively). *, P <0.05; **, P < 0.01; ***, P < 0.005; *****, P < 0.0005. Significance is compared with placebo. N=25.

5.4 DISCUSSION

The current study is the first to systematically investigate *Panax ginseng's* acute, chronic and superimposed effects on behaviour and subjective mood in healthy volunteers. Additionally, *Panax ginseng's* gluco-regulatory properties were further investigated. With regards to behavioural performance the results of the present study revealed improvements in working memory following a single acute dose of *Panax ginseng,* whereas, following chronic dosing (i.e. 29 and 57 days, 200 mg/d) results revealed both improvements and decrements in certain aspects of cognition and mood. Interestingly, and for the first time, the results also revealed a superimposed relationship between acute and chronic ingestion. This pattern of results highlights that an observed effect on a performance measure following chronic ingestion (i.e. pre-dose testing on day 29 and 57), can be further modulated by that day's acute dose (i.e. post-dose testing on day 29 and day 57). Results failed to reveal any acute effect on circulating blood glucose levels after participants had consumed a 'normal' breakfast. There was also no effect found on any longer-term indicator of glucose regulation (i.e. HbA1c).

With regards to the effects on circulating blood glucose levels the present result is inconsistent with the hypoglycaemic properties of *Panax quinquefolius* reported following a 25g glucose challenge, during a 120 minute glucose tolerance test, in diabetic patients who had ingested 3g, 6g and 9g (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b), and healthy participants administered 1g, 2g and 3g of *Panax quinquefolius* (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2001). The results of the present study are somewhat consistent with those glycaemic results reported in chapter 2 - 200mg G115 did not lower circulating blood glucose levels following 25g glucose drink, in fact results suggested further rises in circulating blood

glucose levels. However, caution should be taken when generalising results across studies as methodological differences (e.g. timings, breakfast vs. glucose drink, participant population) will add to the error, especially when the mechanisms responsible for ginsengs hypoglycaemic effects are not fully understood (see section 1.2.5 of this thesis). With regards to the results for HbA1c, again the present result is inconsistent with those of Sotaniemi *et al.*, (1995) who reported reductions in fasted blood glucose levels and glycated haemoglobin following 8 weeks administration of 100 mg and 200 mg/day of an unspecified ginseng extract in 18 participants with type 2 Diabetes Mellitus; With, Tetsutani (2000) who reported that 24 months of treatment with 3 - 4.5g/day of Korean red *Panax ginseng* decreased HbA_{1c} in 34 type 2 diabetics compared with controls; With Vuksan *et al.*, (2000) who reported a decrease in fasted blood glucose and HbA_{1c} in 24 type II diabetic patients following 8 weeks administration of 1g of a proprietary ginseng extract, taken 40 minutes before each meal. However, again caution should be taken as methodological differences between studies make generalising results difficult.

With regards to the acute behavioural effects of *Panax ginseng* the results of the present study revealed that a single 200 mg (G115) dose significantly improved accuracy of working memory performance 3 hrs post-dose. This results is somewhat in line with the reported improvements in mental arithmetic performance (a task not traditionally thought of as a working memory task but will require working memory resources, as well as many other higher cognitive processes) following 200 mg (see chapter 2 and chapter 3), and with a significant improvement in the accuracy and speed of working memory performance following 400mg revealed in Chapter 4. However, the present result is in contrast

with the repeated failure to find acute effects of single doses (i.e. 200mg; 400mg; 600mg), on any parameter of working memory performance previously investigated (see: Kennedy *et al.*, 2003) and is in direct contrast with the recent impairments on the speed of working memory performance following the same 200 mg (G115) dose reported in Chapter 4 and with impaired mental arithmetic performance (Scholey and Kennedy, 2002) for the same dose. It should be noted that the present study is the first study to assess ginseng's behavioural effects 3 hours post-dose and that this alone may explain some of the patterns in the present results. This possible explanation for gains support from the fact that attentional performance (as assessed by the CRD computerised assessment battery) was only affected when tested 90 minutes post-dose (Sünram-Lea et al., 2004) as opposed to 60, 150, 240 and 360 minutes post-dose (see Kennedy and Scholey, 2003).

With regards to the chronic effects of *Panax ginseng,* the initial analysis revealed either "simple" chronic effects (indicative of a main effect of treatment); differential chronic effects of 29 and 57 days ingestion (indicative of a significant treatment x week interaction); or superimposed effects (indicative of a significant treatment x pre/post ingestion) which were then further delineated through the statistical comparison between treatment and placebo at each testing session.

In relation to working memory performance, a significant superimposed treatment effect was revealed by the ANOVA on the primary outcome measure of working memory (Figure 5.2a). This pattern of results suggests that chronic ingestion of *Panax ginseng* (i.e. pre-dose testing - means summed across on day 29 and 57,) led to improvements in working memory performance, however, these improvement were somewhat attenuated following the ingestion of an acute dose.

Planned comparisons (Figure 5.2d) comparing each treatment with placebo at each time point supported this pattern. Results revealed that chronic ingestion of *Panax ginseng* led to significantly improved working memory performance at pre-dose on day 29 and 57, as compared with placebo, however, following that day's acute dose this significant chronic improvement in working memory performance was weakened.

Inspection of the individual tasks (i.e. the numeric working memory task and spatial working memory task), that load onto the CDR primary working memory factor, revealed the same general pattern of results (Figure 5.2b) although the ANOVA revealed a non-significant trend only for the spatial working memory task ($P = 0.06$).

In contrast with the non-significant spatial working memory results revealed for the CDR task (i.e. the spatial working memory task) there was, however, a significant treatment effect revealed on the non-CDR computerised spatial working memory task (i.e. the Corsi-Block task). On this task the ANOVA revealed a 'simple' chronic effect. The pattern of results would suggest that ginseng improves spatial working memory performance irrespective of length of treatment time (i.e. 29 or 57 consecutive days of ginseng ingestion) and irrespective of the acute dosing on each testing day (Figure 5.2d). Planned comparisons revealed that ginseng led to significantly better spatial working memory performance at both testing sessions on day 29 but only the pre-dose testing session on day 57 (performance was better at the post dose-testing session on day 57 following ginseng but not statistically significant so probably due to improvement seen in the placebo group rather then a fall in performance in the ginseng group (see Figure 5.2, f and table 5.1).

The present results reveal inconsistencies between measures purported to assess similar cognitive domains/processes i.e. working memory performance. For example, improvements in verbal working memory performance were found following chronic ingestion of ginseng as assessed by the CDR battery. However, no such chronic improvements were reported on the non-CDR verbal working memory tasks (N-back or Alphabetic Working Memory). The latter non-CDR task was manipulated by the author to try and decrease strategic chunking by volunteers and has not been validated. Therefore, no further reference will be made to this exploratory working memory task. Conversely, improvements in spatial working memory performance were reported following chronic ingestion of ginseng as assessed by the non-CDR spatial working memory task (Corsi block task). However, this time, no significant improvements were apparent on the CDR spatial memory task. These differences in task sensitivity may simply have been due to uncontrolled factors such as task order, or unmeasured changes in fatigue, boredom or motivation. Alternatively, these inconsistencies (i.e. improvements on one working memory task but not on another) may be due to the tasks themselves (further exploration as to the sensitivity of the measurement tools may be needed); the underlying cognitive processes needed to complete each task; the anatomical location and the underlying biological mechanisms affected by ginseng's many active constituents or more probably a complex relationship between all factors and the added complication of time. Speculation will be made later in the discussion which is aimed to further understand ginseng's cognitive effects and underlying mechanisms of action. Specific arguments will be made for further fractionated assessment of previously identified independent cognitive processes through methodological design and

also the need for specific reference to cognitive processes assessed by each measurement tool.

When consideration is taken of the four working memory tasks utilised in the present study it is apparent that there is a distinction between the CDR tasks and the non-CDR task. Simplistically, the non-CDR tasks are cognitively more demanding but additionally these non-CDR tasks require the involvement of the Central Executive component of working memory for their successful completion (see later). A speculative suggestion may be that human cognitions in healthy volunteers may only be susceptible to 'ginseng' when 'resources' are failing as a result of fatigue or increased demand (as seen in chapter 2 and chapter 3) or have become impaired due to insult (Kennedy and Scholey, 2003). An additional speculation may be that ginseng is having its effect on previously un-tested cognitions. Historically, these cognitions were believed to be involved in the completion of specific and common working memory tasks. Baddely and Hitch (1972) first grouped these cognitions and termed them 'Central Executive Processes' and believed they were responsible for the control of all 'higher order' cognitions. Recently at least three independent central executive processes have been identified (Miyake *et al.*, 2000). The areas predominantly involved in these processes are located in the frontal and prefrontal cortex (Miyake *et al.*, 2000).

By definition a central executive task is one that requires the concomitant storage and processing of information and would therefore be a relatively difficult and 'demanding' task. Therefore, a central executive task intuitively fits the speculation that ginseng preferentially affects a task that is more 'demanding' (Kennedy and Scholey, 2003; Chapter 2; Chapter 3; Chapter 4).

One distinguishing difference between the CDR and non-CDR working memory tasks utilised is that the latter group of tasks require the simultaneous storage, sequential memory updating and sequential recall of information for successful completion. Whereas the former group of tasks only require the solitary maintenance process for successful completion, hence the non-CDR tasks will involve Central Executive processes (Miyake *et al.*, 2000).

Therefore, the disparity in results between the two spatial working memory tasks utilised in the present study (and possibly between studies) may be accounted for by the 'demands' that each task places on working memory processes or may be due to other central executive cognitions not previously tested (see later). It is possible that the Corsi block task makes increased 'demands' on a spatial system relative to the CDR spatial task. These higher 'demands' may, for example, require more concentration, lead to fatigue or may even be dependent upon the local delivery and use of metabolic substrates and/or neurotransmitter communication. This suggestion may also explain the acute verbal working memory improvements found in the present study (as opposed to the non significant CDR acute working memory effects) and the improved performance on the more demanding verbal working memory task only (Chapter 4) and on the more difficult version on a serial subtraction task (Chapter 2) and on an easier version (chapter 3) but only when concomitant fatigue levels are high. However, such speculation would not explain the non-significant acute findings revealed for the Corsi block task in the present study (there may be differences in ginseng's biological effect when administered acutely which favours verbal memory) or the non-significant findings relating to the chronic effects on the N-back task in the present study. Although, it could be argued that the CDR verbal working memory

task (numerical working memory task) does require sequential memory encoding, storage and retrieval. Therefore this task may involve executive processes (Miyake *et al.*, 2000).

The effect of *Panax ginseng* on central executive proficiency, in any population, has not been investigated directly (except in a limited context in chapter 4). However, previous tasks utilised in the ginseng literature may allow some discussion of these processes in an exploratory and indirect manner. In chapter 4 of this thesis a commonly used central executive tool was utilised (the random number generation method) to investigate the effect of acute and sub-chronic ginseng ingestion. Results revealed that ginseng had no effect. In chapter 4 and chapter 5 of this thesis the N-back task was used to assess verbal working memory. This task will draw heavily on the central executive, as it requires the concomitant storage and processing of information (Miyake *et al.*, 2000). It was reported that ginseng improved only the most demanding version of this task following an acute dose (Chapter 4 and Chapter 5). We know that the two verbal tasks (random number generation and N-back) draw upon two different central executive processes (inhibition and memory updating respectively – see Miyake et al 2000) and we have seen, in this thesis, effects on N-back (only acutely and only in the most demanding version) and no effect on Random Number Generation. This may suggest differential effects on independent central executive processes (it could be argued that the non-verbal equivalent central executive memory updating process underlies the corsi-block performance). This speculation may further be supported when one considers the non-significant effects reported previously on working memory tasks (i.e. these tasks are simple maintenance based tasks and will not involve Central Executive processes

Baddely, 2000). Systematic investigation of the Central Executive system could benefit the ginseng literature. It may be difficult to imagine a differential effect on a fractionated system, which is believed to be located in the same cortical region and therefore presumably would be susceptible to the same neurotransmitter pathways and reliant on the same blood capillary network. However, there is evidence from MDMA poly-drug users that such selectivity (albeit impairments) of effect can be achieved, presumably through neuro-chemical toxicity (Reay et al., 2006).

The chapter making this thesis and the previously documented results, following acute ingestion, can be taken as further support into the speculative suggestion of ginseng's domain-specific effects. For example, in the present study *Panax ginseng* improved working memory but impaired attention following chronic dosing. In previous acute studies there has been reports of improved secondary memory performance (episodic) but no effect on either working memory or attentional performance (Kennedy et al., 2003); improvements in attention but no effect on working memory or secondary memory when post-dose testing times are changed (Sünram-Lea et al., 2004) and improvements in working memory but no effect on secondary memory (Chapter 4). The studies suggest a complex relationship between the cognitive domain tested, the task utilised, the dosing regimen and the post-dose testing times. More research is needed to further delineate these factors. The mechanisms underlying ginseng's effects are not know, are highly complex and are probably the result of the many active ginsenosides of ginseng acting by numerous mechanisms on target cells. A speculative suggestion would be that the cognitive effects reported are a result of ginseng's diverse psychopharmacological actions over time and the cortical

location underlying the cognitions. For instance, the pre-frontal cortex is rich in dopamine and 5-HT projection and is believed to be integral to working memory performance, whereas the hippocampus is densely populated with insulin receptors and ACh projections and is believed to be heavily involved in memory consolidation and secondary memory. All of these may be sensitive to delivery and use of metabolic substrates. Ginseng or its individual ginsenosides have been shown to have excitatory and inhibitory effects on these neurotransmitter systems, regulate glucose levels and promote insulin secretion (see general introduction). To understand ginseng's mechanisms of action, further research should evaluate ginseng's (the specific extract under investigation) propensity for physiological effect in vitro and in vivo and then relate any subsequent behavioural change to these physiological effects.

In relation to attention, a chronic effect of *Panax ginseng* was revealed for primary outcome measure of speed of attention (Figure 5.3a). The initial ANOVA revealed a differential effect of 29 and 57 days of ginseng ingestion. The pattern of results indicates that *Panax ginseng* led to slower performance of attentional tasks following 29 days of treatment, which was further impaired by day 57. Planned comparisons revealed that *Panax ginseng* led to significantly slower attentional performance at pre and post-dose testing session on both day 29 and day 57. When examining the individual tasks (i.e. simple and choice reaction time) that load the speed of attention factor the results reveal that the same pattern (Figure 5.3d). However, it was a non-significant trend for simple reaction time ($P = 0.058$). The chronic effect is consistent with the recent acute studies that have reported significant slowing of attentional tasks (Kennedy *et al.*, 2001) and modest, but significant, reductions in the speed of performing a serial subtraction

task (Scholey and Kennedy, 2002) and significant slowing of working memory (Chapter 4). These decrements in speed of task performance contrast with recent findings for the same 200 mg dose of improved speed of information retrieval, attention and arithmetical performance (Kennedy *et al.*, 2004; Chapter 2 and Chapter 3), significantly shortened latency of the P300 component of auditory evoked potentials (Kennedy *et al.*, 2003), faster attentional performance following 400mg (Sünram-Lea et al., 2004) and faster working memory performance (Chapter 4).

With regards to secondary memory performance the results of the present study revealed a significant superimposed effect on delayed picture recognition reaction time. The pattern of results suggests that the placebo group appears to be speeded in their performance at pre-dose, which is then attenuated post-dose (possibly due to fatigue). Planned comparisons revealed that *Panax ginseng* led to significantly slower reaction times at the pre-dose testing session on day 29 and day 57.

With regards to subjective self-report ratings of contentedness the initial ANOVA revealed differential chronic effect of 29 and 57 days of treatment. The pattern of results suggests that following 29 days of treatment content ratings were worse, however, following these ratings were ameliorated by day 57. The planned comparisons supported this pattern showing that ginseng led to significantly worse mood ratings at the pre and post-dose testing sessions on day 29 but these significant differences had disappeared by day 57 (Figure 5.5).

On the subjective self-reported dimensions of confusion, tension and depression the ANOVA revealed superimposed effects (Figure 5.6). In all three cases, the pattern of results suggests that following chronic ingestion of ginseng (i.e. pre-dose testing sessions) subjective mood was worse, as compared to placebo.

However, after the ingestion of that day's dose these negative moods were somewhat ameliorated. Planned comparisons supported this pattern, however, these mood effects were only significantly ameliorated following an acute dose on day 57 (Figure 5.6).

A number of studies deal with the more generalised question of 'quality of life' or 'well-being.' *Panax ginseng's* effect on these parameters has been investigated in a number of placebo controlled trials administered alone (Wiklund et al., 1999; Sotaniemi et al., 1995; Ellis and Reddy 2002; Cardinal and Engles, 2001) and in conjunction with vitamins and minerals (Wiklund et al., 1994; Neri et al., 1995; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000). Ginseng's effects have been evaluated using dosages ranging from 80 to 400 mg in patient (Wiklund et al., 1994, Sotaniemi et al., 1995, Neri et al., 1995) and healthy populations of various ages and stress levels (Wiklund et al., 1994; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000; Ellis and Reddy 2002; Cardinal and Engles, 2001). Study duration has spanned from 2 to 9 months (See Coleman et al., 2003 for review). Improvements in the measures pertaining to 'quality of life' or 'well-being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations have been demonstrated, although findings of this nature are by no means unequivocal (see Kennedy and Scholey, 2003). As an example and even when considering only those studies that have used supplementation of *Panax ginseng* alone in a healthy cohort, results have still been mixed results. For instance, Ellis and Reddy (2002) report improvements in measures of 'quality of life' after 4 weeks of 200 mg G115/day, as compared to placebo, in a small cohort of 30 healthy young adults but these

improvements were attenuating by the 8-week end point. Conversely, Cardinal and Engels (2001), using a similar but slightly larger cohort of 83 healthy young adults, reported no significant differences on the Positive Affect–Negative Affect Scale (PANAS) or Profile of Mood States (POMS) at their 8-week end point following 200 mg G115 or 400 mg G115 daily as compared to placebo. Chapter 4 of this thesis did however report acute improvements in calmness ratings and chapter 2 and chapter 3 did report significant improvements in subjective self-report ratings of mental fatigue.

In conclusion the results of the current study are the first to systematically investigate *Panax ginseng's* acute, chronic and superimposed effects on behaviour and subjective mood in healthy volunteers. Additionally, *Panax ginseng's* gluco-regulatory properties were further investigated. With regards to behavioural performance the results of the present study revealed improvements in working memory performance following a single acute dose of *Panax ginseng,* whereas, following chronic dosing (i.e. 29 and 57 days, 200 mg/d) results revealed both improvements and decrements in certain aspects of cognition and mood. Interestingly, and for the first time, the results also revealed a superimposed relationship between acute and chronic ingestion. This pattern of results highlights that an observed effect on a performance measure following chronic ingestion (i.e. pre-dose testing on day 29 and 57) can be further modulated by that day's acute dose (i.e. post-dose testing on day 29 and day 57). Results failed to reveal any acute effect on circulating blood glucose levels after participants had consumed a 'normal' breakfast. There was also no effect found on any longer-term indicator of glucose regulation (i.e. HbA1c). Caution is advised when generalising results because of methodological differences between

studies. This is especially true when the underlying mechanisms for ginseng's behavioural and glucoregulatory effects are not fully understood.

CHAPTER 6. AN INVESTIGATION INTO THE ACUTE, CHRONIC AND SUPERIMPOSED EFFECTS OF A NON-STANDARDISED KOREAN GINSENG EXTRACT ON COGNITIVE PERFORMANCE, MOOD AND INDICES OF GLUCOSE REGULATION: A 20 WEEK TRIAL IN HEALTHY VOLUNTEERS.

6.1 Introduction

Chapter 5, of this thesis, was the first study to systematically investigate *Panax ginseng's* (G115) acute, chronic and superimposed effects on behaviour, subjective mood, capillary blood glucose levels and indices of glycaemic regulation in healthy volunteers. There were no effects of acute or chronic ingestion on any physiological measure (blood glucose levels; HbA1c or insulin). However, there were behavioural and mood effects of acute and chronic ingestion of *Panax ginseng* (G115). Working memory performance was significantly improved 3 hours post-dose following 200 mg (G115). A differentiated pattern of chronic and superimposed effects was also found. Working memory was improved by chronic dosing but attenuated by an acute dose. Conversely, *Panax ginseng* (G115) impaired the speed of attentional performance following chronic ingestion. Subjective self-report ratings of contentedness were significantly worse following 29 days, however, these were attenuated by day 57. Additionally, self-reported ratings of confusion, tension and depression were significantly worse following chronic ingestion of (i.e. pre-dose testing sessions), as compared to their respective placebos, however, after the ingestion of that day's acute dose these negative mood states were somewhat ameliorated. These data are the first to report chronic effects of *Panax ginseng* (G115) and also the first to report the superimposed effects. In chapter 4 it was shown that 6 consecutive days of *Panax*

ginseng (G115) ingestion had no effect on behavioural measures, however, following day 7's acute dose, improvements were reported on working memory up to 4 hours post-dose. Conversely, in chapter 5 there were chronic behavioural effects following both 29 and 57 days. Further research is needed to investigate when these effects first emerge and the extent to which they persist past 57 days. Additionally, further investigation of the superimposed effects is needed.

The mechanisms by which ginseng might modulate human cognitive performance are not yet well understood (Kennedy and Scholey 2003) however, it is believed that behavioural change following ginseng ingestion is due to the ginsenoside content (ginseng's psychoactive chemicals) of the extract.

There is an abundance of *in vitro* and *in vivo* animal work demonstrating the physiological effects and behavioural change following whole ginseng ingestion or its individual ginsenosides (see introduction). Interpretation and conclusions relating to ginseng's behavioural and physiological effects is difficult as studies have often investigated different ginseng species, used different doses and investigated different behavioural parameters. Additionally, human studies into the effects of ginseng have been criticised for lack of control (Kennedy et al., 2003; Scholey et al., 2004). These methodological differences alone may account for some of the variability and inconsistent data patterns previously reported. However, the variability in the active ginsenoside content both between and within species must also play an important role. As a practical example, Seivenpiper et al (2003) demonstrated that a batch of *Panax quinquefolius* with a depressed ginsenoside profile did not affect post-prandial glycaemia as opposed to the hypoglycaemic properties demonstrated earlier using the same species, same methodology and the same cohort but enhanced ginsenoside content

(Vuksan et al 2000; 2001a; 2001b). This could suggest that differences in the ratio of the individual active constituents that make ginseng may account for the differences in behavioural effects previously reported. As a further practical example, Rb1 and Rg1 (the two main ginsenosides of the protopanaxatriol and protopanaxadiol groups respectively) have shown to have opposing effects in vitro and differ in the magnitude of effect on a number of different physiological responses in vitro and in vivo (see general introduction). The physiological effects of the individual ginsenosides may be somewhat clearer, although by no means unequivocal, however, these results tell us nothing of the effects of whole ginseng extracts, which may contain at least another 30 different active molecules each having potential inhibitory, excitatory and possibly synergistic behavioural effects. Even when considering a standardised extract (e.g. G115 standardised 4% total ginsenoside content) the results are still somewhat inconclusive (although there is some consistent data pertaining to the acute behavioural effects, for example, see chapter 2, chapter 3 and Kennedy et al., 2003) as studies have used different methodologies, investigated different responses and it is still possible that small differences in the ratio of protopanaxatriol : protopanaxadiol may produce differing results. This being one possible explanation for the somewhat inconsistent results of the present thesis, although, methodological differences between studies, the cognitive structures investigated and individual differences must play a role.

The ginseng literature would benefit greatly if non-standardised extracts were profiled with regards to; ginsenoside content; physiological effect on potentially relevant parameters and then assessed for behavioural change utilising standardised methodologies and testing platform. The present study investigated

the cognitive, mood and gluco-regulatory effects of a non-standardised *Panax ginseng* extract (Korean origin). The extract was subjected to HPLC to quantify the active ginsenosides. Additionally, gluco-regulation and behavioural performance were assessed utilising methodologies and testing platforms that have been shown to be sensitive to the effects of herbal supplements.

6.2. Subjects and methods

6.2.1 Participants

Thirteen female and 5 male volunteers recruited through local media advertisements, participated in the study. Two female participants failed to complete all study sessions of the study, stating time constraints as the reason, leaving 16 volunteers (mean age 38.31 years; SD 10.3). The study was approved by the Northumbria University Division of Psychology Ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation each participant gave informed consent and completed a medical health questionnaire (see appendix 30). All participants reported that they were in good health and were not taking any illicit social drugs. Additionally, they were free from 'over the-counter' or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. All participants overnight fasted and were alcohol and caffeine free for 12 hours prior to all assessment days, and abstained from products containing these substances on the days of testing. Volunteers were paid £150 for participation. Treatment order was counterbalanced and participants were randomly allocated to a treatment regimen (see appendix 31).

6.2.1.1. Treatment compliance

Participants were asked to keep a record of the days that they failed to take their treatment. Only one participant reported a failure to take one day's dose when in the active treatment condition (day 17 of the treatment regimen) due to unforeseen working commitments. This participant was included in all

subsequent analysis. Participants were blind to their treatment condition throughout the study.

6.2.2 Behavioural and mood assessment

6.2.2.1 Subjective self-reported assessment of mood

Subjective self-reported mood was assessed using the same measurement tools as used in chapter 5 (section 5.2)

6.2.2.2 Behavioural assessment

The assessment battery and running order of the tasks were identical to that in chapter 5 (section 5.2).

6.2.3 Physiological measures:

Measurements and timings were identical to that in chapter 5 (section 5.2)

6.2.4 Treatments

Active treatments and placebo capsules, matched for size, colour, opacity and odour were provided by the manufacturer (Korea ginseng corporation, Seoul, Korea). Prior to the commencement of the study, a disinterested third party, who had no other involvement in the study prepared the treatments for each individual participant (treatment order was counter-balanced and participants were randomly allocated to a treatment regime) and sealed them in containers marked only with the participant code and study day number. On each study day, participants received two capsules. The individual capsules contained either an inert placebo,

or 100 mg of *Panax ginseng* extract. Depending on the condition to which the participant was allocated on that particular day, the combination of capsules treatments corresponded to a dose of 0 mg (placebo) or 200 mg ginseng.

6.2.4.1 Ginsenoside profile

HPLC was used to investigate the ginsenoside profile of this non-standardised *Panax ginseng* extract. Results were inconclusive (see appendix 32)

6.2.5 Procedure

The study commenced with a practice day that was identical to subsequent study days with the exception that no treatment was offered, no blood samples were taken and the behavioural data acquired was not entered into any analysis (except to check that it fell within established norms). Participants received each treatment (placebo, and 200 mg) for 57 days in total, with a wash-out period of 27 days between treatments. The order of presentation of the treatments was counterbalanced by random allocation to a treatment regimen. Participants were assessed on the first day (day 1), the halfway point (day 29) and last day (day 57) of each treatment period (i.e. 6 assessment days in total across the two treatments (see Diagram 7). On each of these days the cognitive/mood testing regime comprised assessments (see chapter 5 for tasks and running order) pre dose, followed immediately be ingestion of the day's treatment, and thereafter at 3hrs post-dose. Testing took place in a suite of laboratories with participants visually isolated from each other. Upon completion of the 3hr post-dose testing session for each treatment period participants were provided with a container containing treatments for each day (days 2 to 28; days 30 to 56) until the next laboratory

visit. Treatments on day 1, day 29 and day 57 of each period were consumed in the laboratory. Capillary finger prick blood samples and whole blood (2 x 2.5 ml) was collected on each study day, in both treatment regimes, immediately prior to the commencement of the pre-dose and post-dose (finger prick sample only) behavioural assessments (see Diagram 8). All participants were in an overnight fasting state during the pre-dose data collection and consumed the same (i.e. across all 6 testing days) self-selected breakfast immediately after treatment ingestion.

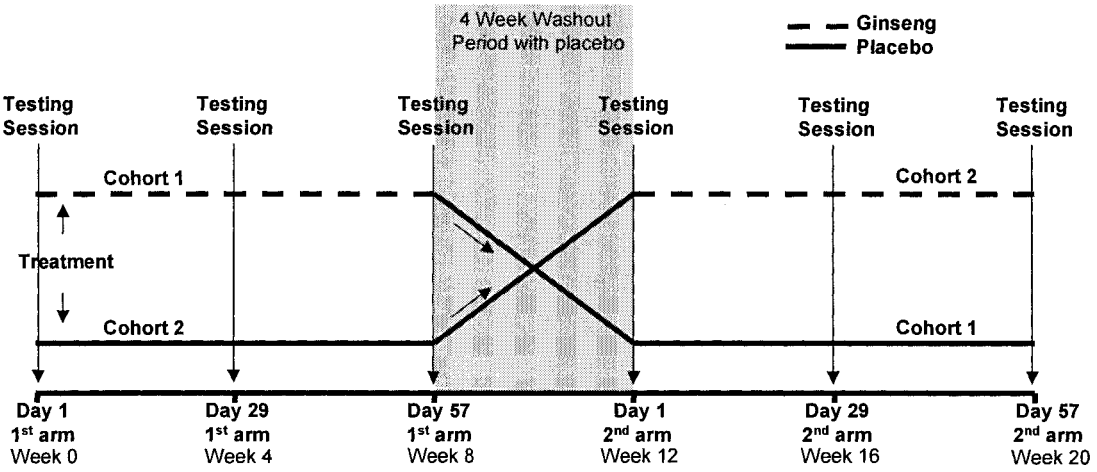

Diagram 7. Timelines of the study. Participants received either placebo or ginseng during the first arm of the study (weeks 0 to 8), and the opposite treatment in the second arm (weeks 12 to 20). All participants received placebo during the washout period (beginning of week 9 to end of week 12). Testing days took place on days 1, 29, and 57 of each arm.

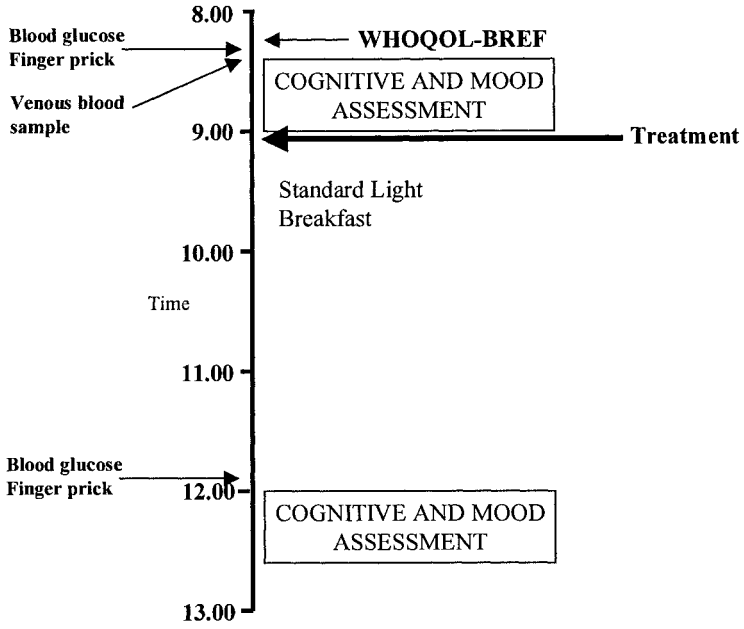

Diagram 8. Running order of each study day (day 1, 29 and 57) of both the 1st and 2nd arms.

6.2.6 Statistics

All data were analysed using the Minitab statistical package version 13.1. An initial one-way repeated measures ANOVA was conducted on day 1 baseline raw score data, for each outcome measure (see appendix 33). For all subsequent analysis, for all outcome measures, 'Change from pre-dose day 1 baseline' scores were used.

6.2.6.1. *Acute effects*

Data obtained from the 3hr post-dose testing session on day 1 (change from day 1 pre-dose baseline) was analysed by one-way repeated measures ANOVA to reveal any acute effects (see appendix 34).

6.2.6.2 *Chronic effects*

When only pre-dose data were obtained (HbA1c; insulin; WHOQOL) on day 29 and 57 (all data was change from day 1 pre-dose baseline), analysis was by two way repeated measures ANOVA (treatment x day) to reveal any chronic effects (see appendix 35).

6.2.6.3 *Using a 2-way ANOVA with treatment and day as the I.Vs*

A significant main effect of treatment would indicate a simple chronic effect summed across both days.

A significant treatment x day interaction would also indicate a chronic effect that is complicated by a differential influence of 29 and 57 consecutive days of treatment.

6.2.6.4 *Chronic and superimposed effects*

Pre and post-dose data obtained from day 29 and 57 (all data was change from day 1 pre-dose baseline) was analysed by three way repeated measures ANOVA (treatment x day x pre/post) to reveal any chronic and super-imposed chronic/acute effects (see appendix 36).

6.2.6.5 *Using a 3-way ANOVA with treatment, day and pre/post as the I.Vs*

A significant main effect of treatment would indicate a simple chronic effect summed across both days and all four testing sessions.

A significant treatment x day interaction would also indicate a chronic effect that is complicated by a differential influence of 29 and 57 consecutive days of treatment.

A significant treatment x pre/post interaction would indicate both chronic (i.e. pre-dose data) and the superimposed (i.e. post-dose data).

6.2.6.6 *Planned comparisons*

On those measures that yielded a significant main effect or interaction with treatment, planned comparisons were made between placebo and the active treatment utilising t tests with MSError as an error term (Keppel, 1991). To ensure the overall protection level, comparisons were strictly planned prior to commencement of the study and only conducted on those outcome measures that reached statistical significance on the initial ANOVA. Additionally, only probabilities associated with planned comparisons were calculated, and all testing was two-tailed (see appendix 37).

6.3. RESULTS

6.3.1 Baseline scores

Prior to analysis of change from baseline data, raw baseline scores for both treatment conditions (placebo, 200mg) for each of the primary outcome measures were subject to one-way repeated-measures ANOVAs. There were no significant baseline differences revealed on any outcome measure. Mean pre-dose baseline raw scores and change from day 1 baseline scores, for each condition, at each post-dose time point on each primary outcome measure and for blood glucose levels, are represented in Table 6.1.

Table 6.1. Effects of 200mg *Panax ginseng* and placebo on task performance, blood glucose levels and indices of glucose regulation. Values represent mean baseline performance/glucose score/levels and mean change from baseline performance score at post-dose on day 1; mean change from baseline performance score at pre-dose and post-dose on day 29; mean change from baseline performance score at pre-dose and post-dose on day 57. Standard errors in italics.

N = 16			Acute effects		Chronic / superimposed effects			
		Baseline se	Day 1 post-dose se	Day 29 pre-dose se	Day 29 post-dose se	Day 57 Pre-dose se	Day 57 post-dose se
Primary Outcome Factors							
Quality of memory (% X 6)	placebo	395.03 *10.40*	-27.100 *11.740*	16.545 *9.869*	-7.606 *16.012*	12.023 *18.804*	-18.413 *13.603*
	200mg	406.51 *15.86*	-47.29 *12.502*	-18.610 *15.960*	-39.957 *13.843*	-15.658 *17.179*	-30.608 *10.104*
Secondary memory (% X 4)	placebo	211.041 *8.454*	-20.63 *10.53*	13.229 *8.690*	-7.710 *13.723*	7.604 *15.451*	-12.397 *10.108*
	200mg	217.291 *15.050*	-42.39 *12.91*	-13.750 *13.415*	-33.437 *12.927*	-13.332 *16.780*	-19.478 *11.514*
Working memory (% X 2)	placebo	183.986 *4.375*	-6.48 *6.93*	3.318 *3.131*	0.104 *3.281*	4.419 *5.069*	-6.017 *5.730*
	200mg	189.221 *2.432*	-4.90 *3.15*	-4.861 *3.896*	-6.520 *3.919*	-2.326 *1.894*	-11.130 *6.135*
Speed of attention (summed ms)	placebo	1134.216 *24.665*	14.08 *12.12*	29.983 *21.514*	19.613 *19.628*	7.085 *20.029*	35.072 *27.603*
	200mg	1152.153 *29.746*	-0.23 *16.57*	5.442 *16.700*	-4.197 *21.123*	66.846 *45.737*	19.263 *16.749*
Speed of memory (summed ms)	placebo	2969.053 *137.018*	-55.94 *58.80*	-12.104 *65.326*	-78.410 *85.725*	-42.086 *59.807*	-59.907 *74.178*
	200mg	2931.286 *160.755*	54.62 *38.10*	45.971 *73.573*	1.352 *76.002*	56.692 *105.175*	6.389 *86.489*
Accuracy of attention (%)	placebo	364.167 *1.973*	-1.67 *2.27*	-0.458 *2.340*	-3.041 *2.398*	-0.396 *2.799*	-1.104 *2.448*
	200mg	364.501 *2.640*	-2.04 *2.92*	2.396 *2.543*	-5.125 *3.011*	-0.146 *3.170*	0.583 *3.115*
Individual CDR tasks							
Simple reaction time (ms)	placebo	282.24 *7.40*	2.46 *4.69*	8.37313 *7.38605*	7.42813 *7.03087*	-3.63375 *7.78309*	12.72125 *10.69625*
	200mg	279.32 *8.03*	6.27 *5.91*	1.29438 *6.28557*	4.58500 *5.54478*	50.42813 *41.57560*	9.62500 *6.29673*
Digit Vigilance							
Accuracy (%)	placebo	96.95 *1.03*	-0.56 *1.38*	-0.41688 *1.31860*	-0.41563 *1.31806*	0.27813 *1.23213*	0.41688 *1.13378*
	200mg	97.78 *0.50*	-0.70 *0.80*	-1.25063 *1.24345*	-2.22250 *1.19986*	-2.08313 *0.74439*	-0.55688 *0.62526*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Reaction time (ms)	placebo	418.13 *8.34*	3.65 *6.46*	9.91313 *6.66287*	4.38125 *7.77289*	1.48250 *8.71528*	1.27313 *7.90793*
	200mg	418.45 *9.37*	-4.93 *7.54*	5.10250 *7.84294*	5.56563 *8.24055*	13.56313 *7.88357*	14.48625 *7.31976*
False alarms	placebo	1.125 *0.272*	-0.043 *0.315*	-0.563 *0.302*	-0.063 *0.335*	-0.313 *0.299*	-0.375 *0.287*
	200mg	0.750 *0.194*	0.023 *0.230*	0.375 *0.300*	0.375 *0.340*	0.875 *0.407*	0.000 *0.274*
Choice reaction time (ms)	placebo	433.85 *13.51*	-0.50 *0.62*	11.69625 *11.65169*	7.80375 *8.83655*	9.23625 *6.50073*	21.07750 *15.68284*
	200mg	454.38 *17.01*	-0.50 *0.90*	-0.95500 *8.05070*	-14.34813 *12.80415*	2.85437 *10.48479*	-4.84813 *12.90844*
Choice RT accuracy (%)	placebo	96.50 *0.56*	7.98 *5.52*	-0.25000 *0.70415*	-0.87500 *0.83604*	-0.25000 *0.89209*	-0.50000 *0.71880*
	200mg	96.38 *0.78*	-1.57 *10.70*	1.00000 *0.82916*	-1.12500 *0.79517*	0.50000 *0.93986*	0.25000 *0.91059*
Spatial Memory							
Targets (%)	placebo	98.047 *1.239*	-5.47 *3.69*	-0.781 *1.383*	-1.563 *1.210*	-1.172 *2.226*	-3.516 *2.613*
	200mg	97.656 *0.967*	1.56 *1.21*	-1.563 *2.344*	-2.734 *2.851*	0.000 *1.614*	-3.906 *2.902*
Distractors (%)	placebo	95.94 *2.00*	-0.31 *3.34*	1.87500 *1.76039*	0.00000 *1.70783*	2.81250 *2.18750*	0.00000 *3.25960*
	200mg	99.06 *0.94*	-1.88 *1.28*	-0.93750 *1.44338*	-2.81250 *1.11512*	-0.93750 *0.67988*	-5.00000 *3.02765*
Sensitivity index	placebo	0.94 *0.03*	-0.06 *0.07*	0.01044 *0.02743*	-0.01463 *0.02042*	0.01650 *0.03938*	-0.03400 *0.05398*
	200mg	0.97 *0.01*	0.00 *0.01*	-0.02556 *0.02851*	-0.05681 *0.03569*	-0.01025 *0.01387*	-0.08875 *0.05650*
Targets RT (ms)	placebo	619.32 *27.41*	-51.59 *16.65*	70.35000 *42.78940*	-17.34875 *28.56501*	4.83000 *26.32217*	26.89000 *40.98493*
	200mg	676.04 *43.26*	-90.26 *29.73*	-24.43750 *26.54182*	-27.53625 *25.93127*	36.97938 *49.13975*	-39.22125 *48.46246*
Distractors RT (ms)	placebo	648.343 *25.851*	3.03 *23.44*	95.198 *36.615*	-9.636 *17.921*	37.474 *20.776*	50.339 *35.227*
	200mg	711.872 *48.907*	-70.76 *22.87*	31.903 *19.994*	26.353 *44.554*	70.121 *61.955*	-30.014 *39.980*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Average RT (ms)	placebo	635.858 *24.434*	-21.85 *18.92*	83.879 *37.390*	-14.094 *19.301*	23.276 *20.133*	40.387 *33.126*
	200mg	696.046 *45.175*	-79.70 *25.04*	7.920 *20.212*	1.417 *33.821*	56.137 *55.177*	-34.729 *43.118*
Numeric Working Memory							
Targets (%)	placebo	93.751 *1.423*	-0.83 *1.05*	1.111 *1.585*	0.694 *1.718*	1.250 *1.518*	-1.529 *1.682*
	200mg	95.556 *1.111*	-3.19 *1.65*	-2.362 *1.543*	-1.111 *0.973*	-1.944 *1.489*	-2.778 *1.323*
Distractors (%)	placebo	96.250 *1.087*	0.14 *1.17*	1.113 *1.074*	0.973 *0.860*	1.529 *0.664*	-0.973 *1.267*
	200mg	96.946 *0.833*	-1.39 *1.27*	0.001 *0.818*	0.138 *1.024*	0.556 *0.745*	0.554 *0.873*
Sensitivity index	placebo	0.903 *0.020*	-0.01 *0.01*	0.022 *0.015*	0.016 *0.020*	0.026 *0.018*	-0.026 *0.023*
	200mg	0.926 *0.017*	-0.04 *0.02*	-0.022 *0.019*	-0.007 *0.011*	-0.011 *0.013*	-0.020 *0.015*
Targets RT (ms)	placebo	623.989 *35.651*	-28.85 *21.73*	-27.053 *25.991*	-37.258 *37.487*	-42.359 *24.142*	-34.681 *19.726*
	200mg	624.873 *49.285*	-16.09 *16.43*	-32.143 *30.537*	-44.409 *23.723*	-55.774 *32.317*	-55.395 *41.256*
Distractors RT (ms)	placebo	704.754 *35.660*	-22.31 *22.96*	-31.385 *26.143*	-40.089 *27.813*	-40.916 *23.790*	-28.943 *21.948*
	200mg	692.333 *53.210*	-6.65 *14.81*	-25.422 *24.152*	-40.787 *24.678*	-31.564 *29.733*	-48.473 *35.580*
Average RT (ms)	placebo	664.640 *34.986*	-25.60 *19.41*	-28.723 *25.411*	-38.541 *31.367*	-41.403 *21.619*	-31.211 *19.697*
	200mg	658.622 *50.732*	-10.74 *14.42*	-28.263 *26.224*	-42.306 *23.482*	-42.794 *29.974*	-51.312 *37.814*
Word Recognition							
Targets (%)	placebo	68.333 *3.776*	1.67 *4.01*	1.667 *4.014*	0.832 *4.977*	4.999 *3.249*	3.750 *4.471*
	200mg	73.334 *3.702*	-2.92 *3.65*	-2.917 *5.034*	-2.917 *3.599*	-5.001 *4.888*	0.000 *4.675*
Distractors (%)	placebo	91.250 *2.083*	-1.67 *2.40*	-2.084 *3.637*	-5.834 *2.974*	2.084 *2.770*	-3.334 *2.582*
	200mg	92.499 *2.097*	-11.25 *3.26*	-2.499 *2.785*	-5.416 *2.295*	-0.415 *1.771*	-5.833 *3.096*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Sensitivity index	placebo	0.649 *0.038*	0.00 *0.04*	0.012 *0.050*	-0.058 *0.061*	0.073 *0.045*	-0.008 *0.048*
	200mg	0.704 *0.034*	-0.15 *0.04*	-0.042 *0.055*	-0.084 *0.028*	-0.029 *0.041*	-0.070 *0.040*
Targets RT (ms)	placebo	772.923 *74.178*	-37.13 *64.20*	-47.445 *50.004*	55.035 *59.039*	-22.791 *48.831*	-37.892 *64.986*
	200mg	753.496 *59.584*	32.92 *18.17*	21.407 *49.121*	-22.028 *40.006*	-5.351 *53.840*	50.078 *75.034*
Distractors RT (ms)	placebo	782.290 *47.126*	3.51 *27.68*	8.149 *27.233*	3.823 *39.122*	28.912 *33.951*	-14.434 *41.758*
	200mg	753.382 *35.829*	75.22 *33.50*	48.251 *33.452*	10.175 *25.839*	7.330 *19.737*	14.773 *39.289*
Average RT (ms)	placebo	776.891 *58.106*	-12.85 *41.36*	-22.084 *29.113*	16.858 *40.697*	3.996 *35.195*	-26.540 *50.147*
	200mg	747.238 *40.976*	62.22 *20.05*	30.899 *34.040*	-1.997 *25.713*	9.076 *26.988*	38.531 *29.850*
Picture Recognition							
Targets (%)	placebo	81.250 *3.276*	2.81 *1.99*	4.375 *2.135*	2.500 *2.993*	1.250 *6.132*	3.125 *3.191*
	200mg	79.375 *5.281*	0.94 *5.70*	0.313 *3.613*	0.625 *5.018*	0.938 *5.849*	0.625 *5.753*
Distractors (%)	placebo	89.375 *1.700*	-0.94 *1.66*	2.188 *1.512*	5.625 *1.281*	-0.313 *5.871*	1.563 *2.127*
	200mg	86.250 *6.098*	3.75 *6.65*	7.188 *1.526*	2.188 *5.737*	6.563 *5.858*	5.313 *6.012*
Sensitivity index	placebo	0.723 *0.035*	0.02 *0.03*	0.065 *0.027*	0.090 *0.032*	0.006 *0.112*	0.044 *0.044*
	200mg	0.664 *0.112*	0.06 *0.12*	0.088 *0.043*	0.032 *0.103*	0.095 *0.110*	0.066 *0.116*
Targets RT (ms)	placebo	885.718 *60.651*	-24.06 *27.20*	-58.600 *46.726*	-42.441 *53.209*	-32.351 *46.182*	-59.380 *42.951*
	200mg	819.054 *44.093*	77.97 *29.30*	36.472 *44.529*	51.112 *31.822*	30.469 *19.869*	64.933 *28.516*
Distractors RT (ms)	placebo	901.210 *49.510*	35.11 *23.08*	-35.659 *33.524*	-39.735 *46.003*	-11.538 *55.000*	-26.836 *38.782*
	200mg	852.368 *47.691*	64.26 *47.98*	31.941 *39.572*	33.374 *41.043*	19.832 *42.178*	30.214 *33.148*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Average RT (ms)	placebo	891.664 *54.268*	4.36 *22.29*	-45.177 *37.943*	-42.633 *47.899*	-27.954 *43.255*	-42.543 *35.535*
	200mg	829.381 *45.836*	82.84 *26.65*	35.414 *40.508*	44.238 *35.256*	34.273 *30.056*	53.899 *25.710*
Bond-lader							
Alert	placebo	52.975 *3.513*	1.74 *3.08*	5.719 *2.775*	6.544 *4.378*	4.838 *4.219*	3.056 *5.234*
	200mg	52.575 *4.219*	-1.90 *2.37*	1.475 *3.847*	1.594 *3.954*	4.375 *3.300*	6.288 *3.534*
Content	placebo	66.363 *3.838*	0.38 *1.12*	1.075 *3.352*	2.175 *3.699*	3.838 *2.779*	2.425 *3.134*
	200mg	65.750 *3.602*	-0.38 *1.37*	1.613 *3.230*	1.588 *2.359*	1.663 *2.525*	2.000 *3.267*
Calm	placebo	63.625 *4.191*	-0.13 *2.30*	3.688 *2.741*	3.844 *2.105*	5.563 *2.731*	2.156 *3.335*
	200mg	70.375 *3.538*	-2.34 *2.18*	-2.906 *2.282*	-5.469 *2.237*	-2.813 *2.825*	-3.750 *2.884*
Immediate Word Recall							
No of words	placebo	7.375 *0.455*	-1.38 *0.53*	0.125 *0.539*	-0.563 *0.563*	-0.188 *0.379*	-0.938 *0.581*
	200mg	7.563 *0.713*	-1.50 *0.51*	-0.813 *0.535*	-1.438 *0.516*	-1.000 *0.780*	-1.000 *0.524*
Errors	placebo	0.188 *0.101*	0.13 *0.13*	0.000 *0.158*	0.250 *0.194*	0.188 *0.209*	0.438 *0.182*
	200mg	0.313 *0.176*	0.44 *0.18*	-0.125 *0.222*	0.063 *0.281*	0.125 *0.287*	0.188 *0.228*
Intrusions	placebo	0.000 *0.000*	0.00 *0.00*	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*	0.000 *0.000*
	200mg	0.000 *0.000*	0.00 *0.00*	0.000 *0.000*	0.063 *0.063*	0.000 *0.000*	0.000 *0.000*
Delayed word recall							
No of words	placebo	5.375 *0.417*	-1.63 *0.35*	0.750 *0.512*	-0.688 *0.435*	0.438 *0.341*	-1.250 *0.371*
	200mg	5.875 *0.735*	-2.63 *0.71*	-1.625 *0.421*	-2.125 *0.688*	-1.000 *0.758*	-1.813 *0.647*
Errors	placebo	0.375 *0.125*	0.13 *0.20*	-0.125 *0.180*	0.125 *0.256*	0.000 *0.224*	0.000 *0.258*
	200mg	0.313 *0.120*	0.38 *0.30*	0.000 *0.225*	0.375 *0.155*	0.125 *0.155*	-0.063 *0.143*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Intrusions	placebo	0.000 *0.000*	0.13 *0.13*	0.000 *0.000*	0.125 *0.085*	0.000 *0.000*	0.125 *0.085*
	200mg	0.000 *0.000*	0.00 *0.00*	0.000 *0.069*	0.063 *0.063*	0.000 *0.000*	0.063 *0.063*
Brunnel scale							
Tension	placebo	0.938 *0.295*	-0.19 *0.23*	-0.500 *0.342*	-0.375 *0.239*	-0.375 *0.256*	-0.500 *0.224*
	200mg	0.688 *0.285*	-0.38 *0.20*	0.313 *0.375*	0.250 *0.403*	0.375 *0.221*	0.125 *0.427*
Depression	placebo	0.875 *0.397*	-0.38 *0.29*	0.000 *0.570*	-0.438 *0.428*	0.000 *0.456*	-0.500 *0.303*
	200mg	1.313 *0.498*	-0.19 *0.19*	0.313 *0.509*	-0.250 *0.504*	-0.688 *0.650*	-1.000 *0.632*
Aggression	placebo	0.375 *0.221*	-0.25 *0.14*	-0.250 *0.214*	-0.313 *0.176*	-0.313 *0.176*	-0.375 *0.221*
	200mg	0.063 *0.063*	0.31 *0.25*	0.500 *0.195*	0.125 *0.155*	0.125 *0.202*	0.250 *0.266*
Vigour	placebo	4.938 *0.772*	0.63 *1.03*	0.500 *0.465*	2.125 *0.638*	0.313 *0.597*	2.000 *0.742*
	200mg	5.250 *1.002*	0.63 *0.78*	-0.250 *0.673*	1.938 *0.994*	-0.188 *0.600*	2.125 *1.147*
Fatigue	placebo	3.500 *0.619*	-0.50 *0.97*	1.188 *0.923*	-1.125 *0.455*	0.250 *0.911*	-1.125 *0.625*
	200mg	4.563 *1.187*	-1.88 *0.55*	-0.250 *1.013*	-1.875 *1.303*	-0.938 *1.352*	-2.188 *1.229*
Confusion	placebo	0.563 *0.341*	-0.06 *0.11*	0.625 *0.523*	0.125 *0.256*	0.125 *0.221*	-0.188 *0.209*
	200mg	0.438 *0.258*	0.06 *0.17*	0.313 *0.341*	-0.063 *0.359*	0.000 *0.387*	0.000 *0.387*
Corsi Block							
Span	placebo	5.238 *0.210*	0.18 *0.23*	0.200 *0.224*	-0.038 *0.268*	0.138 *0.140*	0.238 *0.193*
	200mg	4.925 *0.168*	0.33 *0.26*	0.038 *0.245*	0.238 *0.240*	0.588 *0.190*	0.400 *0.221*
Alphabetic Working Memory							
Targets	placebo	13.354 *0.271*	-0.21 *0.22*	-0.021 *0.259*	0.438 *0.327*	0.500 *0.265*	0.125 *0.235*
	200mg	13.646 *0.244*	0.21 *0.28*	-0.229 *0.320*	-0.104 *0.282*	-0.438 *0.310*	-0.042 *0.349*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Distractors	placebo	14.104 *0.227*	1.08 *0.79*	0.250 *0.387*	-2.102 *0.681*	0.479 *0.182*	0.500 *0.188*
	200mg	14.313 *0.151*	0.19 *0.19*	0.292 *0.614*	-1.859 *0.613*	0.146 *0.146*	0.271 *0.167*
Targets RT (ms)	placebo	0.740 *0.041*	-0.02 *0.02*	-0.017 *0.023*	-0.032 *0.025*	0.025 *0.035*	-0.049 *0.030*
	200mg	0.714 *0.033*	-0.01 *0.02*	0.002 *0.022*	0.004 *0.028*	0.001 *0.028*	-0.004 *0.021*
Distractors RT (ms)	placebo	0.937 *0.108*	-0.12 *0.12*	-0.136 *0.119*	-0.187 *0.125*	-0.119 *0.119*	-0.153 *0.119*
	200mg	0.932 *0.133*	0.01 *0.18*	-0.129 *0.139*	0.045 *0.153*	0.020 *0.185*	-0.177 *0.131*
Error	placebo	1.646 *0.271*	0.21 *0.22*	0.021 *0.259*	-0.438 *0.327*	-0.500 *0.265*	-0.125 *0.235*
	200mg	1.354 *0.244*	-0.21 *0.28*	0.229 *0.320*	0.104 *0.282*	0.438 *0.310*	0.042 *0.349*
False alarms	placebo	0.896 *0.227*	-0.33 *0.25*	-0.458 *0.237*	2.000 *0.662*	-0.479 *0.182*	-0.500 *0.188*
	200mg	0.688 *0.151*	-0.19 *0.19*	-0.292 *0.595*	1.688 *0.568*	-0.146 *0.146*	-0.271 *0.167*
N Back							
Targets	placebo	10.313 *0.778*	0.69 *0.60*	-0.188 *0.807*	-0.625 *0.752*	0.625 *0.682*	0.500 *0.577*
	200mg	10.000 *0.612*	0.75 *0.50*	0.563 *0.773*	0.313 *0.568*	0.000 *0.524*	-0.125 *0.584*
Error	placebo	3.438 *0.741*	-0.44 *0.60*	0.375 *0.752*	0.750 *0.756*	-0.375 *0.657*	-0.375 *0.547*
	200mg	3.938 *0.588*	-0.75 *0.50*	-0.688 *0.790*	-0.313 *0.568*	0.063 *0.512*	0.000 *0.524*
Distractors	placebo	25.188 *0.802*	0.00 *0.81*	0.500 *0.532*	-0.750 *1.010*	-0.563 *0.871*	0.188 *0.909*
	200mg	24.063 *1.105*	0.31 *0.55*	-0.563 *0.984*	0.625 *0.651*	0.750 *0.864*	0.625 *0.598*
False alarms	placebo	2.250 *0.595*	0.56 *0.72*	-0.188 *0.502*	0.875 *0.816*	0.750 *0.750*	0.000 *0.736*
	200mg	3.750 *1.051*	-0.63 *0.57*	0.500 *0.842*	-0.500 *0.606*	-0.938 *0.814*	-0.563 *0.547*

			Acute effects		Chronic / superimposed effects		
			Day 1	Day 29	Day 29	Day 57	Day 57
		Baseline se	post-dose se	pre-dose se	post-dose se	Pre-dose se	post-dose se
Targets RT (ms)	placebo	0.840 *0.062*	-0.14 *0.06*	-0.059 *0.058*	0.019 *0.074*	-0.086 *0.060*	-0.094 *0.070*
	200mg	0.876 *0.069*	-0.05 *0.06*	-0.076 *0.064*	-0.146 *0.042*	-0.169 *0.056*	-0.146 *0.060*
Distractors RT (ms)	placebo	0.825 *0.044*	-0.06 *0.04*	-0.029 *0.043*	-0.059 *0.030*	-0.026 *0.048*	-0.067 *0.044*
	200mg	0.860 *0.068*	-0.04 *0.04*	-0.006 *0.031*	-0.084 *0.043*	-0.155 *0.049*	-0.118 *0.049*
Average RT (ms)	placebo	0.832 *0.047*	-0.09 *0.04*	-0.041 *0.045*	-0.047 *0.031*	-0.050 *0.048*	-0.082 *0.046*
	200mg	0.868 *0.067*	-0.05 *0.05*	-0.037 *0.031*	-0.107 *0.036*	-0.163 *0.048*	-0.133 *0.049*
Sensitivity index	placebo	0.696 *0.054*	0.01 *0.04*	0.008 *0.048*	-0.062 *0.053*	0.011 *0.042*	0.031 *0.044*
	200mg	0.618 *0.058*	0.07 *0.03*	0.011 *0.057*	0.036 *0.051*	0.045 *0.050*	0.009 *0.041*
Finger prick blood glucose levels	placebo	5.648 *0.126*	-0.162 *0.199*	-0.254 *0.145*	-0.489 *0.218*	-0.292 *0.166*	-0.346 *0.200*
	200mg	5.434 *0.148*	-0.171 *0.147*	0.092 *0.176*	-0.326 *0.304*	0.114 *0.195*	-0.024 *0.256*
Quality of life							
Physical Health	placebo	12.57 *0.35*		0.03571 *0.23600*			0.000 *0.261*
	200mg	12.43 *0.46*		-0.07143 *0.24564*			0.071 *0.329*
Psychological Health	placebo	13.67 *0.48*		-0.125 *0.204*			-0.083 *0.321*
	200mg	13.29 *0.49*		0.250 *0.296*			0.375 *0.251*
Social relations	placebo	15.42 *0.68*		-0.500 *0.363*			-1.000 *0.463*
	200mg	14.50 *0.82*		0.667 *0.512*			0.333 *0.375*
Environmental	placebo	15.41 *0.50*		-0.344 *0.335*			-0.156 *0.397*
	200mg	15.16 *0.58*		0.156 *0.329*			-0.375 *0.212*

				Acute effects			Chronic / superimposed effects				
		Baseline	se	Day 1 post-dose	se	Day 29 pre-dose	se	Day 29 post-dose	se	Day 57 Pre-dose	se	Day 57 post-dose	se
Insulin	placebo	13.98	*1.00*			0.584	*1.381*					-0.863	*0.754*
	200mg	13.99	*1.32*			-0.875	*1.427*					-0.428	*1.936*
HbA1c	placebo	5.46	*0.09*			-0.183	*0.066*					-0.325	*0.059*
	200mg	5.48	*0.10*			-0.100	*0.056*					-0.175	*0.056*

6.3.2 Acute effects

6.3.2.1 CRD tasks

6.3.2.2 Delayed word recognition (*SI*)

The ANOVA revealed a significant main effect of treatment on delayed word recognition (SI) [F (1, 15) = 12.39, P = 0.003; d=0.9]. *Panax ginseng* led to significantly poorer accuracy (mean = - 0.15) than placebo (mean = - 0.003) on delayed word recognition (Figure 6.1 and Table 6.1).

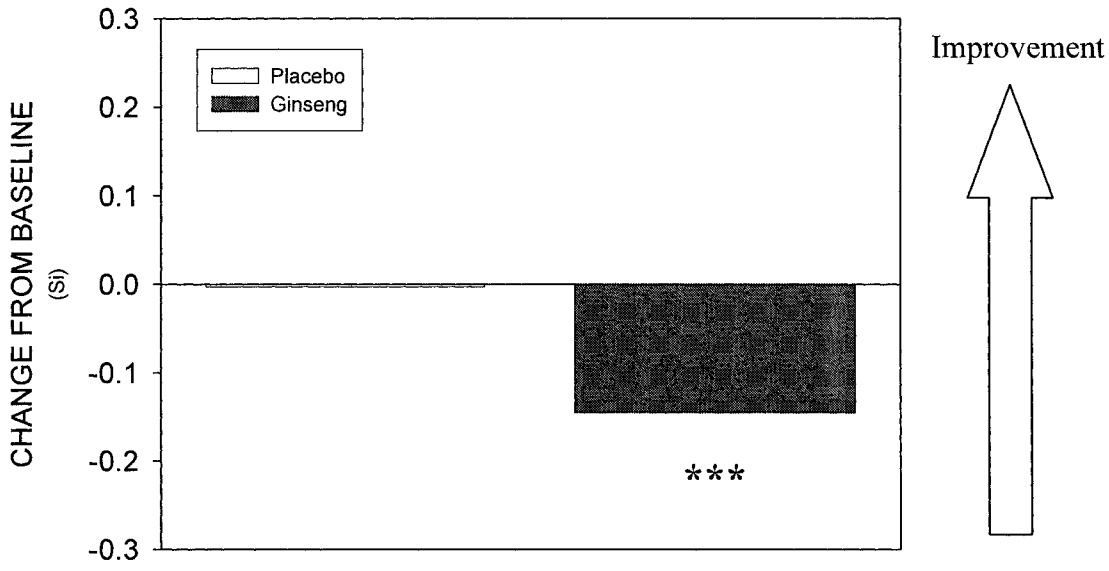

Figure 6.1. The effect of 200 mg *Panax ginseng* on delayed word recognition (si) 3 hours post-dose in healthy volunteers (***, P< 0.005). N = 16

6.3.2.3 Delayed picture recognition reaction time

The ANOVA revealed a significant main effect of treatment on delayed picture recognition reaction time [F (1, 15) = 6.61, P = 0.021; d=0.7]. *Panax ginseng* led to significantly quicker reaction times (mean = - 82.84) than placebo (mean = - 4.36) on delayed picture recognition (Figure 6.2 and Table 6.1).

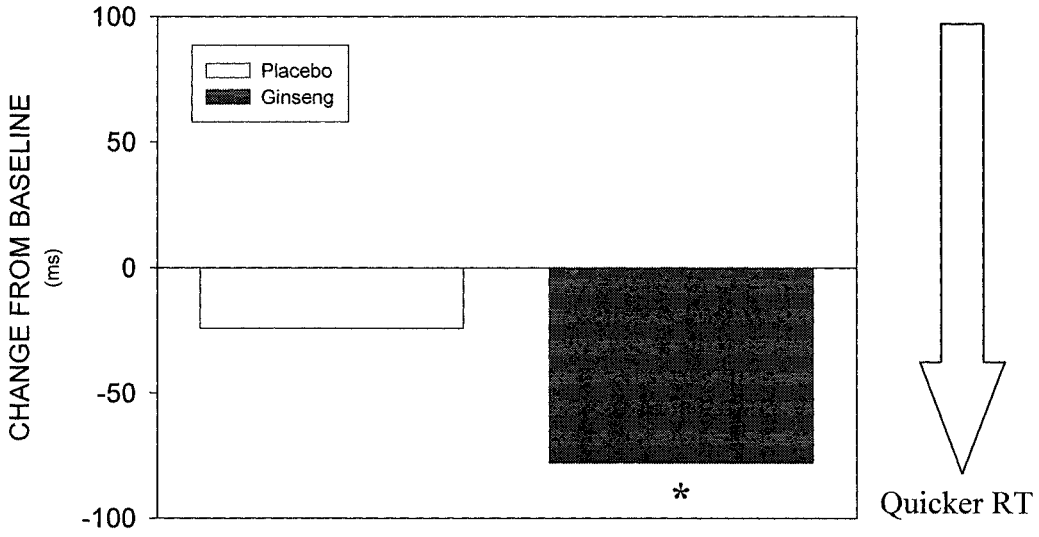

Figure 6.2. The effect of 200 mg *Panax ginseng* on delayed picture recognition reaction time 3 hours post-dose in healthy volunteers (*, P< 0.05). N= 16.

6.3.2.4 Non –CDR tasks

There were no significant acute effects found on the non-CDR tasks (see Table 6.1)

6.3.3 Chronic effects

6.3.3.1 CDR Tasks

6.3.3.2 Delayed picture recognition reaction time

The ANOVA revealed a significant main effect of treatment on delayed picture recognition reaction time indicative of a simple chronic effect [F (1,15) = 5.26, P = 0.037]. *Panax ginseng* led to significantly slower reaction times (mean = 41.96) than placebo (mean = -39.58) on delayed picture recognition (Figure 6.3a and Table 6.1). Planned comparisons comparing treatment with placebo at each testing session revealed that *Panax ginseng* led to significantly slower reaction times on day 29 at the pre-dose testing session (mean = 35.41) as compared with

placebo (mean = - 45.18) [t (15) = 4.71, P = 0.0003; d=0.5] and at the post-dose testing session (mean = 44.23) as compared to placebo (mean = - 42.63) [t (15) = 5.08, P = 0.0001; d=0.4]. Additionally, *Panax ginseng* led to significantly slower reaction times on day 57 at the pre-dose testing session (mean = 34.27) as compared to placebo (mean = - 27.95) [t (15) = 3.64, P = 0.002; d=0.4] and at the post-dose testing session (mean = 53.98) as compared to placebo (mean = - 42.54) [t (15) = 5.64, P = 0.00005; d=0.7] (Figure 6.3b and Table 6.1).

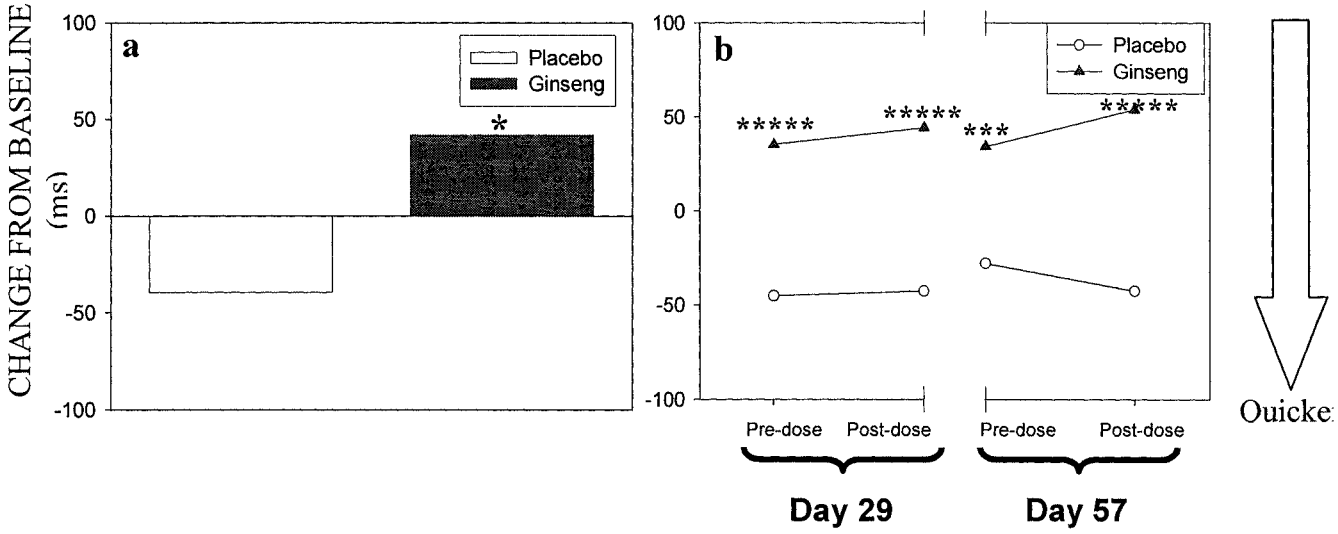

Figure 6.3 depicts the chronic effect of *Panax ginseng* (200 mg/d for 57days) on delayed picture recognition reaction time (a). Figure also depicts planned comparisons between treatment and placebo at pre-dose and post-dose testing sessions on day 29 and day 57 (*, P< 0.05; ***, P< 0.005; *****, P< 0.0005). Significance is compared with placebo. N=16

6.3.3.3 Digit vigilance false alarms

The ANOVA revealed a significant main effect of treatment on the number of false alarms committed during the digit vigilance task indicative of a simple chronic effect [F (1,15) = 6.37, P = 0.023]. *Panax ginseng* led to a significantly greater number of false alarms being committed on the digit vigilance task (mean

= 0.41) than placebo (mean = -0.32) (Figure 6.4a). Planned comparisons comparing treatment to placebo at each testing session revealed that *Panax ginseng* led to a significantly greater number of false alarms being committed on day 29 at the pre-dose testing session (mean = 0.375) as compared to placebo (mean = - 0.56) [t (15) = 2.206, P = 0.043; d =0.8]. Additionally, *Panax ginseng* led to a significantly greater number of false alarms being committed on day 57 at the pre-dose testing session (mean = 0.875) as compared to placebo (mean = - 0.312) [t (15) = 2.79, P = 0.013 ;d=0.9] (Figure 6.4b and Table 6.1).

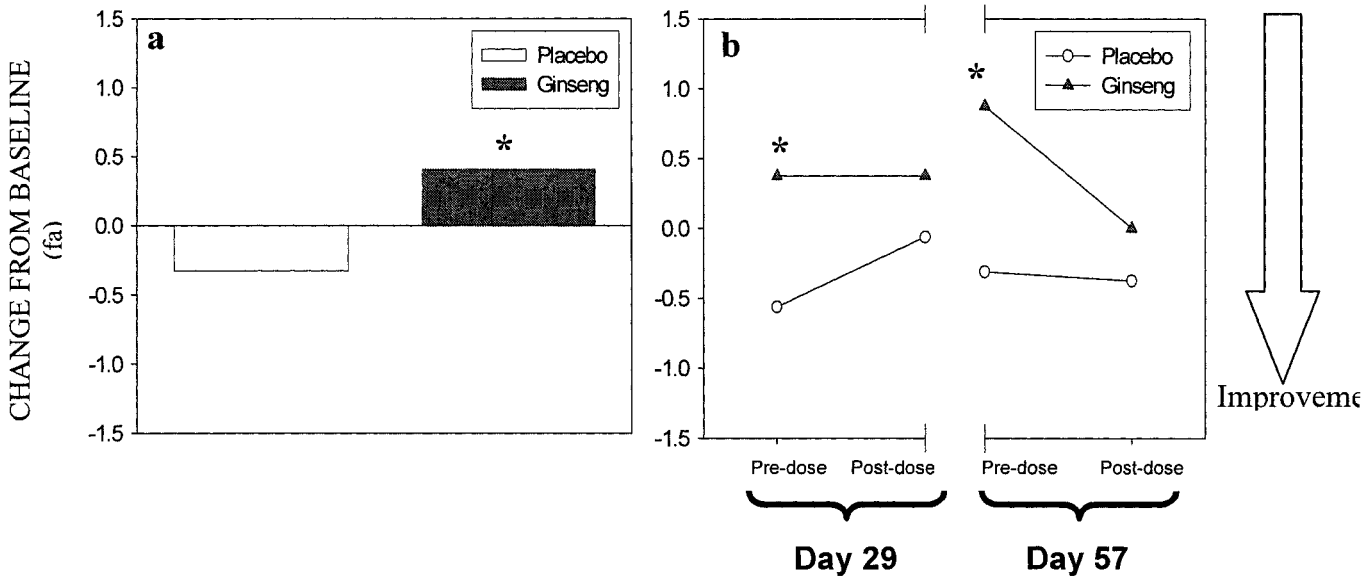

Figure 6.4 depicts the chronic effect of *Panax ginseng* (200 mg/d for 57days) on the number of false alarms committed during a digit vigilance task (a). Figure also depicts planned comparisons between ginseng and placebo at pre-dose and post-dose testing sessions on day 29 and day 57 (*, P< 0.05). Significance is compared with placebo. N =16

6.3.3.4 Non-CDR tasks

6.3.3.5 N-back reaction time

The ANOVA revealed a significant treatment x day x session interaction [F (1,15) = 8.19, P = 0.012]. Planned comparisons comparing treatment to placebo at

each testing session revealed that *Panax ginseng* led to significantly faster reaction times at the post-dose testing session on day 29 (mean = -0.11) as compared to placebo (mean = 0.05) [t (15) = 2.892, P = 0.011; d=0.4] on the N-back task. Additionally, *Panax ginseng* led to significantly faster reaction times on day 57 at the pre-dose testing session (mean = -0.16) as compared to placebo (mean = - 0.05) [t (15) = 5.404, P = 0.0001; d=0.6] and post-dose (mean = -0.13) as compared to placebo (mean = -0.08) [t (15) = 2.403, P = 0.030; d=0.3] on the N-back task (Figure 6.5 and Table 6.1).

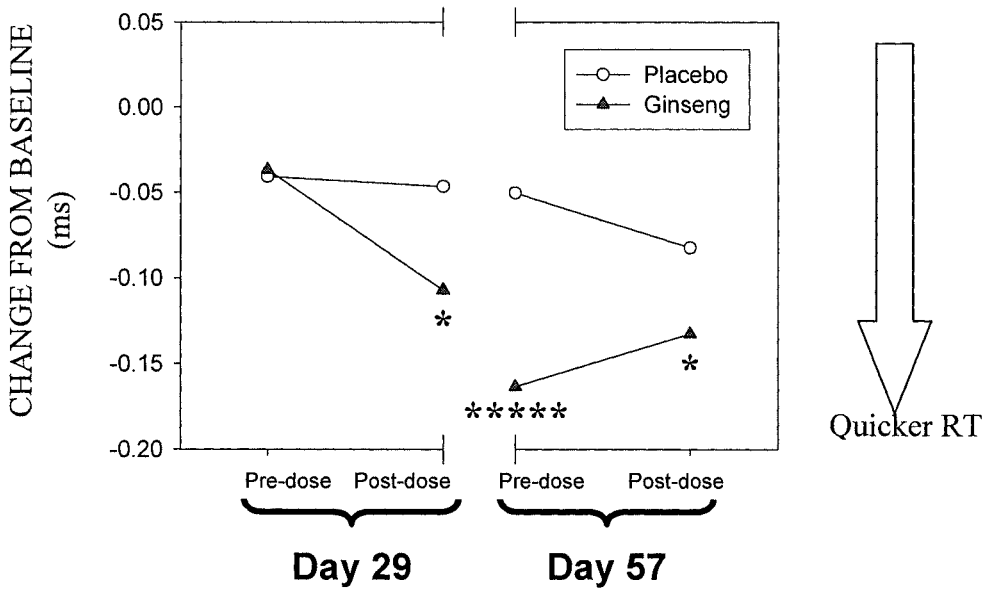

Figure 6.5 depicts planned comparisons between treatment (200 mg/d *Panax ginseng* for 57days) and placebo at pre-dose and post-dose testing sessions on day 29 and day 57 on N-back reaction time (*, P< 0.05). Significance is compared with placebo. N=16

6.3.3.6 Quality of life and mood effects

6.3.3.7 Social relations

The ANOVA revealed a main effect of treatment on the quality of life domain - social relations indicative of a simple chronic effect [F (1,15) = 5.95, P = 0.028].

Panax ginseng led to significantly higher self-report ratings (mean = 0.5) than placebo (mean = -0.75) (Figure 6.6a). Planned comparisons comparing treatment to placebo at each testing session revealed that *Panax ginseng* led to significant better self-report ratings on day 29 (mean = 0.6) as compared to placebo (mean = -0.5) [t (15) = 2.52, P = 0.023; d=0.8] and day 57 (mean 0.3) as compared to placebo (mean = -0.1) [t (15) = 2.88, P = 0.0114; d=0.7] (Figure 6.6b and Table 6.1).

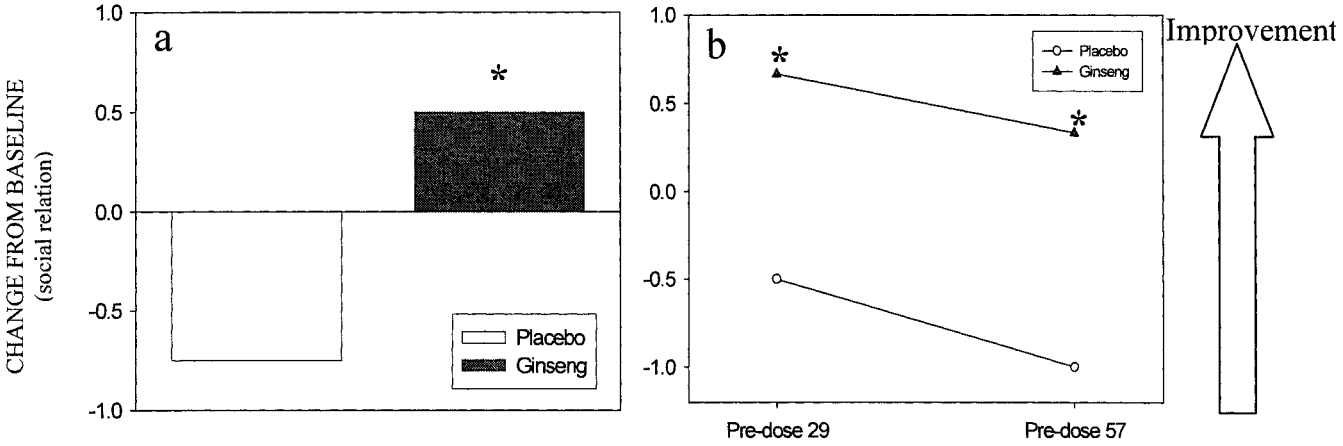

Figure 6.6 depicts the chronic effect of *Panax ginseng* (200 mg/d for 57days) on the social relations domain of quality of life (a). Figure also depicts planned comparisons between treatment and placebo on day 29 and day 57 (b) (*, P< 0.05). Significance is compared with placebo. N=16.

6.3.3.8 Calm

The ANOVA revealed a significant main effect of treatment on self-report ratings of calmness indicative of a simple chronic effect [F (1,15) = 5.05, P = 0.04]. *Panax ginseng* led to significantly worse ratings of calmness (mean = - 3.74) than placebo (mean = 3.81) (Figure 6.7a and Table 6.1). Planned comparisons comparing treatment to placebo at each testing session revealed that *Panax ginseng* led to significantly worse ratings of calmness on day 29 at the pre-dose

testing session (mean = - 2.91) as compared to placebo (mean = - 3.69) [t (15) = 2.60, P = 0.020; d=0.6] and at the post-dose testing session (mean = - 5.47) as compared to placebo (mean = 3.84) [t (15) = 3.667, P = 0.0023; d=1]. Additionally, *Panax ginseng* led to significantly worse ratings of calmness on day 57 at the pre-dose testing session (mean = - 2.81) as compared to placebo (mean = 5.56) [t (15) = 3.30, P = 0.005; d=0.8] and at the post-dose testing session (mean = - 3.75) as compared to placebo (mean = 2.16) [t (15) = 5.64, P = 0.035; d=0.4] (Figure 6.7b and Table 6.1).

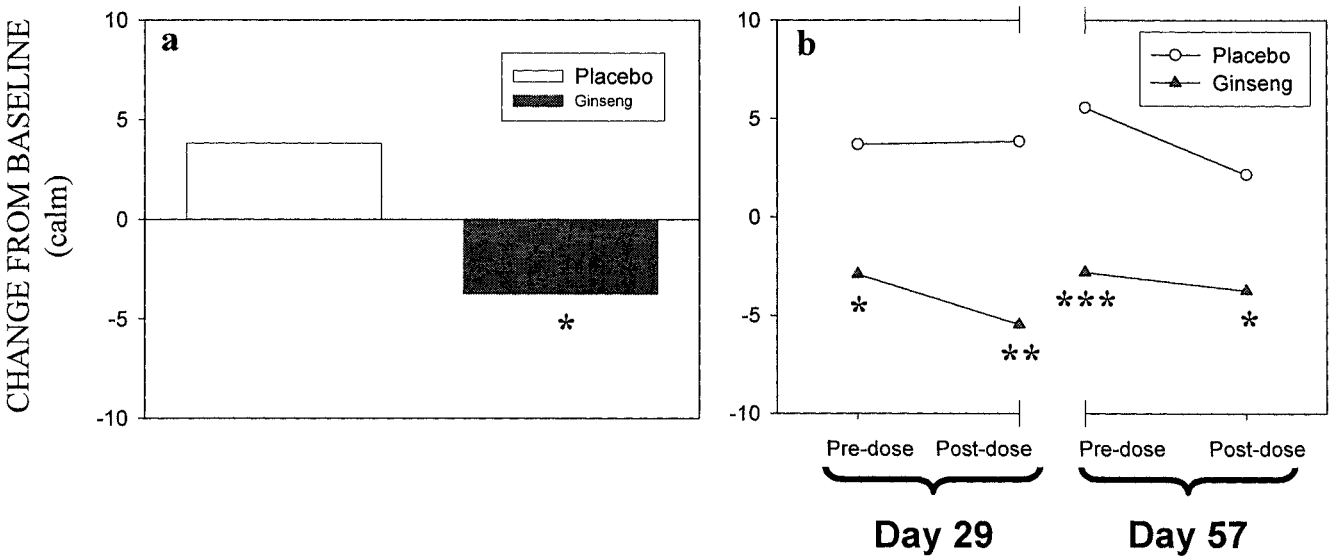

Figure 6.7 depicts the chronic effect of *Panax ginseng* (200 mg/d for 57days) on self-report ratings of calmness (a). Figure also depicts planned comparisons between treatment and placebo at pre-dose and post-dose testing sessions on day 29 and day 57 (*, P< 0.05; **, P<0.01; ***, P< 0.005). Significance is compared with placebo. N = 16

6.3.4 Physiological results

6.3.4.1 *HbA1c*

There were no significant results.

6.3.4.2 *Insulin*

There were no significant results.

6.3.4.3 *Finger prick blood glucose levels*

There were no significant results.

6.4 Discussion

This study was designed to assess the behavioural effects of a non-standardised *Panax ginseng* extract (Korean origin) in healthy volunteers. A secondary intended objective, of the study was to attempt to profile the extract's ginsenoside content and to investigate its glycaemic properties. Unfortunately, the ginsenoside profiling was inconclusive (see appendix 12). The extract had no effect on any physiological measure of glycaemic regulation investigated. However, the results did reveal positive and negative acute and chronic behavioural and mood effects. The results are fundamentally different from those of chapter 5, which investigated a standardised *Panax ginseng* (G115) extract utilising exactly the same, methodology and testing platform in a group of healthy volunteers.

With regard to the 3hour post-dose acute effects the results of the present study revealed that *Panax ginseng* led to improvements in the speed of delayed picture recognition but impairments in the accuracy of delayed word recognition. Although, there are some fundamental differences between the two above-mentioned tasks (the most obvious being that one is visually encoded and the other is verbally encoded) they are both thought to tap into secondary memory (in particular episodic memory). Therefore, the results suggest improvements in the speed but impairments in the accuracy of secondary memory (see later for further discussion of task specific sensitivity) as assessed by different tasks therefore precluding any speed accuracy trade-off.

These acute effects are dissimilar to those reported in chapter 5 following the ingestion of a standardised *Panax ginseng* extract (G115). The results of chapter 5 demonstrated that the standardised extract had no effect on secondary memory but rather improved working memory performance 3 hours post-dose. The most

parsimonious explanation would be that the different effects are down to differences in active constituents of the two *Panax ginseng* species (unfortunately the HPLC data was inconclusive for the non-standardised Korean extract). However, the acute modulation of secondary memory performance, revealed in the present study, is somewhat similar to the results reported in a series of acute trials documenting consistent improvements in secondary memory performance following single doses of the standardised extract (G115) in healthy volunteers (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2001b; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004). Further investigation is clearly needed to delineate the importance of ginsenoside content in acute behavioural modulation.

Secondary memory modulation in the present study was earlier suggested to represent *Panax ginseng's* opposing effects on speed and accuracy of secondary memory performance (if indeed these performance measures are separate entities). The results preclude any speed / accuracy trade –off, as improvements and decrements in performance of secondary memory were limited to specific tasks. As a result it is speculated that these results may represent the differential pharmacological impact on potentially independent cognitions as assessed by specific task (e.g. picture vs. word recognition). One possible explanation may be that these results represent differences in visual (pictures) and verbal (words) encoding, consolidation and/or retrieval processes. Research has indeed suggested that the involvement of the left or right prefrontal cortices in episodic memory is determined largely by the modality and/or type (i.e. verbal or non-verbal) of processing within episodic memory. Thus, verbal material (i.e. word lists) disproportionately activates the left prefrontal cortex while non-verbal material (i.e. pictures) disproportionately activates the right prefrontal cortex (Klingberg

and Poland, 1998). Additionally, Aggleton and Brown (1999) provide evidence suggesting that the modality of stimuli presentation (verbal vs.' non verbal) is important for distinct hippocampal (an area implicated in memory consolidation) activation. They suggest that the left hippocampal region mediates verbal learning whereas the right hippocampal region mediates non-verbal learning. It is therefore possible that ginseng is affecting different processes located in different cortical locations by some unknown mechanism (possible candidates could be neurotransmitter communication, blood flow, NO and/or glucose utilisation). However, clearly more research is needed to support such a speculation.

With regards to chronic effects of *Panax ginseng* revealed in the present study the ANOVA revealed a simple chronic effect on delayed picture recognition (episodic secondary memory). Planned comparisons comparing treatment to placebo at each testing session revealed that *Panax ginseng* led to significantly slower performance of this task at all testing sessions. The acute post-dose improvements observed in this task following a single dose (Figure 6.2) appear to be lost when dosing is continued for 29 and 57 consecutive days.

A simple chronic effect was also revealed on the accuracy of performing the digit vigilance task. Planned comparisons revealed that *Panax ginseng* led to a significantly greater number of errors being committed at the pre-dose testing session on day 29 and 57. This is the first report of a chronic effect of *Panax ginseng* on a vigilance task. Chapter 5 failed to report any effect of *Panax ginseng* (G115) on any measure of vigilance or the more global domain of attention, however, one previous acute trial has demonstrated improvements in attentional measures following 400 mg G115 (Sünram-Lea et al., 2004).

Although, there was no significant superimposed relationship revealed by the ANOVA digit vigilance task (i.e. no significant treatment x testing session interaction although there was a non-significant trend P = 0.06), there was a pattern in results suggesting that an acute dose alleviates the decrements in performance seen at the pre-dose testing session (this pattern is more evident on day 57). This superimposed relationship was apparent in chapter 5 albeit on different outcome measures. Further research is needed to investigate this pattern of results.

The results of the present study also revealed significant improvements in working memory performance following chronic *Panax ginseng* ingestion. *Panax ginseng* was associated with speeded N-back performance at all but the first testing session on day 29. Improvements in working memory performance are consistent with the chronic working memory improvements demonstrated in chapter 5 (although see later for further discussion) and the acute improvements demonstrated in chapter 4 and chapter 5 and the improved mental arithmetic performance revealed in chapter 2 and chapter 3.

With regards to these working memory effects it is apparent that again there are some inconsistencies in the working memory measures affected between the present study and that of chapter 5. In chapter 5 the chronic working memory improvements demonstrated were only apparent on a different dependent measure of working memory (i.e. CDR tasks), with no effect revealed on the N-back task. The opposite relationship is true for the present study. Additionally, chapter 5 also revealed acute verbal working memory effects and chronic non-verbal working memory effects but also an additional pattern of results that is suggestive of a superimposed relationship. It was speculated in chapter 5 that *Panax ginseng*

(G115) might be affecting independent working memory processes underlying these complex working memory tasks in a disproportionate way through unknown mechanisms. It is possible that the ginseng used in the present study may be consistent with this suggestion as we are seeing only improvements on a task that will require executive processes (see chapter 5 discussion). However, there are no improvements seen on the non-verbal executive working memory task in the present study. As this is the first study to assess the effects of this non-standardised extract (and the simple fact that it is a non-standardised extract) further discussion of theses processes will not be made until these results are replicated and investigated further.

The present study also demonstrated chronic effects on measures pertaining to human 'quality of life' and 'mood'. The results of the present study revealed that *Panax ginseng* significantly improved ratings of social relations following 29 days and 57 days of ginseng treatment (Figure 6.6). Conversely, self-report ratings of calmness were significantly worse following 29 days and 57 days of ginseng (Figure 6.7). Improvements in the measures pertaining to 'quality of life' or 'well-being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations have been demonstrated, although findings of this nature are by no means unequivocal (see Kennedy and Scholey, 2003). Indeed, even within the chapters of this current thesis the present improvements in quality of life is inconsistent with the results of chapter 5, which reported no effect of 29 and 57 consecutive days of *Panax ginseng* (G115) treatment on any dimension of quality of life. Additionally, the present decrements in calmness ratings are inconsistent with the improvements in calmness ratings following

acute dosing reported in chapter 4. The present study also failed to find an effect on contentedness, depression, tension or confusion as reported in chapter 5. The most parsimonious explanation would be that the different ginsenoside content and testing regimens of the extracts utilised in the present thesis and in previous research account for some of this discrepancy (see chapter 5 discussion).

In conclusion the results of the present study suggest that a non-standardised Panax ginseng extract (of Korean origin) can modulate secondary memory performance acutely. Chronic administration improved measures pertaining to working memory and self-report ratings of quality of life in healthy adults. The results are fundamentally different to those found for a standardised extract G115, which may suggest an active role of the specific ginsenosides content of any given extract in the modulation of human behaviour and mood. The present extract failed to modulate indices of gluco-regulation at least as measured here. These results further highlight the need for caution when generalising across studies utilising different methodologies and different extracts. The present study utilised the same methodology and type of ginseng (*Panax ginseng*) as used in chapter 5, however, the behavioural results reported are fundamentally different.

CHAPTER 7. DISCUSSION

The results of the five studies making this thesis suggest that both acute and chronic ingestion of *Panax ginseng* is capable of modulating mood and cognitive performance in healthy young volunteers. Chapters 2 and 3 also demonstrate, for the first time, *Panax ginseng's* ability to modulate blood glucose levels following a single dose in overnight fasted healthy volunteers; however, chapters 3, 5 and 6 failed to replicate ginseng's acute effects on blood glucose levels when a glucose drink (chapter 3) or a standard breakfast (chapter 5 and 6) was consumed on the morning of testing. Ginseng's ability to modulate blood glucose levels is one physiological effect (there are many others) that may partially explain the acute improvements in cognitive performance and subjective mood. Chapters 4 and 5 revealed for the first time *Panax ginseng's* positive effects on direct measures of working memory, thus raising the possibility that previous failures to find working memory effects may have been due to poor task selection. This raises the more general issue of ensuring appropriate tasks selection and the difficulty of interpreting non-significant effects in this field. For example, previous research has concluded that working memory gains no benefit from the acute ingestion of *Panax ginseng*; however, the results of the present thesis cast doubt on these previous conclusion, suggesting that working memory can be improved following acute doses. Chapter 5 revealed an unexpected relationship between chronic and acute ingestion of *Panax ginseng* on subjective mood and cognitive performance (suggestive of a psychological dependence). The study making chapter 5 of this thesis was the first to assess the effects of *Panax ginseng* on cognitive performance and subjective mood following chronic dosing (4 and 8 weeks),

utilising controlled methodology. The results clearly demonstrate the need for further investigation of such dosing regimes. The final chapter documents a different profile of behavioural effects following the ingestion of a non-standardised *Panax ginseng* extract, thus highlighting the need for caution when generalising findings and conclusions across ginseng types and beyond the specific parameters of the methodologies utilised in any given study. Methodological differences between studies may go some way in explaining the inconsistent data patterns reported between studies, research groups and ginseng extracts. These data further highlight the need for well-controlled studies utilising standardised ginseng extracts and the need for the integration of 'theory driven' research in order to fractionate any behavioural effects of herbal extracts. Such methodologies will inevitably lead to greater consistency between behavioural studies, at least in the first instance within the restricted population of volunteers utilised in the present thesis (see table 7.1 for a summary of the methodologies and research finding pertaining to the acute effect of *Panax ginseng).*

The studies described in the present thesis used two major methodologies i.e. acute vs. chronic trials. These differences may prove, given further research, to be of primary importance when considering the applied use of ginseng and the theoretical interpretations of results - in terms of behavioural modulation and underlying mechanisms of action. The remainder of this discussion will start with an initial commentary on the pattern of results revealed following acute ingestion, followed by a commentary on how these acute findings fit with the previous literature. Following this will be a discussion pertaining to the pattern of results revealed following the first controlled chronic trials. There will be integrated discussion of ideas for further research. Finally, there will be limited discussion

of possible mechanisms of action and some methodological considerations for future projects.

7.1 ACUTE INGESTION

In chapter 2, a single dose of *Panax ginseng* (G115) administered to overnight fasting healthy young volunteers caused significant reductions in circulating blood glucose levels (blood glucose was measured at 60, 90 and 120 minutes post-dose) with concomitant improvements in mental arithmetic performance. Ginseng also ameliorated the increase in subjective feelings of mental fatigue experienced by participants during sustained intense cognitive processing (see Figure 2.1 and Tables 2.1 and 2.2). Both 200mg and 400mg of *Panax ginseng* (G115) led to significant reductions in circulating blood glucose levels up to 120 minutes post-dose whilst only 200mg improved performance of a mental arithmetic task (this was restricted to the most difficult task employed in the study) (see Figure 2.1 and Table 2.2). This improved speed of performance was not associated with more errors, precluding the possibility of any treatment specific "speed/accuracy trade-off" (see Table 2.2). The finding of concomitant improvements in cognitive performance and reductions in circulating blood glucose levels was unexpected. However, one possible explanation for this specific pattern of results could be that *Panax ginseng* is promoting the cellular uptake of glucose for use in metabolic reactions, resulting in a fall in circulating blood glucose levels, whilst improving cognitive performance. This tentative hypothesis was addressed in chapter 3 of this thesis (See section 3.4 of this thesis for further discussion).

Chapter 3 attempted to investigate a hypothesis, proposed in chapter 2, that ginseng promotes the cellular uptake of glucose resulting in acute improvements in cognitive performance and mood and lower blood glucose levels. It was hypothesised that if this was indeed a potential mechanism, underlying ginseng's acute cognitive effects, one might expect to see a synergistic relationship between acute ginseng ingestion and exogenously administered glucose on cognitive performance and mood. For example, one might expect to see faster reductions in blood glucose levels, once an initial peak had been reached, coupled with improved cognitive performance. The results of chapter 3 revealed, for a second time, significant reductions in blood glucose levels following 200mg of *Panax ginseng* measured 60 minutes post-dose (in overnight fasted volunteers who ingested saccharine 30 minutes after ginseng) (see Figure 3.1 and Table 3.1). This reduction in blood glucose was again coupled with improved mental arithmetic performance (albeit a previously subjectively-rated easier version) (se Figure 3.3 and Table 3.1) and significant ameliorations in subjective feelings of mental fatigue reported by participants during sustained, intense cognitive processing, following the same 200mg dose (see Figure 3.5 and Table 3.1). However, the study failed to find any cognitive evidence to support a synergistic relationship between ginseng and glucose ingestion and thus failed to support the hypothesis that ginseng may promote the uptake of glucose into the cell (see Figure 3.3; 3.4; 3.5 and Table 3.1). In spite of the inability of the data to support such a tentative hypothesis it should be noted that the study making chapter 3 was the first study to test this hypothesis and the fact still remains that ginseng causes blood glucose levels to fall significantly, in overnight fasted healthy volunteers. It has been established that the pre-prandial administration time of American ginseng may be

an important factor for the post-prandial glycaemic effects (see Vuksan *et al.*, 2002). Therefore, further research should investigate the effect of different pre-prandial administration times of *Panax ginseng*, as chapter 3 only assessed the impact of one pre-prandial time point (i.e. 30 minutes before glucose administration) on post-prandial glycaemic levels (measured 60 and 120 min post ginseng dose), cognitive performance and subjective mood in healthy volunteers.

Chapter 3 also revealed a significant 2-way interaction between the administration of ginseng and a glucose drink (see Figure 3.2 and Table 3.1). This interaction is further evidence of *Panax ginseng's* acute glucose modulating properties as reported in chapter 2. The pattern of results revealed that, in the absence of a glucose load (i.e. in overnight fasting participants) a single dose of *Panax ginseng* (200mg G115) resulted in a post-dose fall in circulating blood glucose levels. However, in the presence of elevated blood glucose levels (i.e. following the consumption of a 25g glucose drink) the addition of ginseng (30 minutes before the ingestion of the drink) was associated with a further rise in blood glucose levels (see Figure 3.2). When this interaction was collapsed into the individual time point and analysed by planned comparisons (utilising the MSerror from the ANOVA) it was revealed that 200 mg (G115) led to significant reduction in circulating blood glucose levels when measured 60 minutes post-dose (consistent to that seen in chapter 2), however, there was no difference between glucose and glucose/ginseng condition at the same time point (see Figure 3.1). As stated above the results of chapter 3 provide no support for the tentative mechanistic hypothesis raised in chapter 2. In fact, the pattern in the results suggests an actual increase in circulating blood glucose levels following ginseng (when glucose levels were elevated following a glucose drink), which is in the

opposite direction to that which was predicted (see Figure 3.1). For example, one might expect to see a steeper/faster reduction in circulating blood glucose levels once an initial peak had been reached if ginseng promotes the uptake of glucose. However, the present results cannot rule out such a speculative mechanism as the results of chapter 2 and chapter 3 did reveal significant reductions in circulating blood glucose levels and concomitant improvements in cognitive performance (mental arithmetic) and fatigue ratings when ginseng was ingested alone (i.e. in overnight fasting volunteers). Therefore, it still remains possible that the reduction in circulating blood glucose levels may be the result of the utilisation of glucose by active cells. However, it should be noted that in both chapter 2 and 3 there was a failure to find any direct relationship between the reduction in blood glucose levels and improved cognitive performance.

Chapter 4 investigated the cognitive and mood effects of *Panax ginseng* (G115) following seven consecutive days of ingestion. There were no 'pure' sub-chronic effects revealed on any performance measure (i.e. performance was no different on the morning of day 8 compared with baseline) (see Table 4.1 and further discussion in section 7.2 of this thesis), however, the results did reveal that acute doses of *Panax ginseng* (both on day 1 and on day 8) improved ratings of calmness as well as improved and impaired aspects of working memory performance in healthy young volunteers (see Figure 4.1 and Table 4.1).

With regards to subjective mood the results indicated a simple acute effect across day 1 and day 8. Planned comparisons revealed that both 200 mg and 400 mg G115 led to significant improvements in subjective self-reports of calmness at 2.5 and 4 hours post-dose on day 1. Additionally, 200mg significantly improved subjective calmness ratings at 1 hour and 4 hours post-dose on day 8, as

compared with placebo. The improvements in the subjective ratings of calmness revealed in chapter 4 may be analogous to ginseng's purported efficacy as an anxiolytic or adaptogenic agent, as the pattern of results across day 1 indicates that participants are becoming less calm as the day progresses (i.e. placebo group). With regards to cognitive performance the results revealed acute improvements and impairments in verbal working memory performance (N back) on day 1 and day 8 (see Figure 4.1). 200 mg led to significant slowing in the performance of a 3 back task (there was no effect on the easier versions of this task) at all but the 4 hour post-dose session on day 8, as compared with placebo (see Figure 4.1 and Table 4.1). Conversely, 400 mg led to significant speeding in the performance of the same task at the 2.5hour session on day 1 and day 8 (see Figure 4.1). The improvements in speed of working memory performance following 400 mg were also associated with significant improvements in the accuracy of performing the same task (see Figure 4.1 and Table 4.1). 400 mg led to significantly improved working memory accuracy at all post-dose testing sessions, as compared to placebo. Conversely, 200 mg led to a significant decrement in the accuracy of working memory performance at 4 hours post-dose on day 8 (see Figure 4.1).

Chapter 5 of this thesis investigated the acute, chronic and superimposed effects of *Panax ginseng* (G115) on cognitive performance, blood glucose levels and indices of glucose regulation (HbA1c). The below commentary discusses only the acute (3 hours post-dose) effects on cognition and blood glucose levels revealed in chapter 5 (the chronic and superimposed effects are discussed in section 7.2 and 7.2.1). The only significant effect revealed in chapter 5 at the three hour post-dose assessment point was improved verbal working memory performance

following a single acute dose of 200 mg G115 (see Figure 5.1 and Table 5.1). There was no effect on blood glucose levels measured 3hours post–dose (see Table 5.1) following 200mg G115 (see section 7.1.1 for further discussion of this non-significant result).

The study making chapter 6 of this thesis was designed to assess the cognitive and gluco-regulatory effects of a non-standardised Korean *Panax ginseng* extract ingested by healthy volunteers. The study utilised the same testing platform and methodology as that used in chapter 5 of this thesis to allow direct comparison. A secondary objective was to profile the ginsenoside content of the non-standardised ginseng extract using HPLC. This objective would allow some discussion of the potential differences in cognitive and physiological effect due to the different ginsenoside content (the active components of ginseng) of the extract under investigation. Unfortunately, the HPLC analysis was inconclusive (see appendix 32).

The results of chapter 6 revealed improvements and impairments in cognitive performance and subjective mood following acute (discussed below) and chronic ingestion (discussed in section 7.2 and 7.2.1 of this thesis) of 200 mg of a non-standardised *Panax ginseng* extract. The results revealed no effect on blood glucose levels when measured 3 hours post-dose; however results did reveal improvements in cognitive performance when assessed at the 3 hour post-dose time point. The cognitive results revealed in chapter 5 and 6 are fundamentally different from one another (discussed below). The results of chapter 6 revealed that the non-standardised Korean *Panax ginseng* extract led to improvements in the speed of delayed picture recognition (see Figure 6.2) but impairments in the accuracy of delayed word recognition (see Figure 6.1) 3 hours post-dose.

Although, there are some fundamental differences between the two above-mentioned tasks (the most obvious being that one is visually encoded and the other is verbally encoded), both tasks are thought to tap secondary (episodic) memory. Therefore, the results would suggest that this non-standardised Korean *Panax ginseng* improves the speed but impairs the accuracy of secondary memory, as assessed by different tasks, therefore precluding any speed accuracy trade-off. Alternatively, it could be suggested that non-verbal episodic memory is improved and verbal episodic memory is impaired. The modality of stimuli presentation may be another important methodological consideration when trying to understand ginseng's behavioural effects (see later for further discussion of task specific sensitivity). These acute effects are dissimilar to those acute effects reported in chapter 5 following *Panax ginseng* (G115) which demonstrated no effect on secondary memory but rather improvements in aspects of working memory performance 3 hours post-dose (verbal working memory as assessed by the N back task) (see Figure 5.1). The most parsimonious explanation would be that of the difference in active constituents between the two *Panax ginseng* species (unfortunately no data is available for the non-standardised Korean extract).

The acute results reported in chapter 6 were suggested, earlier in this discussion, to represent ginseng's opposing effects on speed and accuracy of secondary memory performance (if indeed these performance measures are separate entities). The results preclude any speed accuracy trade–off as improvements and decrements in performance of secondary memory were limited to specific tasks (see Figures 6.1 and 6.2 and Table 6.1). As a result it is speculated that these results may represent the differential pharmacological impact on potentially

independent cognitions as assessed by specific task (e.g. picture vs. word recognition). One possible explanation may be that these results represent differences in visual (pictures) and verbal (words) encoding, consolidation and/or retrieval processes. Research has indeed suggested that the involvement of the left or right prefrontal cortices in episodic memory is determined largely by the modality and/or type (i.e. verbal or non-verbal) of processing within episodic memory. Thus, verbal material (i.e. word lists) disproportionately activates the left prefrontal cortex whereas non-verbal material (i.e. pictures) disproportionately activates the right prefrontal cortex (Klingberg and Poland, 1998). Additionally, Aggleton and Brown (1999) provide evidence suggesting that the modality of stimuli presentation (verbal vs.' non verbal) is important for distinct activation of the hippocampus (an area implicated in memory consolidation). They suggest that the left hippocampal region mediates verbal learning whereas the right hippocampal region mediates non-verbal learning. It is therefore possible that ginseng (more specifically the individual ginsenosides) is affecting different processes located in different cortical locations by some unknown mechanism (possible candidates could be neurotransmitter communication, blood flow, NO and/or glucose utilisation). However, clearly more research is needed to support such a speculation.

7.1.1 Acute finding and the extant literature

The studies making chapters 2 and 3 of this thesis are the first studies to demonstrate *Panax ginseng's* (G115) acute hypoglycaemia inducing properties in healthy, overnight fasting human volunteers. However, in contrast, chapter 3, 5 and 6 failed to support *Panax ginseng's* gluco-regulatory properties, when

glucose (chapter 3) or a standard breakfast (chapter 5 and 6) was also ingested. In line with ginseng's ability to modulate blood glucose levels reported in chapter 2 and 3, previous research has reported gluco-regulatory effects following chronic ingestion of *Panax ginseng* (Sotaniemi *et al.*, 1995; Vuksan *et al*, 2000) and *Panax quinquefolius* (American ginseng) (Tetsutani *et al.*, 2000). Additionally, a series of acute studies has reported the hypoglycaemic effects of American ginseng on post prandial glycaemic response following a glucose challenge in healthy and diabetic populations (Vuksan *et al.*, 2000b; Vuksan *et al.*, 2001; Sievenpiper *et al.*, 2003). The acute hypoglycaemic effect of *American ginseng,* following a 25g glucose load, has been reported in both diabetic patients following 3g, 6g and 9g American ginseng (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2000b), and non-diabetics administered 1g, 2g and 3g (Vuksan *et al.*, 2000a; Vuksan *et al.*, 2001). This effect, however, was restricted to a batch of American ginseng with a specific total ginsenosides content (3.54%) and protopanaxadiol: protopanaxatriol ratio (2:4) (Sievenpiper *et al.*, 2003). Additionally, Sievenpiper *et al* (2003) reported that there was no effect of eight other widely used ginseng types (*Sanchi, Siberian, American, Asian, Korean red, Japanese, wild American, and Vietnamese*) on indices of glycaemic control following a 75g oral glucose tolerance test. However, an extract of *Panax ginseng,* was associated with increased blood glucose levels and insulin response following the glucose load (Sievenpiper *et al.*, 2003), which is somewhat consistent with the pattern of results suggesting increased blood glucose levels reported in chapter 3 and no effect on post prandial glycaemic response following glucose drink (chapter 3) and a standard breakfast (chapter 5 and 6). It appears that *American ginseng* can lower post prandial blood glucose levels but has no effect on fasting blood

glucose levels. Whilst *Panax ginseng* lowers fasting blood glucose levels but has no effect on post prandial glucose levels – in fact *Panax ginseng* appears to increase post prandial glucose levels.

In relation to the cognitive effects, following single doses of *Panax ginseng*, revealed in the present thesis it appears that working memory performance is modulated. This is particularly curious as previous studies have failed to show effects of acute ingestion of *Panax ginseng* on direct measures of working memory (see Kennedy and Scholey 2003)., The improved speed of performing a mental arithmetic task (verbal working memory) following 200mg (G115) and 400mg (G115) in chapter 2 (see Figure 2.1) and following 200 mg (G115) in chapter 3 (see Figure 3.3) is consistent with the recent findings of faster memory, attention, and serial subtraction task performance (Kennedy et al., 2004), and decreased latency of the P300 component of auditory evoked potentials following the same dose (Kennedy *et al.,* 2003). The accumulation of findings documenting improvements in the speed of task performance does suggest that the original observation of slower attention (Kennedy *et al.*, 2001) and Serial 7s task performance (verbal working memory) (Scholey and Kennedy, 2002) may have been anomalous; However, 200mg (G115) did result in significantly slowed N back performance (verbal working memory) in chapter 4 (see Figure 4.1) of this thesis when assessed 3hours post-dose, which in itself, contrasts with the improved accuracy of N back performance (verbal working memory) following the same dose in chapter 5 (see Figure 5.1) and following 400mg in chapter 4 (see Figure 4.1) of this thesis. The improved accuracy following 200mg (G115) (chapter 5) and 400mg (G115) (chapter 4) of performing N back task (verbal working memory) is consistent with the improved accuracy of performing a serial

subtraction task (Scholey and Kennedy, 2002) and faster responses on an attentional task (Sünram-Lea et al., 2004). The significant slowing in N back performance (verbal working memory) following 200mg (G115) (chapter 4) is consistent with the significant slowing reported in other cognitive domains following the same 200mg (G115) dose (Kennedy et al., 2001).

The ability of *Panax ginseng* (G115) to modulate indirect (chapter 2 and 3) (see Figure 2.1 and 3.3 respectively) and direct measures of working memory (chapter 4 and 5) (see Figure 4.1 and 5.1 respectively), following acute ingestion, are in direct contrast to the repeated failures to elicit any effect, of any dose, on any purported direct measure of working memory performance previously utilised i.e. numeric working memory task (verbal working memory) and spatial working memory task (non-verbal working memory). These tasks are incorporated in the CDR computerised assessment battery (see: Kennedy and Scholey 2003). However, acute improvements and decrements have been reported in the performance of mental arithmetic tasks following single doses of *Panax ginseng* G115 (Scholey and Kennedy, 2002; Chapter 1 and Chapter 2). Although this arithmetic task is not traditionally thought of as a measure of working memory, this task will load heavily on verbal working memory processes. These processes involved in the completion of this mathematical task may draw upon different cognitive processes than those that the CDR working memory task utilise (see later for further discussion). To date, the most consistent findings, following an acute single dose of *Panax ginseng* G115, was that of improved secondary (episodic) memory performance. Working memory performance has continually been unaffected by an acute dose of *Panax ginseng* (200 mg, 400 mg and 600mg G115 tested at 1, 2.5, 4 and 6 hours after ingestion). This effect (or lack of) has

been reported following G115 alone (Kennedy *et al.*, 2001a; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004), and in combination with both *Ginkgo biloba* (Kennedy et al., 2001b; Kennedy *et al.*, 2002) and guaraná (*Paullinia cupana*) (Kennedy *et al.*, 2004). The previous failure to educe an affect on working memory performance may have been due to the 'under-loaded' nature of the working memory tasks utilised in previous studies. Indeed, this suggestion is supported by the observation that, in chapter 4 and 5, ginseng only exerted its effects on the most cognitively demanding level on the N Back task (3 back) (see Figure 4.1; Table 4.1 and Figure 4.1; Table 4.1) and the more demanding version of the serial subtraction task (Chapter 2) (see Figure 2.1), or on an easier version of the subtraction task but only when concomitant subjective self reported mental fatigue was at its greatest (Chapter 3) (see Figure 3.3). Further investigation is needed to delineate the effects of *Panax ginseng* (following acute and chronic dosing) on working memory performance with specific consideration of all the individual processes involved.

Chapter 6 revealed, for the first time in this thesis, acute improvements in secondary (episodic) memory (see Figure 6.2). Although, this is a first for this thesis these results are somewhat similar to a series of acute trials reporting consistent improvements in secondary (episodic) memory performance, albeit following single doses of the standardised extract (G115) in healthy volunteers (Kennedy *et al.*, 2001a; Kennedy et al., 2001b; Kennedy *et al.*, 2002; Kennedy *et al.*, 2004).

With regards to *Panax ginseng's* effects on subjective reports of mood. It was found that the 200 mg dose led to a significant amelioration in the participants' subjective feelings of mental fatigue at all post-dose time points (except the first

post-dose battery completion) in chapter 2 (see Figure 2.1) and at the last two post-dose time points in chapter 3 (see Figure 3.5). The 400 mg dose led to a significant reduction in ratings of mental fatigue after the third battery completion only (see Figure 2.1). These are the first reported human studies that have examined the effect of *Panax ginseng* on subjective feelings of mental fatigue associated with intense cognitive processing. However, one study has demonstrated *Panax ginseng's* ability to ameliorate fatigue associated with night shift working in a group of nurses (Wesnes *et al.*, 2003) and others have shown improvements in measures pertaining to 'quality of life' or 'well being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations. Chapter 4 (Figure 4.1) also demonstrated that 200mg and 400mg (G115) improved subjective reports of calmness up to 240 minutes after ingestion. This improved calmness rating may be in keeping with the acute anti-fatigue properties of ginseng reported in chapter 2 (see Figure 2.1) and 3 (see Figure 3.5), as well as the findings that ginseng can attenuate the effects of fatigue in night nurses (Wesnes *et al.*, 2003) as it appears ginseng is ameliorating the progressive worsening in mood on day 1 (which may be associated with successive completions of the test battery throughout the day or through unmonitored stressors) (see Figure 4.1). Findings of this nature are by no means unequivocal and interpretation is made more difficult due to methodological differences between studies. For example, ginseng's effects on such parameters have been evaluated using doses ranging from 80 to 400 mg in patient (Wiklund et al., 1994, Sotaniemi et al., 1995, Neri et al., 1995) and healthy populations of various ages and stress levels (Wiklund et al., 1994; Caso Marasco et al., 1996;

Ussher et al., 1995; Ussher et al., 2000; Ellis and Reddy 2002; Cardinal and Engles, 2001) whilst study duration has ranged from 2 to 9 months (See Coleman et al., 2003 for review). Since both improvements in performance and amelioration of mental fatigue were associated with the same 200 mg dose, in both chapter 2 (see Figure 2.1) and 3 (see Figure 3.3; 3.4 and 3.5), it is possible that the effects on either measure were secondary to those on the other. Unfortunately, the studies making chapters 2 and 3 were not designed to address this potential cause and effect issue. Similarly, previous studies investigating ginseng's acute effects on behavioural performance (for a review see Kennedy and Scholey 2003) have not directly measured performance related fatigue (although they have included measures of mood), and it is possible that the previously demonstrated cognitive effects are as a consequence of altered fatigue levels.

It is difficult, if not impossible, with the present knowledge pertaining to the mechanisms underlying ginseng's behavioural effects to explain the disparity in the results obtained from study to study, following *Panax ginseng* or even the non-linear dose response relationship. However, it should be noted that the differences in study methodology may go some way in explaining some of these inconsistencies. Fore example, the study making chapter 5 of this thesis is the first study to assess *Panax ginseng's* acute behavioural effects 3 hours post-dose. This methodological difference may explain some of the patterns in the data due to the pharmacokinetics of *Panax ginseng* (see section 1.2.4 of this thesis). As a practical example of the potential importance and confounding factor of post-dose testing time, Sünram-Lea *et al* (2004) reported improvements in attentional performance following 400 mg G115 when assessed 90 minutes post-dose. This

was the first time attentional performance had been affected by the acute ingestion of *Panax ginseng* but was also the first time *Panax ginseng's* acute effects had been investigated 90 minutes post-dose (previous acute studies had assessed ginseng's behavioural performance using the same attentional tasks, as that utilised by Sünram-Lea (2004), at 60, 150, 240 and 360 minutes post dose. (for a review of these studies see Kennedy and Scholey, 2003).

Additionally, in chapter 4 the slowing in performance, following the 200 mg dose was associated with a concomitant increase in subjective reports of calmness (see Figure 4.1). This improved state of calmness may be accountable for the slowed speed of performance as one might expect to see the same pattern after ingestion of a sedative (although this pattern was not evident for the 400 mg dose). Unfortunately, the study was not designed to delineate any cause/effect relationship between mood and cognitive performance. Therefore, the most parsimonious explanation is that of a simple cohort effect (all studies report data from the same healthy young cohort). Alternatively, whilst the extract used is standardised to total ginsenoside content, it is possible that even minor differences in the levels of single ginsenosides, or groups of ginsenosides (e.g. the ratio of protopanaxadiols to protopanaxatriols), may have exerted an affect. Additionally, while the effects may appear curious, previous ginseng research, both in humans and animals, is replete with dose-specific effects and non-linear dose response profiles (for review see: Kennedy and Scholey, 2003). The literature pertaining to the fractionation of working memory processes is growing (see Myake *et al.*, 2001). Future research should endeavour to choose appropriate testing platforms and methodologies that will allow conclusions to be formulated

regarding these fractionated cognitive systems. Such research would further the literature into the understanding of ginseng's effects on working memory

The acute finding of chapters 2, 3, 4, 5 and 6 open some interesting avenues for further research. One question that needs addressing is whether the hypoglycaemic effect, seen in overnight fasted healthy volunteers, is dose dependent. Additionally, further studies investigating the concomitant ingestion of glucose and ginseng need to be carried out. These results will have important practical application, especially for those who suffer difficulties in glucose regulation. The speculative hypothesis suggesting the cellular uptake of glucose by ginseng also deserves further investigation. One avenue would be to use an *in vitro* model to investigate the mechanism for ginseng's glycaemic effect (uptake of glucose?) and then follow these results with an *in vivo* behavioural trial. Additionally, fMRI could give an indication of the brain areas involved and allow good temporal and spatial resolution.

7.2. CHRONIC INGESTION

Below will be a discussion relating to the sub-chronic and chronic results revealed in the present thesis.

Chapter 4 revealed no effect of 7 consecutive days of ginseng ingestion on working memory (verbal and non-verbal), episodic memory and mood. Results revealed that performance was no different on the morning of day 8 (before that day's treatment) in comparison to baseline (performance on the morning of day 1). Although, these results are non-significant they provide insight into the fact that 7 days of ginseng administration does not modulate cognitive performance in

healthy volunteers. This result leads the way for some interesting speculation and ideas for further research. For example, this result may provide indirect evidence that ginseng's acute and chronic effects are modulated by different biological mechanisms - the latter mechanism taking longer than 7 days to take effect. It also raises questions and gives some insight into the length of time acute effects last (i.e. the previous days dose is having no effect). These results also open the obvious question – how long does *Panax ginseng* need to be ingested before chronic effects will be observed in cognitive performance and mood. Evidence from chapters 5 and 6 (discussion to follow) suggests that chronic effects are present following 4 and 8 weeks of daily *Panax ginseng* ingestion; however, chapter 5 revealed that these chronic effect can be further modulated by an addition acute dose (see later).

The study that makes chapter 5 of this thesis was the first study to systematically investigate *Panax ginseng's* (G115) acute, chronic and superimposed effects on cognitive performance, subjective mood and blood glucose levels in healthy volunteers. The results pertaining to the acute effects revealed in chapter 5 are discussed in section 7.1 and 7.1.1 of this thesis. The remaining commentary will only discuss those chronic and superimposed effects revealed in chapter 5. Results revealed both positive and negative effects following chronic dosing (i.e. following 29 and 57 days of 200 mg G115 per day) on memory (see Figures 5.2; 5.4) and attentional performance (see Figure 5.3). Additionally, superimposed effects were revealed for the first time on measures of mood and cognitive performance (see Figures 5.2; 5.5; 5.6). The pattern of results underlying the superimposed results suggests that an effect on cognition and mood following chronic dosing (i.e. pre-dose testing on day 29 and 57) is further modulated

following a subsequent acute dose (i.e. post-dose testing on day 29 and day 57). Results also reveal that there was no effect revealed on any physiological measure investigated (HbA1c, insulin and circulating blood glucose levels) (see Table 5.1). With regards to glucose regulation, chapter 5 and 6 found no effect on HbA1c levels following 4 or 8 weeks of *Panax ginseng* (200mg per day) in healthy non-diabetic sample (see Table 5.1 and 6.1 respectively). Additionally, chapter 5 and 6 did not find any effect on fasting blood glucose levels over the same time period (see Table 5.1 and 6.1 respectively). In contrast to the present result Xie et al (2005) demonstrated that both oral (150 and 300 mg/kg) and intraperitoneal injection (100 and 200 mg/kg) of total ginsenosides resulted in a significant reduction in fasting blood glucose levels after 12 days of treatment and also improved glucose tolerance in diabetic mice. With regards to human participants, a reduction in fasting blood glucose levels and glycated haemoglobin were reported following 8 weeks administration of 100 mg and 200 mg/day of an unspecified extract in 18 participants with type 2 Diabetes Mellitus (Sotaniemi *et al.*, 1995). Similarly, Tetsutani (2000) reported that 24 months of treatment with 3 - 4.5g/day of Korean red *Panax ginseng* decreased HbA_{1c} (an index of average blood glucose levels over approximately the previous month) in 34 type 2 diabetics compared with controls. With regards *Panax quinquefolius*, a decrease in fasted blood glucose and HbA_{1c}, has been reported in 24 type II diabetic patients following 8 weeks administration of 1g of a proprietary ginseng extract, taken 40 minutes before each meal (Vuksan *et al.*, 2000). It should be noted that previous effects on measures of glycated haemoglobin and indices of glucose regulation (fasting blood glucose levels) following repeated ingestion of ginseng was reported in a diabetic population. These previous studies have used non-

diabetics as controls and therefore the results of the present thesis are in keeping with the previously reported effects in that population.

With regards to the chronic effects of *Panax ginseng* reported in chapter 5, the results revealed either "simple" chronic effects or chronic effects dependent upon the length of treatment (i.e. 29 and 57 days). Chapter 5 also revealed, for the first time, a pattern of results highlighting a superimposed effect of chronic and acute ingestion of *Panax ginseng* G115.

In relation to working memory performance there was a superimposed treatment effect on the primary outcome measure of working memory as measured by the CDR computerised assessment battery (see Figure 5.2). The pattern of results suggests that chronic ingestion of *Panax ginseng* (i.e. pre-dose testing - means summed across on day 29 and 57) led to improvements in working memory performance, however, these improvement were attenuated following the ingestion of an acute dose (i.e. 200 mg G115). Planned comparisons revealed that *Panax ginseng* led to significant improved working memory performance at pre-dose on day 29 and 57, as compared with placebo, although this difference was ameliorated following an acute dose (see Figure 5.2). When examining the individual tasks (i.e. numeric working memory and spatial working memory) that load the CDR primary working memory factor, the ANOVA revealed the same superimposed pattern (although the ANOVA only revealed a non-significant trend for the spatial working memory task $P = 0.06$) (see Table 5.1).

In contrast with the non-significant spatial working memory results revealed for the CDR task (i.e. the spatial working memory task) there was, however, a significant treatment effect for the non-CDR computerised spatial working memory task utilised in chapter 5 (a computerised Corsi-Block tapping task) (see

Figure 5.2). Here the ANOVA revealed a 'simple' chronic effect. The pattern of results suggesting that *Panax ginseng* G115 improved spatial working memory performance irrespective of treatment duration (i.e. 29 or 57 days) and independently of, and with no further effect of acute dosing (see Figure 5.2 and Table 5.1). Planned comparisons revealed that *Panax ginseng* led to significantly improved spatial working memory performance at both testing sessions on day 29 but only the pre-dose testing session on day 57 (this non-significant result on day 57 doesn't appear to be a result of a fall in performance in the ginseng group but rather improvements seen in the placebo group) (see Figure 5.2).

The results reported in chapter 5 revealed some inconsistencies which may reflect different sensitivities of the measurement tools purported to assess the same cognitive processes, i.e. working memory performance/processes. For example, improvements in verbal working memory performance were reported following chronic ingestion of ginseng as assessed by the CDR battery (see Figure 5.2), however no such improvements were reported on the non-CDR working memory task (N-back task or alphabetic working memory task) also utilised in chapter 5 (see Table 5.1). The latter of these two non-CDR verbal working memory tasks was manipulated by the author to try and decrease strategic chunking by volunteers and therefore has not been validated in this form. As a result no further reference will be made to this exploratory working memory task. Conversely, improvements in spatial working memory performance were reported following chronic ingestion of ginseng as assessed by the non-CDR spatial working memory task (Corsi block task) (see Figure 5.2), however, no improvements were apparent on the CDR spatial memory task (see Table 5.1). These differences in task sensitivity may simply have been due to uncontrolled factors such as task

order, or unmeasured rises in fatigue, boredom / motivation. However, these inconsistencies (i.e. improvements on one working memory task but not on another) may be due to the tasks themselves; the underlying cognitive processes; their anatomical location; the underlying biological mechanism affected by ginseng's many active constituents or more probable a complex relationship between all factors and the added complication of time.

When consideration is taken of the four working memory tasks utilised in chapter 5 it is apparent that there is a distinction between the CDR and the non-CDR tasks. Simplistically, the non-CDR tasks are cognitively more demanding but additionally these non-CDR tasks require the involvement of the central executive component of working memory for their successful completion (see later). A speculative suggestion may be that human cognitions in healthy volunteers may only be susceptible to ginseng's effects when 'resources' are failing as a result of fatigue or increased demand (as seen in the acute studies reported within chapter 2 and chapter 3 of this thesis) or have become diminished through natural ageing or disease. An additional speculation may be that *Panax ginseng* is affecting previously un-tested cognitions. These cognitions were once grouped and believed to be involved in the completion of certain working memory tasks. Baddely and Hitch, (1972) first grouped these cognitions and termed them 'central executive processes' and argued that they were responsible for the control of higher order cognitions. Recently at least 3 independent central executive processes have been identified (Miyake *et al.*, 2000). These cognitions are also believed to be located in the frontal and prefrontal cortex (Miyake *et al.*, 2000). A central executive task, by definition, is one that requires the concomitant storage and processing of information, therefore is a difficult and 'demanding

task'. The speculation made in this thesis regarding the possibility that ginseng may preferentially affect 'demanding tasks' (Chapter 2; Chapter 3; Chapter 4) would therefore fit this definition of an executive task quite well. As a result of such a speculative suggestion there are distinguishing differences between the CDR tasks and the non-CDR tasks that have previously been utilised in the present chapter of this these. The latter group of tasks (non-CDR) require the simultaneous storage, as well as sequential memory updating and sequential recall of information as opposed to the solitary maintenance requirements (which would not draw upon executive processes) of the CDR spatial tasks (Miyake *et al.*, 2000). Therefore, the disparity in results between two spatial working memory tasks utilised in the present thesis may be accounted for by the 'demands' that each task place on working memory processes or may be due to other central executive cognitions not previously tested (see later). It is possible that the non-CDR spatial task (computerised Corsi block tapping task) makes increased demands on a spatial system relative to the CDR spatial task. These higher demands may, for example, require more concentration, lead to fatigue or may even be dependent upon the local delivery and use of metabolic substrates and/or neurotransmitter communication. This suggestion may also go some way in explaining the acute improved verbal working memory performance seen in this thesis when assessed by the non-CDR tasks (as opposed to the non-significant effects reported on verbal working memory when assessed by the CDR tasks) (see Figure 5.2) and the improved performance on the more demanding verbal working memory task only (Chapter 4) (see Figure 4.1) and on the more difficult version on a serial subtraction task (chapter 2) (see Figure 2.1) and on an easier version (Chapter 3) but only when concomitant fatigue levels are

high (see Figure 3.3). However, such speculation would not explain the non-significant acute findings revealed for the Corsi block task in chapter 5 (see Table 5.1) (although a speculative suggestion may be that when administered acutely *Panax ginseng* may favour verbal memory performance through unknown mechanisms which may include the involvement of specific brain regions related to the storage of differently presented stimuli) or the non-significant findings relating to the chronic effects on the N-back task (see Table 5.1). However, it could be argued that the CDR verbal working memory task (numerical working memory task) requires the sequential memory encoding, storage and retrieval leaving it dependent on executive control (see Miyake *et al.*, 2000). Further research is needed to delineate the impact of acute and chronic dosing on potentially independent working memory processes.

The effect of *Panax ginseng* on central executive proficiency, in any population, has not been investigated directly (except in a limited context in chapter 4 utilising the random number generation task). However, previous tasks that have been utilised in the ginseng literature may allow some discussion of these processes in an exploratory and indirect manner. In chapter 4 of this thesis a commonly used central executive tool was utilised (the random number generation method) to investigate the effect of acute and sub-chronic ginseng use. It was reported that ginseng had no effect on random number generation (see Table 4.1). In chapter 4 and 5 of this thesis the N-back task was used to assess verbal working memory. This task will draw heavily on the central executive processes, as it requires the concomitant storage and processing of information. It was reported that ginseng only improved the most demanding version of the N back task acutely (Chapter 4 and Chapter 5) (see Figures 4.1; 5.1 respectively). It

is recognised that the two verbal tasks (random number generation and the N-back task) draw upon two independent central executive processes (inhibition and memory updating respectively – see Miyake *et al.,* 2000) and we have seen, in this thesis, effects on N-back (only acutely and only in the most demanding version) and no effect on random number generation. This pattern may suggest differential effects on independent central executive processes (it could be argued that the non-verbal equivalent central executive memory updating process underlies the corsi-block performance). This idea may further be supported when considering the non-significant effects reported previously on individual measures purported to assess different aspects of working memory. These tasks (i.e. numeric working memory and spatial working memory tasks comprising the CDR computerised assessment battery) can be regarded as simple maintenance based tasks that asses the memory capacity of the 'slave' systems in Baddely's model of working memory (Baddely 2000). Successful completion of these slave system tasks would not involve central executive processes. Future research could address the systematic investigation of the central executive system following ginseng ingestion. It may be difficult to imagine a differential effect on a fractionated cognitive system, which is believed to be located in the same cortical region (frontal cortex) and therefore presumably would be susceptible to the same neurotransmitter pathways and reliant on the same blood capillary network. However, there is evidence from MDMA poly-drug users that such selectivity (albeit impairments) of effect can be achieved, presumably through neuro-chemical toxicity (Reay et al., 2006).

Process or domain specific effects may suggest the need to consider cognitive fractionation in study methodology. Fractionation may also be further supported

by the present data and previous acute studies. For example, in the present study *Panax ginseng* improved working memory but impaired attention following chronic dosing (see Figures 5.2; 5.3 respectively). Additionally, previous acute studies have reported improved secondary memory performance but no affect on either working memory or attentional performance (Kennedy et al., 2002); improvements in attentional performance but no effect on working memory or secondary memory (Sünram-Lea et al., 2004) and improvements in working memory but no affect on secondary memory (chapters 4 and 5) (See Tables 4.1 and 5.1 respectively). Taken together it may be suggested that acute studies are starting to suggest a complex relationship between the cognitive domain tested, the task used to assess this domain, the dosing regimen and the post-dose testing time. More research is needed to further delineate these factors. The mechanisms underlying ginseng's behavioural effects are not know, are highly complex and are probably the result of the many active constituents of ginseng acting on numerous processes. A speculative suggestion is that the fractionation of cognitive effect is a result of the pharmacology and cortical location underlying these cognitions. For example, the pre-frontal cortex is rich in dopamine and 5-HT projection and is believed to be responsible for working memory, whereas the hippocampus is densely populated with insulin receptors and ACh projections and is believed to be heavily involved in memory consolidation and secondary memory. All of which may be sensitive to delivery and use of metabolic substrates. Ginseng or its individual ginsenosides have been shown to have excitatory and inhibitory effects on these neurotransmitter systems (see Kennedy and Scholey, 2003) (see later for further discussion of the possible underlying mechanism). To gain further insight into the underlying mechanisms responsible

for ginseng's behavioural effects it is suggested that future research should first evaluate ginseng's (the specific extract under investigation) physiological effect using an *in vitro model.* Once the physiological effect has been documented the next stage should be to run a controlled human trial, thus attempting to relate behavioural change to the known (specific to that extract) cellular effects.

In relation to attentional performance assessed in chapter 5, a chronic effect of *Panax ginseng* G115 was revealed on the primary outcome measure of speed of attention (see Figure 5.3). The initial ANOVA revealed a differential effect of 29 and 57 days of ginseng ingestion. The pattern of results suggests that ginseng led to slower performance of attentional tasks following 29 days of treatment, and were slowed further by day 57 (see Figure 5.3). Planned comparisons revealed that *Panax ginseng* G115 led to significantly slower attentional performance at pre and post-dose testing session on both day 29 and day 57 (see Figure 5.3). When examining the individual tasks (i.e. simple and choice reaction time) that load the speed of attention factor the results reveal the same pattern (see Figure 5.3 and Table 5.1). However, the ANOVA results for simple reaction time revealed a non-significant trend ($P = 0.058$). This slowing in attentional performance is consistent with other studies that have reported slowing in other domains of performance. For example the present results is consistent with the recent acute studies that have reported significant slowing of attentional tasks (Kennedy *et al.*, 2001), modest, but significant, reductions in the speed of performing a serial subtraction task (Scholey and Kennedy, 2002) and significant slowing of working memory (Chapter 4) (see Figure 4.1). These decrements in speed of task performance contrast with recent findings for the same 200 mg dose of improved speed of information retrieval, attention and arithmetical

performance (Kennedy *et al.*, 2004; chapter 2 and chapter 3), significantly shortened latency of the P300 component of auditory evoked potentials (Kennedy *et al.*, 2003) and faster attentional performance following 400mg (Sünram-Lea et al., 2004) and faster working memory performance (Chapter 4). However, caution is advised when generalising results across studies as a better understanding of the behavioural effects of *Panax ginseng* will be achieved if one limits comparisons to those studies that have reported effects on the same cognitive domains. However, as the above results all appear to reflect slowing of performance across a number of fractionated cognitive domains, such generalisation may be informative.

With regards to subjective ratings of mood an initial ANOVA revealed a differential chronic effect of 29 and 57 days of treatment on content ratings (see Figure 5.5). The pattern of results suggests that following 29 days of ginseng, content ratings were worse, however, by day 57 these negative content ratings were ameliorated (see Figure 5.5). Planned comparisons revealed *Panax ginseng* led to significantly worse subjective ratings of contentness at the pre and post-dose testing sessions on day 29, however, by day 57 these differences were non-significant (see Figure 5.5). With regards to the subjective self-reports of confusion, tension and depression the respective ANOVAs revealed a superimposed relationship between chronic and acute dosing. In all three cases the pattern of results suggests that following chronic ingestion (i.e. pre-dose testing sessions) subjective mood was worse. However, these negative mood states were somewhat ameliorated after the ingestion of that day's acute dose (see Figure 5.6). Planned comparisons revealed the same pattern, although, these negative mood states were only significantly ameliorated by the post-dose session

on day 57 (see Figure 5.6). This type of pattern may suggest some kind of psychological "addiction" to *Panax ginseng*. Thus, before taking that day's acute dose participants are reporting negative mood states, however, following their acute dose these negative moods are ameliorated somewhat. Further research is needed to investigate this relationship. An integration of qualitative research methodologies (as well as questionnaire type quantitative methodology as employed here) may provide a useful tool in such circumstances. A number of studies deal with the more generalised question of 'quality of life' or 'well being.' *Panax ginseng's* effect on these parameters has been investigated in a number of placebo controlled trials administered alone (Wiklund et al., 1999; Sotaniemi et al., 1995; Ellis and Reddy 2002; Cardinal and Engles, 2001) and in conjunction with vitamins and minerals (Wiklund et al., 1994; Neri et al., 1995; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000). Ginseng's effects have been evaluated using dosages ranging from 80 to 400 mg in patient (Wiklund et al., 1994, Sotaniemi et al., 1995, Neri et al., 1995) and healthy populations of various ages and stress levels (Wiklund et al., 1994; Caso Marasco et al., 1996; Ussher et al., 1995; Ussher et al., 2000; Ellis and Reddy 2002; Cardinal and Engles, 2001). Study duration has spanned from 2 to 9 months (See Coleman et al., 2003 for review). Improvements in the measures pertaining to 'quality of life' or 'well being' have been reported in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002), although findings of this nature are by no means unequivocal (see Kennedy and Scholey, 2003). For example, when considering only those studies that have used supplementation of *Panax ginseng* alone in healthy volunteers the results are somewhat inconsistent. For instance, Ellis and

Reddy (2002) report improvements in measures of 'quality of life' after 4 weeks of 200 mg G115/day, as compared with placebo in a small cohort of 30 healthy young adults but these improvements were attenuating by the 8-week end point. Conversely, Cardinal and Engels (2001), using a similar but slightly larger cohort of 83 healthy young adults, reported no significant differences on the Positive Affect–Negative Affect Scale (PANAS) or Profile of Mood States (POMS) at their 8-week end point following 200 mg G115 or 400 mg G115 daily as compared to placebo. Chapter 4 however did show acute improvements in calmness ratings (see Figure 4.1) and chapter 2 and chapter 3 showed significant improvements in self-ratings of mental fatigue (see Figures 2.1 and 3.5 respectively).

Chapter 6 With regards to the effect of this non-standardised extract on indices of glucose regulation (HbA1c, insulin and blood glucose) the results revealed no effect on any physiological measure taken (see Table 6.1). The failure to report an effect on any physiological measure is consistent with that of chapter 5 (see Table 5.1). However, due to the fact that the actives in the extract utilised in chapter 6 are unknown, coupled with the fact that chapter 6 is the first study to use this specific extract in a controlled behavioural study, further discussion of the physiological effect (or lack of) is somewhat meaningless.

With regards to chronic effects of the non-standardised Korean *Panax ginseng* extract used in chapter 6 the results revealed a simple chronic effect on delayed picture recognition (episodic secondary memory). Planned comparisons revealed that ginseng led to significantly slower performance of this task at all pre and post-dose testing sessions on both day 29 and 57 (see Figure 6.3). The acute 3

hour post-dose improvements in secondary memory (se Figure 6.2) appear to be lost when dosing is continued for 29 and 57 consecutive days. The relationship between acute and chronic improvements and decrements in cognitive performance require further investigation using standardised extracts (or extracts which know ginsenoside content) to allow insight into the behavioural effects of ginseng.

A simple chronic effect was also revealed on the accuracy of performing the digit vigilance task (see Figure 6.4). Planned comparisons revealed that ginseng led to a significantly greater number of errors being committed at the pre-dose testing session on day 29 and 57 (see Figure 6.4). This result is the first to report a chronic effect of *Panax ginseng* on a vigilance task. Chapter 5 failed to report any effect of *Panax ginseng* (G115) on any measure of vigilance or the more global domain of attention, however, one previous acute trial has demonstrated improvements in attentional measures 90 minutes post-dose following 400 mg G115 (Sünram-Lea et al., 2004). This again highlights the differences between dosing regimes and extracts, and the need for further research to delineate these effects. Although, there was no significant superimposed relationship revealed on any outcome measure (i.e. no significant treatment x testing session interaction; there was a non significant trend ($P = 0.06$) on this vigilance task, suggesting a pattern in results indicating that an acute dose alleviates the decrements in performance seen at the pre-dose testing session (this pattern is more evident on day 57) (see Figure 6.4). This superimposed relationship was apparent in chapter 5, albeit on different outcome measures. Further research is needed to investigate these patterns.

There were significant improvements in working memory performance following chronic ingestion of a non-standardised *Panax ginseng* extract. Ginseng was associated with speeded working memory performance at all but the first testing session on day 29 (see Figure 6.5). Improvements in working memory performance are consistent with the chronic working memory improvements demonstrated in chapter 5 (see Figure 5.2) (although see later for further discussion) and the acute improvements demonstrated in chapter 4 (see Figure 4.1) and chapter 5 (see Figure 5.1) and the improved mental arithmetic performance in chapter 2 (se Figure 2.1) and chapter 3 (see Figure 3.3). However, there are some inconsistencies in the working memory effects reported in chapter 5 and chapter 6. In chapter 5 the chronic verbal working memory improvements were only apparent on the CDR task with no effects revealed on the non-CDR verbal working memory task (i.e. N-back task) (see Figure 5.2 and Table 5.1). The opposite is revealed in chapter 6 (see Figure 6.5 and Table 6.1). Moreover, chapter 5 revealed chronic effects on non-verbal working memory performance and a superimposed relationship (see Figure 5.2). Additionally, chapter 5 revealed acute improvements in the accuracy of verbal working memory (see Figure 5.1). It was speculated in chapter 5 that *Panax ginseng* (G115) might be affecting independent working memory processes underlying these complex working memory tasks in a disproportionate way through unknown mechanisms. It is possible that the non-standardised ginseng extract used in chapter 6 may be consistent with this suggestion as results again show improvements on a task that will require executive processes (see Figure 6.5). However, chapter 6 failed to reveal any effect on non-verbal working memory performance (see table 6.1). As this is the first study to assess the effects of this non-standardised extract (and the

simple fact that it is a non-standardised extract) further discussion of theses processes will not be made until these results are replicated and investigated further.

The results in chapter 6 also revealed chronic effects on measures pertaining to human 'quality of life' and 'mood'. Ginseng led to significant improved ratings of social relations following 29 days and 57 days of ginseng treatment (see Figure 6.6). Conversely, self-report ratings of calmness were significantly worse following 29 days and 57 days of ginseng (see Figure 6.7). Improvements in the measures pertaining to 'quality of life' or 'well being' in pathological (Sotaniemi *et al.*, 1995; Neri *et al.*, 1995; Tode *et al.*, 1999) and healthy (Marasco *et al.*, 1996; Wiklund *et al.*, 1994; Ellis and Reddy 2002) human populations have been demonstrated, although findings of this nature are by no means unequivocal (see Kennedy and Scholey, 2003). Indeed, even within the chapters of this current thesis the present improvements in quality of life is inconsistent with the results of chapter 5, which reported no effect of 29 and 57 consecutive days of *Panax ginseng* (G115) treatment on any dimension of quality of life (see Table 5.1). Additionally, decrements in calmness reported in chapter 6 (see Figure 6.7) are inconsistent with improved calmness following acute dosing of G115 reported in chapter 4 (see Figure 4.1) and the anti-fatigue properties reported in chapters 2 (see Figure 2.1) and 3 (see Figure 3.5). The results in chapter 6 also failed to find an effect on contentedness, depression, tension or confusion as reported in chapter 5 following *Panax ginseng* G115 (see Figures 5.5 and 5.6 respectively). The most parsimonious explanation would be that of the different ginsenoside content and testing regimens of the extracts utilised in the present thesis and in previous research

It has consistently been suggested throughout this thesis that methodological differences between studies do not help in the understanding of the behavioural effects of ginseng. Future research must build upon the results of the present thesis utilising consistent methodologies and address the notion that specific fractionated cognitive processes may be affected by different doses and extracts of *Panax ginseng*, ingested at different time points.

7.3. MECHANISMS OF ACTION

The results outlined in the chapters of this thesis do not, in themselves, comprise an adequate platform for identifying ginseng's underlying biological mechanisms of action. However, the extant literature may suggest a number of speculative possibilities. Additionally, chapters 2 and 3 may provide evidence to suggest a role of glycaemic modulation as a partial explanation for ginseng's acute cognitive and mood effects. What follows is limited discussion of ginseng's possible mechanisms of action.

7.3.1. Neurotransmission

Neurotransmitters are integral to the efficient communication between cells. Glutamate and GABA are the two most widely excitatory and inhibitory transmitters (respectively) in the human brain; however, there are dozens of other neuro-modulators, all with specific tracts/pathways, involved in many bodily functions. The synthesis, release, breakdown and uptake of these chemicals are stringently controlled; therefore, any drug that affects these steps may have a direct influence on cognition and mood. For example, the main synthetic treatment for

dementia of the Alzheimer's type is to block the breakdown of Acetylcholine by inhibiting the action of the cholinesterase enzyme. Similarly, a successful group of drugs known as selective serotonin reuptake inhibitors (SSRIs) used in the treatment of depression, block the reuptake of 5HT. Both treatments have been found to aid memory and improve mood respectively. Additionally, dys-regulation of the dopamine system has been linked to Parkinson's (too little), Schizophrenia (too much) and with impaired higher cognitions function. In line with this suggestion is that reduced concentrations of Dopamine, in the frontal lobes, have been link to ADHD (Roman et al., 2004). It is perhaps somewhat unrealistic to speculate that the modulation of one system underlies ginseng's effects, especially when one considers the plethora of evidence documenting ginseng's ability to modulate numerous physiological and neuro-chemical systems (see Kennedy and Scholey 2003). Additionally, it is also somewhat unrealistic to suggest that any one system acts independently of others. However, the possible role of the Dopamine (DA), Serotonin (5HT) and Acetylcholine (ACh) deserves some discussion.

Ginseng and its constituent parts have been reported to modulate a number of monoamines. For example, increased levels of dopamine and serotonin have been reported in the cortex following 50 mg/kg ginseng administration (Petkov, 1997). Others have reported both facilitated and inhibited monoamine metabolism dependent on the period of dosing (100 mg/kg for 2 or 7 weeks), the monoamine under investigation and the discrete brain area involved (Itoh et al., 1989). The total saponin content of ginseng has been shown to inhibit serotonin receptor subtypes (Min et al., 2003) and modulate dopamine activity at both pre-synaptic and post-synaptic dopamine receptors and block behavioural sensitisation induced by psychostimulants, for example, nicotine (Kim et al., 2005). It has been

suggested that these effects are mediated by the inhibition of drug-related dopamine release by the action of ginseng total saponins on presynaptic dopamine terminals (Shim et al., 2000) and postsynaptically by the binding to DA D(2) receptors (Kim et al 2005). It appears that ginseng can modulate the DA and 5HT systems. Both neurotransmitters project widely throughout the brain; however, they both project to the frontal cortex (an area of the cortex thought to be involved in working memory). It is possible that the effects on working memory and mood revealed in the present study can be explained by DA and 5HT system modulation. The cholinergic system has been suggested, for many years, to be important in human cognitive function. Evidence supporting this view comes from both healthy and patient populations. For example, evidence suggests that the level of acetylcholine (ACh) is greatly reduced in the brains of Alzheimer's patients with some cognitive functions been maintained (or at least the time scale for cognitive decline slowed) by acetylcholinesterase inhibitors (see Pinel, 2002). Additionally, modulation of the cholinergic system, either directly or indirectly, has been shown to impact upon cognitive function in young healthy volunteers (for example see Kennedy *et al.,* 2004) and older healthy populations (for example see Scholey *et al.*, under review). Therefore, modulation of the cholinergic system may underlie the behavioural effects of ginseng (see Lewis *et al*, 1999). Numerous studies have identified cholinergic properties of whole ginseng extracts and individual ginsenosides (see Kennedy and Scholey 2003). For example, *Panax ginseng* extracts and *Panax quinquefolium* extracts have been reported to reverse scopolamine induced memory impairments in rodents, with the latter extract increasing choline uptake in synaptosomal preparations (Bao et al., 2005). Additionally, *Panax ginseng* has been shown to exhibit an affinity for both

nicotinic and muscarinic receptors in human brain cerebral cortex membranes (Lewis et al., 1999). Wang et al., (2006) demonstrated that beta-amyloid memory impairments were reversed by 5 days pre-treatment with total ginseng saponins (80 mg/kg/day) and that this pre-treatment completely protected the animal against beta-amyloid-induced reduction of hippocampal ACh release. Conversely, *Panax ginseng* has been shown to inhibit ACh-stimulated release of catecholomines *in vitro* (Tachikawa and Kudo, 2004). Individual ginsenosides have also been shown to modulate the cholinergic system. For example, Rg_2 has a direct interaction with nicotinic receptor subtypes (Sala et al., 2002; choi et al., 2002), whilst, Rb_1 modulated ACh release and uptake, and also up-regulated the number of uptake sites in the hippocampus, and to a lesser extent in the cortex (Benish, 1992).

The cholinergic system has for many years been implicated as important for long-term memory, with studies in the 1970's showing poorer long-term memory performance when the cholinergic system is blocked (Drachman and Leavitt, 1974; Peterson, 1977), while sparing working memory performance, except in certain conditions where processing demands were made particularly high (Rusted and Warburton, 1989). Such patterns have been interpreted as reflecting a disruption of information transfer from short term into long-term memory (Feldman *et al,* 1997; Ghonheim and Mewaldt, 1977). However, other studies have suggested that the cholinergic system is also involved in attentional performance (Rusted and Warburton, 1989; Wesnes and Warburton, 1983; 1984) with further evidence suggesting memory effects are a secondary consequence of the regulation of attentional processes (Blokland et al., 1996). Similarly, others have suggested that cholinergic modulation of central executive functioning is the primary factor (Rusted, 1988; Rusted *et al*, 1991; Rusted and Warburton, 1988; 1991).

It therefore may be suggested that the modulation of the cholinergic system would be expected to produce a pattern of preferential improvements both in the performance of attentional performance, and in long term memory, rather than working memory. However, if the working memory tasks required executive control or resources, then one might expect to see improvements in these working memory tasks too, in comparison to those working memory tasks that only require slave systems (Baddely, 2002). You would also expect to see modulation of these tasks by DA and 5HT.

With consideration of the limited available behavioural data it is apparent that any firm conclusions, regarding a neurotransmitter mechanism or action, would be difficult to justify. For example, following single doses of *Panax ginseng* in young healthy volunteers, improvements have been reported in secondary memory performance (see Kennedy et al., 2003), however, both deficits (see Kennedy et al., 2003) and improvements (Sünram-Lea et al., 2004) have been reported in attentional performance. However, it may be the case that ginseng's ability to improve memory (long term memory) may be related to the modulation in cholinergic parameters specific to hippocampal tissue (Benishin 1992; Benishin *et al*, 1991). Indeed this may explain some of the effect found in the present thesis (chapter 6); although, a complex relationship between other systems and methodological differences must play a role in the behavioural patterns.

The behavioural data revealed in the chapters of the current theses present no clearer support for such a neurotransmitter hypothesis. For example, in chapter 2 and 3 the results revealed improvements in mental arithmetic performance (a task which would require working memory, attentional processes and possibly long term memory depending and the strategy used by the participants to complete the

task) but not in attentional performance *per se* (RVIP task). It may indeed be the case that central executive resources underpin the improvements in mental arithmetic performance, which may be sensitive to modulation of DA, 5HT or ACh, consequently leading to the cognitive modulation (Rusted, 1988; Rusted *et al*, 1991; Rusted and Warburton, 1988; 1991). The picture is no clearer when considering chapter 4, as the results revealed only improvements in working memory performance with no affect on secondary (episodic) memory. Interestingly, the acute behavioural results revealed in chapter 2, 3, and 4 appear to have one common denominator – that of central executive involvement and it may indeed prove, given further research, that it is this domain that is affected through neurotransmitter modulation by ginseng.

The possible role of a neurotransmitter involvement in ginseng's cognitive effects is further complicated, and no clearer, when the behavioural results of chapters 5 and 6 are considered. It should be noted that there might be differences in the underlying mechanisms of action associated with acute and chronic dosing and also between different ginseng extracts that will inevitably contain different ratios of active ginsenosides (and actually different ginsenosides). Therefore, the cognitive results of chapters 2, and 3 (acute studies) may not be comparable to those 4, or chapter 5 and 6 (chronic studies). Additionally, the difference in the extract used between chapter 5 (standardised extract) and 6 (non-standardised extract) may also render comparisons between those chapters somewhat meaningless. As a practical example to highlight these possible confounding factors Rg_2 has been shown to have a direct interaction with nicotinic receptor subtypes (Sala et al., 2002; Choi et al., 2002), whilst, Rb_1 modulated ACh release and uptake, and also up-regulated the number of uptake sites in the hippocampus,

and to a lesser extent in the cortex (Benish, 1992). Well-controlled studies that implement behavioural task that fractionate hippocampal memory consolidation, attentional performance, non-executive and executive working memory processes may provide a useful insight into the underlying mechanisms of action and provide somewhat more consistent behavioural results.

7.3.2. Cellular metabolism:

The oxidised breakdown of glucose is essential to meet the energy requirements of the body to allow 'normal' function (see Pinel, 2002). fMRI studies have demonstrated increased utilisation of glucose in areas of the brain, when volunteers are engaged in cognitive processing (see Pinel, 2002). Behavioural studies have documented that the modulation of peripheral blood glucose levels can impact upon cognitive function (see Messier, 2004). It is therefore possible that ginseng's effects on cellular metabolism may go some way to explaining its behavioural effects. The animal literature provides some evidence that ginseng can modulate glucose levels - lowering blood glucose levels acutely or improving parameters of glucose regulation following chronic ingestion (e.g. HBA1c) (see section 1.2.5.6 of this thesis). The mechanism responsible for ginseng's gluco-regulatory effect is unknown but may relate to the modulation of glucose disposal, glucose uptake and/or insulin secretion. The present thesis was interested in glucose uptake (although this process in not mutually exclusive of the other two proposed mechanisms – indeed glucose transport is reliant upon insulin in some cases i.e. the GLUT 4 transporter). *In vitro* studies have shown individual ginsenosides are capable of promoting cellular uptake of glucose, for example, glucose uptake has been shown following ginseng by GLUT 1 (Hasegawa *et al.,* 1993), GLUT 2

(Ohnishi *et al.,* 1996) and GLUT 4 (Lai *et al.,* 2006). Additionally, *in vivo* studies have documented significant reductions in circulating blood glucose levels by whole ginseng extracts in both diabetic and non-diabetic populations, with the authors suggesting increased uptake as one possible explanation (see Vuksan *et al.,* 2002). A specific aim of the present thesis was to relate any acute modulation of blood glucose levels, as a result of ginseng ingestion, with any behavioural effects, in the hope that glucose uptake may partially explain the acute behavioural effects following ginseng ingestion. Interestingly, the results of chapters 2 and 3 report improvements in cognitive performance and concomitant reductions in circulating blood glucose levels. Additionally, in chapters 4 and 5 the results report improvements on only the most difficult working memory task utilised (task difficulty has been frequently reported as being an important factor for a glucose facilitation effect. Therefore, the observations in chapters 4 and 5, that only the most difficult version of a working memory task was improved by ginseng ingestion may be taken as indirect evidence that glucose utilisation may be involved in the performance of this task). However, in the present thesis there was no direct relationship between the change in blood glucose levels and the change in behavioural performance. However, the failure to find a direct relationship between blood glucose levels and cognitive performance is not uncommon in the glucose literature – see later). Additionally, in chapters 5 and 6 there were no effects reported on any measure of glycaemic regulation; although, the mechanism underlying any chronic behavioural effects of ginseng may be different from those mechanisms underlying ginsengs acute effects. Given this, a speculative suggestion could be that the acute behavioural improvements described in chapters 2 and 3 may well be most parsimoniously attributed to improved delivery and utilisation of

circulating blood glucose. In line with this suggestion there is an extensive literature suggesting cognitive improvements following a glucose drink (as a result of increased local provision of acetyl-coA or increased utilisation of glucose as a the source of neuronal energy), however, these results are by no mean unequivocal and the underlying mechanism responsible for a glucose facilitation effect is not fully understood (see Messier *et al.,* 2004). Additionally, there are studies that fail to report any direct relationship between glucose levels following a glucose drink and cognitive performance (Sünram-Lea *et al,* 2002; Sünram-Lea (2002). However, Sünram-Lea *et al* (2001) and Sünram-Lea *et al* (2002) did report significant positive correlations (one tailed) between blood glucose levels and some memory tasks, when the results were analysed across all groups (these correlations were lost when analysed for the glucose group alone). In these studies, glucose levels significantly explained between 12% (r = .34) and 22% (r =.47) of the variance in cognitive performance. Additionally, it has been suggested that circulating blood glucose levels may not be a true reflection of central plasma glucose levels (see McNay *et al.,* 2001) and Sünram-Lea *et al,* (2002) state that *"blood glucose levels should not be seen as an absolute measure of central glucose regulation and should not be used as an indicator of how much glucose is successfully transferred to and used in the brain".*

Examples of cognitive improvements by glucose ingestion include improved attentional performance (Benton *et al,* 1994; Owens and Benton, 1994), executive performance (Donohoe and Benton, 1999; Martin and Benton, 1999; Sünram-Lea *et al,* (2002), spatial working memory (Sünram-Lea *et al,* (2002), long term verbal (Sünram-Lea *et al,* 2001; Sünram-Lea *et al,* 2002) and non-verbal memory (Sünram-Lea *et al,* (2001) and mental arithmetic performance (Kennedy and

Scholey, (2000). Foster *et al,* (1998) demonstrate mnemonic enhancement, with clear facilitation of declarative memory performance, and little or no enhancement of working memory tasks. Although, only a limited number of working memory processes were assessed using forward and backward span tasks, which would assess verbal slave systems and verbal memory up-dating executive processes respectively.

It has been suggested that the underlying mechanism of enhanced attentional performance and declarative memory is the direct increased local provision of acetyl-CoA, and therefore increased acetylcholine synthesis, through the direct oxidative breakdown of glucose (e.g. Gold, 1995, Sünram-Lea and Foster, 2002; Wenk, 1989), a process that, by its nature, could benefit directly from increased delivery of either or both metabolic substrates.

Cholinergic modulation is only one possible mechanism underlying glucose's facilitatory effects (e.g. Sünram-Lea and Foster, 2002), and it has also been suggested that increased delivery of glucose may also preferentially enhance performance on cognitively demanding 'fuel limited' tasks i.e. tasks that are facilitated by the simple delivery of the metabolic substrates necessary to increase localised neuronal activity, and thereby raise the usual ceiling of performance (Kennedy and Scholey 2000; Scholey *et al* 2001; Sünram-Lea *et al,* 2002). This suggestion is somewhat consistent with the reports of chapter 2 showing improvements on only the most difficult arithmetic tasks and with Chapter 3 showing improvement restricted to the subjectively easier (see Kennedy and Scholey, 2000) arithmetic subtraction task but only when concomitant fatigue ratings were at their greatest. Similarly, the acute results of Chapters 4 and 5 are also somewhat in line with the above, as improvements were only seen on the most

difficult of working memory tasks utilised. In support of the suggestion that behavioural performance may be improved through the increased neuronal activity leading to greater need for the oxidized breakdown of glucose to create energy in the form of ATP (although it should be noted that a by-product of this process would be increased local provision of acetyl-CoA, which could be used in the synthesis of ACh), it has been established that fluctuations in the levels of circulating blood glucose levels can modulate cognitive performance (see Messier, 2004 for review). Perhaps the greatest evidence for the latter hypothesis comes from those studies that have utilised task that are thought to be independent of the cholinergic system – working memory tasks (see later). Therefore, the hypothesis suggesting glucose facilitation results from the increased synthesis of ACh would be less pertinent and evidence could point towards the increased utilisation of glucose as a basic fuel. However, as discussed in the cholinergic section above (see section 7.3.1 of this thesis), working memory tasks that were once thought to be independent of cholinergic systems may in fact be reliant upon this system if the task requires central executive involvement. Therefore, chapters 2 and 3 of this thesis could be explained by either hypothesis.

Two studies presenting strong evidence for the latter hypothesis is that of Kennedy and Scholey (2000), and Scholey *et al* (2001). In the former, the authors report a positive association between the rate at which a person's blood glucose levels falls, following an initial peak, and the level of cognitive performance, particularly during periods of cognitive demand. In the latter, results show that compared with placebo, a glucose drink improved performance during intense mental processing, which, in turn, led to a measurable reduction in blood glucose levels (Scholey *et al.*, 2001). One explanation for such findings of Scholey et al (2001) and that of

chapter 2 and 3 is that increased uptake of blood glucose results in better performance and concomitant measurable reduction in blood glucose levels. However, it should be noted that there was no direct relationship between the post-dose change in blood glucose levels and the post-dose change in behaviour in any chapter of this thesis. However, as discussed above this may not be a direct indicator of central blood glucose levels.

This raises the possibility that the acute cognition enhancing properties of *Panax ginseng* is attributable partly to two mechanisms. Firstly, a simple increased delivery of metabolic substrates, with a subsequent increase in acetylcholine production, leading to a pattern of cognitive improvements in line with the 'cholinergic' pattern outlined in the previous section; and secondly, increased delivery and utilisation of metabolic substrate (glucose) in active neural tissue during localised demand, leading to improved performance on 'cognitively demanding' tasks.

The mechanisms underlying ginseng's behavioural effects are poorly understood. The suggestions above are 'best guesses' based on the profile of results, and the literature dealing with laboratory investigations of possible mechanisms. The suggestions are by no means exhaustive. Given the complex nature of the multiple active components in ginseng extracts it is highly unlikely that cognitive modulation is as a result either of the action of a single active component, or as a result of action on a single physiological parameter.

7.4. POTENTIAL METHODOLOGICAL LIMITATIONS

The chapters of the present thesis are somewhat independent of each other in terms of methodology. Therefore, the acknowledgement of potential methodological problems will follow.

The first potential methodological limitation is the nature of the statistical analysis utilised throughout this thesis. It is notable that the available data relating to the behavioural effects of ginseng is week at best (especially when considering the chronic use of ginseng). Therefore, the current thesis concentrated on novel methodological design and experimentation whilst utilising comprehensive computerised assessment tools previously shown to be sensitive (either in earlier chapters of this thesis or the limited data available in peer reviewed journals). As a result the general philosophy underlying the design, analysis and interpretation of the results making up this thesis is that of a *tabula rasa* approach. The approach is therefore necessarily exploratory, and as such the statistical analysis of the individual studies has been balanced between the need to demonstrate the statistical significance of any findings with a desire not to obscure potentially important findings that can be taken forward into more focused research.

The chapters making this thesis have utilised the same well-used approach to the analysis of multiple dose, multiple time point studies (see Kennedy *et al.,* 2003), and have adopted strictly planned comparisons assessing the limited number of questions of true relevance i.e. the effect of each treatment versus placebo at each discrete post-dose time point. Although this holds true for chapters 2, 3 and 4, as these chapters utilised multiple doses and multiple post-dose time points, it does not hold true for chapters 5 and 6 (there were multiple post-dose time points though). However, as the studies making chapters 5 and 6 are the first studies to

investigate the chronic behavioural effects of ginseng utilising a comprehensive assessment battery, a standardised ginseng extract (in the case of chapter 5) and placebo controls, these results are truly exploratory in nature. Therefore, it was felt that to correct the alpha level associated with the planned comparisons in chapters 5 and 6, allowing the family wise error rate to equal 0.05 and therefore control for the possibility of increased type 1 error (although by virtue increasing the possibility of committing type 2 errors), would hinder interpretation of these studies and would fail to initiate further research in this area. However, other measures were taken to control for the risk of type 1 errors (see later).

The statistical approach adopted in each chapter of the present thesis is that advocated by Keppel (1991), who states that in the case of experiments specifically designed with a hypothesis in mind (i.e. does ginseng have an effect over that of placebo) *'most researchers conduct analyses relevant to these hypotheses directly without reference to the omnibus F test. (Although the omnibus test may be computed, its significance or non-significance does not modify this particular course of action)'*. In line with this the omnibus Analysis of Variance has been calculated in each case, but reference to it has then been eschewed in favour of planned comparisons using t tests incorporating MS Error from the ANOVA (reference was made to the ANOVA results in chapter 5 as novel and interesting interactions were revealed. Additionally, in chapters 4, 5 and 6, planned comparisons were restricted to those outcome measures that revealed significant main effects of treatment or interaction with treatment in an attempt to minimise type 1 error). While this is not a controversial approach to analysis it does raise the question of the inflated chance of Type I errors, and on this matter Keppel (1991) notes that '*The most widely used strategy is to evaluate the planned comparisons in*

the normal way – at the usual PC, or α, level – and to exercise control of the FW rate for post-hoc comparisons through special evaluation procedures designed to cope with the problem'.

On the question of inflated Type I errors Keppel suggests that the number of comparisons that should be made without any kind of correction is arbitrary, depending on the nature of the investigation (however, the number of comparisons should be restricted to K-1). He also goes on to propose a modified Bonferroni test that can be used to adjust the probability level that will be taken as significant if desired (in the case of the relevant studies the most conservative interpretation would lead us to set the significance level on any given comparison at p=0.0125), but he also suggests that results that fall between this modified significance and P=0.05 should still be reported, but that judgement should be 'suspended'. In the case of the relevant chapters here the comparisons have been left uncorrected, but interpretation has concentrated on those results that fall into patterns, with notes where relevant, in the text urging caution in the over-interpretation of single significant differences. Additionally, it should be noted that the probability of two, three or four significant data points appearing for a specific dose, or doses, on a specific measure, are far lower than the probability of the type I error which we would wish to protect against. Interpretation on the data in the present thesis was restrained to these situations and for chapters 4, 5 and 6 planned comparisons were restricted to those outcome measures that revealed significant main effects of treatment or interaction with treatment.

It is also noteworthy that Keppel also states, again with specific reference to planned comparisons, *'I am in agreement with Davis and Gaito (1984), who argue that an over concern for Type I error in any particular experiment may actually*

impede progress in that area of research'. In the case of the relevant chapters of the current thesis the investigations include the first study of the acute effects of ginseng on blood glucose levels, experimental fatigue, and the first studies of the chronic cognitive effects of ginseng. As such it is necessary to consider the possibility that there is a relationship between both dose and time course, and the experiments have been designed accordingly, with several doses and three or four time points. To be over cautious at this early stage in investigating the effects of ginseng utilising standardised extracts and adequate methodological design, would only serve to confuse potentially important findings that will drive research forward and ultimately lead to a better understanding of the behavioural effects of ginseng.

Another area offering potential for confounding the results is the use of change from baseline data. It is possible that any post-dose results may simply represent regression to the mean. To control for this the present thesis preformed one-way ANOVA between conditions on all baseline data. If there was a significant effect or a trend towards effect caution was advised in the text. Whilst the possibility of statistical anomalies exists, it seems unlikely that they have had an undue influence on the results reported here.

7.5. CONCLUSIONS

The relevant chapters of this thesis suggest that the administration of acute doses of *Panax ginseng* can modulate circulating blood glucose levels, mood and cognitive performance. The results also suggest that 7 days of ginseng ingestion has no effect on secondary or working memory performance whereas an acute dose on day 1 and day 7 can modulate cognitive performance. Conversely, the results within this thesis suggest that ginseng is capable of modulating mood and cognitive performance following 29 and 57 days. The results also suggest a superimposed relationship.

The findings of this thesis are novel. There is little in the way of previous research into the cognitive effects of ginseng, despite the fact that it is the second most commonly taken herbal product (after ginkgo) for memory problems (Hartman Group's Natural Products Census Supplement Report: July 1998 - July 1999) and was recently found to be the most popular taken psycho-active herbal product in the US (Barnes et al., 2004) However, a series of acute trials have demonstrated consistent secondary memory improvements following 400mg G115 (Kennedy et al., 2003). With regards the effect following prolonged ingestion the research is even more scars and often the nature of the findings are contaminated from methodological shortcomings and a lack of standardisation in ginseng products (Bahrke and Morgan, 1994; 2000).

On this last point it is notable that the term 'ginseng' covers a huge collection of commercial products, which vary dramatically in overall ginsenoside content. With the exception of research using standardised extracts (G115 being the most commonly used), there is rarely any way of assessing what the experimental treatment actually contains in the way of active ingredients.

By convention, but unsupported by any empirical evidence, there has also been an assumption that ginseng takes some weeks to manifest its effects. The studies in the current thesis are the first to utilise methodological appropriate designs to investigate the effect of prolonged ginseng use in humans and also further highlight the acute behavioural effects.

The results suggest that *Panax ginseng* exerts a potent hypoglycaemic effect following single doses and has a specific beneficial effect on working memory processes. However, results also suggest ginseng can be associated with decrements in cognitive performance and subjective ratings of mood following chronic ingestion. Further research into the effects of ginseng is warranted, and a number of specific questions are raised both by the extant literature and the studies described in this thesis. These include: further investigation into the glycaemic modulating properties of different doses, taken with and without food/glucose drink and clarification of any interactions with pre and post-prandial time points; clarification of the optimum acute dosage; investigation of the underlying cognitive processes; investigation of the comparative effects of acute and chronic dosage; an elucidation of individual differences in response to ginseng; a comparison of the effects of ginseng extracts varying in the ratio of protopanaxadiols to protopanaxatriols; an assessment of the acute effects of ginseng in populations with pathology related memory decrements; and whether the chronic administration of the combination of ginkgo and ginseng confers any additional advantage over and above the single extracts. The above is by no means an exhaustive list of possible directions of enquiry. However, it is hoped that the results of the relevant chapters in this thesis might encourage further research into some of these questions.

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Table 7.1 Summary of the studies and results following acute administration of *Panax ginseng*

	Sample	Methods	Physiological Measurement	Treatment	Cognitive Measurement	Cognitive domains Assessed	Assessment points	Results
Chapter 2	N=30. Overnight fasted healthy young volunteers. Mean age 22.6 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 10 minute cognitive demand battery (CDB) at baseline and then continuously for 60 min, commencing 60 min after ginseng ingestion.	Finger prick blood glucose level was measured at 60, 90 and 120 min post ginseng ingestion	Placebo 200 mg (G115) 400 mg (G115)	CDB Serial subtraction RVIP task Mental fatigue (VAS)	Verbal Working memory Attention Subjective fatigue	Performance on each task was assessed once per CDB completion.	200mg and 400mg significantly lowered blood glucose levels at all post-dose measurement points. 200mg significantly improved serial subtraction performance (working memory) and subjective ratings of mental fatigue.
Chapter 3	N=27. Overnight fasted healthy young volunteers. Mean age 21.89 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 10 minute cognitive demand battery (CDB) at baseline and then continuously for 60 min, commencing 60 min after ginseng ingestion. 30 min after ginseng ingestions volunteers ingested either glucose (25g) or placebo (30mg saccharine) drink	Finger prick blood glucose level was measured at 60 and 120 min post ginseng ingestion	Placebo (0mg ginseng and 30mg saccharine) Ginseng (200 mg(G115 and 30mg saccharine) Glucose 0mg G115 and 25g glucose drink Combination (200mg G115 and 25g glucose drink)	CDB Serial subtraction RVIP task Mental fatigue (VAS)	Verbal Working memory Attention Subjective fatigue	Performance on each task was assessed once per CDB completion.	200mg G115 significantly lowered blood glucose levels 60 minutes post ginseng dose in the ginseng condition. Ginseng condition and glucose condition improved serial subtraction performance (working memory) and subjective ratings of mental fatigue.

Table 7.1 Summary of the studies and results following acute administration of *Panax ginseng*

	Sample	Methods	Physiological Measurement	Treatment	Cognitive Measurement	Cognitive domains Assessed	Assessment points	Results
Chapter 4	N=30. Overnight fasted healthy young volunteers. Mean age 22.87 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 60, 150 and 240 min post ginseng ingestion on day 1 and following 7 consecutive days of ginseng ingestion (day 8). Participants consumed a light breakfast; however their last testing session preceded lunch. Between each treatment regime there was a 6 day placebo washout period.	None	Placebo 200 mg (G115) 400 mg (G115)	Immediate word recall Delayed word recall Delayed word recognition Corsi block N Back (0,1,2,3) Random Number generation Bond lader (VAS)	Episodic memory Episodic memory Episodic memory Non-verbal working memory Verbal working memory Central executive Alert, calm, content	Participants were assessed 60, 150 and 240 min post dose on day 1 and day 8.	Only acute effects were found. There was no effect following 7 consecutive days of ginseng ingestion. 200mg and 400mg significantly improved subjective ratings of calmness up to 4hrs post dose on day1 and day 8. 200mg significantly slowed working memory performance 400mg significantly improved the accuracy of working memory performance
Chapter 5	N=25. Overnight fasted healthy volunteers. Mean age 35.28 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 180 min following ginseng ingestion on day 1. Participants consumed a light breakfast; however post dose testing preceded lunch.	Finger prick blood glucose levels measured 180 min post ginseng ingestion	Placebo 200mg (G115)	Cognitive drug research computerised assessment battery N back (3) Cosi block Brunel scale WHOQOL bref	Episodic memory, verbal and non-verbal working memory, attention, alert, calm, content Verbal working memory Non-verbal working memory Anger, confusion, depression, vigour fatigue, tension,	Participants were assessed 180 min post-dose	No effect on blood glucose levels. 200mg Improved the accuracy of the N back task performance (working memory)

Table 7.1 Summary of the studies and results following acute administration of *Panax ginseng*

	Sample	Methods	Physiological Measurement	Treatment	Cognitive Measurement	Cognitive domains Assessed	Assessment points	Results
Chapter 6	N=18. Overnight fasted healthy volunteers. Mean age 38.31 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 180 min following ginseng ingestion on day 1. Participants consumed a light breakfast; however post dose testing preceded lunch. .	Finger prick blood glucose levels measured 180 min post ginseng ingestion	Placebo 200mg Korean *Panax ginseng*	Cognitive drug research computerised assessment battery N back (3) Cosi block Brunel scale WHOQOL bref	Episodic memory, verbal and non-verbal working memory, attention, alert, calm, content Verbal working memory Non-verbal working memory Anger, confusion, depression, vigour fatigue, tension,	Participants were assessed 180 min post-dose	There was no effect of blood glucose levels 200mg significantly impaired the accuracy of delayed word recognition 200mg significantly improved the speed of delayed picture recognition

Table 7.2 Summary of the studies and results following chronic administration of *Panax ginseng*

	Sample	Methods	Physiological Measurement	Treatment	Cognitive Measurement	Cognitive domains Assessed	Assessment points	Results
Chapter 4	N=30. Overnight fasted healthy young volunteers. Mean age 22.87 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 60, 150 and 240 min post dose on day 1 and day 8. Participants consumed a light breakfast; however their last testing session preceded lunch. Between each treatment regime there was a 6 day placebo washout period.	None	Placebo 200 mg (G115) 400 mg (G115)	Immediate word recall Delayed word recall Delayed word recognition Corsi block N Back (0,1,2,3) Random Number generation Bond lader (VAS)	Episodic memory Episodic memory Episodic memory working memory memory Central executive Alert, calm, content	Participants were assessed baseline 60, 150 and 240 min post dose on day 1 and day 8.	No significant difference between pre-dose session on day 8 and baseline on day 1. 7 days of Panax ginseng ingestion had no effect of mood and cognitive performance. There were no superimposed effects of sub-chronic and acute ingestion
Chapter 5	N=18. Overnight fasted healthy volunteers. Mean age 38.31 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 180 min following ginseng ingestion on day 1, day 29 and day 57. Participants consumed a light breakfast; however post dose testing preceded lunch. .	Finger prick blood glucose levels measured pre-dose and 180 min post ginseng ingestion HbA1c measured at pre dose on day 1, day 29 and day 57	Placebo 200mg Korean *Panax ginseng*	Cognitive drug research computerised assessment battery N back (3) Cosi block Brunel scale WHOQOL bref	Episodic memory, verbal and non-verbal working memory, attention, alert, calm, content Verbal working memory Non-verbal working memory Anger, confusion, depression, vigour fatigue, tension,	Participants were assessed pre-dose and 180 min post-dose on day 1, day 29 and day 57	No effect on HbA1c Significantly improved verbal and non-verbal working memory Significantly impaired attention and episodic memory Significantly less content on day 29. Significantly worse ratings of tension, depression and confusion following chronic ingestion, however these mood states improved following that day's acute dose

Table 7.2 Summary of the studies and results following chronic administration of *Panax ginseng*

	Sample	Methods	Physiological Measurement	Treatment	Cognitive Measurement	Cognitive domains Assessed	Assessment points	Results
Chapter 6	N=18. Overnight fasted healthy volunteers. Mean age 38.31 years.	Placebo-controlled, balanced, cross-over design. Participants completed a 30 min computerised assessment battery at baseline and then 180 min following ginseng ingestion on day 1, day 29 and day 57. Participants consumed a light breakfast; however post dose testing preceded lunch. .	Finger prick blood glucose levels measured pre-dose and 180 min post ginseng ingestion HbA1c measured at pre dose on day 1, day 29 and day 57	Placebo 200mg Korean *Panax ginseng*	Cognitive drug research computerised assessment battery N back (3) Cosi block Brunel scale WHOQOL bref	Episodic memory, verbal and non-verbal working memory, attention, alert, calm, content Verbal working memory Non-verbal working memory Anger, confusion, depression, vigour fatigue, tension,	Participants were assessed pre-dose and 180 min post-dose on day 1, day 29 and day 57	No effect on HbA1c Significant improvements in the speed of delayed word recognition (episodic memory) Significant decrements in the accuracy of digit vigilance Significant improvements in the speed of N back (working memory) Significantly improved social relations Significant worsening in calmness