SEROTONIN AND ATTENTION

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1 Preface

1.1 Acknowledgements

This body of research was conducted in order to further investigate the relationships between neurotransmitter function and cognition. It is borne out of a desire to understand the biological bases of cognitive function through pharmacological manipulation, and an interest in integrating cognitive psychological and neuro-pharmacological science. Without the efforts and support of a large number of individuals and organisations, the research outlined in this thesis would not have been possible. I would like to thank all those involved for their time and understanding. The supervision of Profs. Scholey and Wesnes has been invaluable in directing the research programme. Drs. Preskorn, Modi and Schmitt are thanked for their critical support of the serotonin selective reuptake inhibitor and monoamine depletion studies respectively, whilst Pfizer, Alza and Euron (Marie-Curie) are thanked for their respective financial support. I would like to thank Ed Pace-Schott and the teams at Harvard and Princeton Universities for encouraging my contribution to their excellent, National Institute of Mental Health funded, programme of research with crack-cocaine users. Also, Cognitive Drug Research and the University of Northumbria are thanked for their ongoing support of this body of research, and specific contributions to the pilot study of 'ecstasy' users. Finally, this research would not have been possible without the continued encouragement of my friends and family.

1.2 Author's declaration

This work has not been submitted for any other award. The thesis is based on collaborative research with a number of research groups. For all studies the author has contributed to the design of the cognitive assessment aspect of each study and has been involved in statistical analyses of data and production of the written material contained in this thesis. For the studies of assessment validation, monoamine depletion and 'ecstasy', the author was responsible for all aspects of the study, including study design/protocol, funding applications and analysis and interpretation of all data. For other studies, the author's input was on study design, aspects of training, data collection, data processing/scoring, and analysis and interpretation of cognitive data.

Chris Edgar

2 Abstract

The serotonergic system along with other brain neurotransmitter systems has been implicated in the modulation of cognitive function. Dysregulation or pathology in neurotransmitter systems is thought to underlie the cognitive impairments associated with normal ageing, a number of disease states and chronic drug abuse. Research into the influence of serotonergic systems on cognition has focussed on the modulation of other neurotransmitter systems by serotonergic input and the importance of serotonergic receptor subtypes for learning and memory. There is evidence supporting an action of serotonin to inhibit attentional processes, perhaps primarily through inhibition of dopaminergic function, but also via other neurotransmitter systems critical to attentional function such as the noradrenergic and cholinergic systems. Studies indicate that the serotonin selective reuptake inhibitors may impair aspects of attention, whilst acute tryptophan depletion to reduce serotonin synthesis and release, may enhance aspects of attention. These data have resulted in several researchers proposing general theories of serotonergic inhibition, particularly in respect to attention/arousal. However, differential effects may be seen from studies of the various serotonergic receptor subtypes, which have so far been targeted, indicating a general theory may not be sufficient to explain the data.

The evidence presented in this thesis demonstrates that some of the paradigms used thus far to support general theories of serotonergic inhibition of attention/arousal may be flawed. Specifically, monoamine depletion studies may not be able to separate serotonergic and dopaminergic influences on cognition, whilst studies of selective serotonin reuptake inhibitors and chronic ecstasy use have not controlled well for influences of sleep on cognition. Furthermore, evidence from studies of the serotonin receptor subtypes may indicate effects specific to neuropsychological processes underlying measures of attention/arousal or differential effects on aspects of cognition, which may contradict a general theory of inhibition.

In conclusion, general theories of inhibition are still sufficient to account for the majority of data. However, in further academic and clinical research, thorough investigation of cognition will be critical to the development of more detailed theory and the development of effective drug treatments for cognitive disorders. Furthermore, the consideration of confounding factors in research such as the influence of sleep on cognition and the competition between monoamines for transport, is critical to the understanding and interpretation of the scientific literature to date.

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3 The serotonergic system

3.1 Introduction

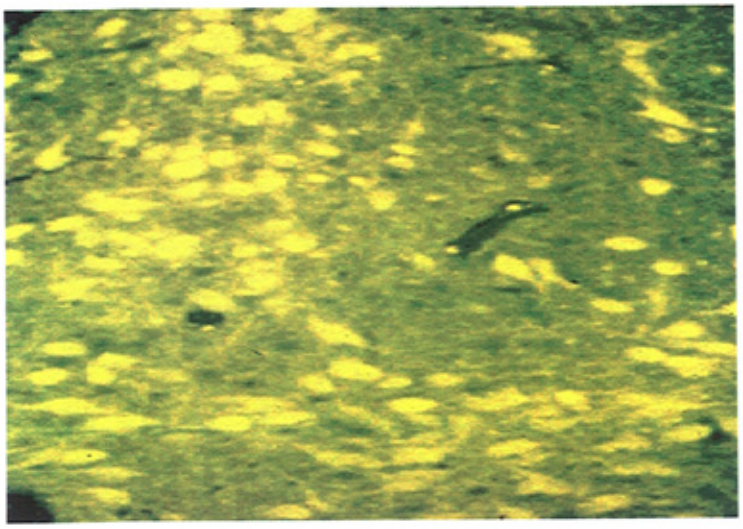
Serotonin [5-hydroxytryptamine (5-HT)] is an indoleamine, which in mammals is found in blood platelets, mast cells, and the enterochromaffin cells of the gut (Figure 1). Serotonin was first identified as the brain substance, which produced peripheral vasoconstriction (Rapport et al., 1948). It was later shown that this substance was identical to an intestinal contractile substance, enteramine (Erspamer and Asero, 1952). The work of the Swedish histochemists Falck and Hillarp (Falck et al., 1962) identified the basic plan of the serotonin pathways in the brain using the fluorescence histochemical technique (Figure 2). The cell bodies, which make serotonin, were found to be located mostly in a series of cell groups in the brainstem collectively known as the raphe nuclei. Each serotonergic neuron gives rise to an axon that branches extensively to contact many hundreds of thousands of neurons (Figure 3). As a result, the thousands of cells that make serotonin contact a sizeable proportion of the billions of neurons that make up the human brain and most areas of the brain receive at least some serotonergic innervation. However, it was not until the late 1970s that understanding of the receptor systems involved in serotonergic function began to grow. This research identified a family of receptors, which have been implicated in a wide range of functional roles, highlighting the clinical potential of drugs, which are able to alter serotonergic function.

The brain serotonin system modulates a diverse range of physiological and behavioural processes, such as appetite, circadian rhythm, cardiovascular regulation, memory and learning, pain sensitivity, respiration, sexual behaviour, the sleep-wake cycle, and thermoregulation. Consequently, 5-HT function has been implicated in a range of neurological and psychiatric disorders including affective disorders, eating disorders, Alzheimer's disease (AD), migraine, aggression, disorders of impulse control, and attention-deficit disorder (for review see Lucki, 1998).

Figure 1: Serotonin molecule

Internet source

Figure 2: Serotonergic neurons of the Dorsal Raphe Nucleus



Fluorescence using Falck-Hillarp method: Internet source

Figure 3: Serotonergic innervation of the rat hippocampus

Orange-brown lines correspond to serotonergic axons stained with the aid of an antibody directed against serotonin: Internet source

3.2 Serotonergic pathways in the CNS

A number of studies have demonstrated that the 5-HT cell bodies located in the rostral raphe nuclear complex of the brainstem central core provide ascending monosynaptic projections to multiple forebrain regions. The serotonergic projections to the hippocampus come mainly from the median raphe, whilst the projections to the striatum are from the dorsal raphe, and both median and dorsal nuclei send projections to the cortex. Innervation of the forebrain by serotonergic fibres is therefore, widespread, including cortex, hippocampus, striatum, amygdala, accumbens, substantia nigra, and hypothalamus. Within these brain regions serotonergic innervation is organised so that identifiable cell groups within the two raphe nuclei send projections to multiple, but functionally related brain regions. Innervation of the hypothalamus, cortex, hippocampus and amygdala has been implicated in processes such as arousal, emotion, and neuroendocrine function, the sleep-wake cycle, and thermoregulation. The SupraChiasmatic Nucleus (SCN) of the hypothalamus regulates the mammalian circadian clock ("day-night cycles"), partially in response to light. Melatonin release is inhibited as a result of the response of the SCN to light. The SCN is densely innervated by serotonergic input from the dorsal raphe nucleus. Serotonin inhibits the response of the SCN (and thus the circadian rhythm) to light, whilst sleep deprivation increases serotonin release in the SCN.

At the axon and terminal level there is also a localised pattern to serotonergic innervation. Density is high in the frontal cortex, and decreases in

more caudal areas. In the cingulate cortex, outer areas of the parietal cortex, and the hippocampus, layers of coarse beaded fibres are found. In other areas of the cortex, fibres are very fine.

A second group of serotonergic neurons (caudal complex) provides major descending projections principally to the spinal cord and brain stem, and has been implicated in the control of motor activity, autonomic functions and nociception (Törk, 1990; Jacobs and Azmatia, 1992). Innervation of the dorsal horn arises predominantly from the raphe magnus and is mainly involved in the modulation of sensory information and nociception. Innervation of the ventral horn arises from the raphe pallidus and obscurus playing an important role in the regulation of motor function. Innervation of the intermediolateral column also comes from these same raphe nuclei and is involved in sympathetic activity.

Therefore, it is clear that the serotonergic system is diverse both in terms of its innervation of brain regions and the localised pattern of innervation. It is investigation of this diversity, which is likely to further understanding of how such a diffuse neurotransmitter arising from two cell bodies is involved in so many brain functions. In addition, research must be careful take account of both forebrain, spinal and peripheral serotonin systems, when investigating the manipulation of the brain serotonin system.

3.3 Synthesis and metabolism of serotonin in the CNS

Levels of serotonin in the CNS represent only around 1-2% of the total amount found in the body. Serotonin cannot cross the blood-brain barrier and is synthesised from the essential amino acid tryptophan (L-tryptophan), by a relatively small number of cells in the raphe nuclei of the brain.

L-tryptophan crosses the blood-brain barrier and neuronal barriers using the same competitive transporter as the other large neutral amino acids. Serotonin synthesis is a 2-step process, the first step of which requires the enzyme tryptophan hydroxylase with oxygen, iron and TetraHydroBiopterin (THB) as co-factors (Koslow and Butler, 1977). The second step requires the enzyme amino acid decarboxylase, which removes a carboxyl group (-COOH) from 5-HTP to produce 5-hydroxytryptamine (5-HT, serotonin). Serotonin concentration in the brain is far more sensitive to the effects of diet than any other monoamine neurotransmitter, and can be increased up to 10-fold by dietary supplementation in laboratory animals. Hydroxylation by tryptophan hydroxylase is the rate-limiting step in the synthesis of serotonin, as the enzyme is not normally saturated. Therefore, an increase in brain tryptophan will enhance serotonin synthesis and vice versa. Reuptake to the surrounding neurons and glial cells from the synaptic cleft by a high affinity transporter, is the principal means by which released serotonin is inactivated. Enzymatic breakdown and metabolism of serotonin occurs in neuronal

and glial mitochondria by monoamine oxidase (MAO), especially MAO-A (Cooper et al., 1996).

Serotonin release takes place via a Ca^{++} dependent process from the serotonin stored in the vescicles. Following its release serotonin is inactivated mainly via re-uptake into the nerve terminals using Na^{+}/K^{+} ATPase dependent carrier. Once re-uptake has taken place serotonin is either stored or metabolised by MAO an enzyme widely distributed throughout the body and which also deaminates dopamine, noradrenaline, adrenaline, tyramine, and tryptamine.

The highest concentration of serotonin is in the pineal body. However, this gland does not use serotonin as a neurotransmitter. Instead, here serotonin is primarily used for synthesis of melatonin. Melatonin is synthesized from serotonin in a 2-step process that takes an acetyl group from acetyl-CoA and a methyl group from SAM (S-AdenosylMethionine). Melatonin is of particular importance for regulating diurnal (circadian), seasonal behaviour, and physiology in mammals.

3.4 Sites of drug action

Within the processes of serotonin synthesis and metabolism there are several steps at which drugs may increase or decrease the neuronal function of serotonin.

Alterations in the availability of tryptophan can be achieved via tryptophan administration ('tryptophan loading') to increase serotonin synthesis, and via low tryptophan diet ('tryptophan challenge') to reduce synthesis. These manipulations are often used experimentally in an attempt to influence serotonin release and either increase or decrease neuronal function of serotonin (Yuwiler et al., 1981).

Inhibition of tryptophan hydroxylase decreases serotonin synthesis (e.g. parachlorophenylalanine). As noted earlier, tryptophan hydroxylase is the rate-limiting step in serotonin synthesis. Therefore, enzyme inhibition produces profound decreases in brain serotonin (Hyttel, 1977).

Certain drugs including riserpine and tetrabenazine prevent intraneuronal storage of serotonin (e.g. Boulton and Juorio, 1977). This exposes serotonin to MAO metabolism and thus reduces serotonin levels. However, in the case of riserpine the effects are not specific to serotonin, affecting intraneuronal amine storage universally.

Several drugs increase serotonin release (e.g. (±)3,4-methylenedioxymethamphetamine (MDMA; 'Ecstasy'), fenfluramine). However, these drugs have differing acute and long-term effects, which impact on there utility as tools for investigation of serotonergic function and this will be discussed more fully when discussing more specifically the effects of serotonergic manipulations on attention.

The monoamine oxidase inhibitors (e.g. tranylcypromine and parygline) reduce metabolism of serotonin resulting in an increase in extracellular serotonin. However, other amines (norepinephrine (NE/NA), and dopamine (DA)) are also

similarly influenced by monoamine oxidase inhibition. Whilst relatively greater 5-HT effect is achieved by the MAO-A specific compound moclobemide, actions on NE and DA are still present and furthermore, safety issues due to potential cardiovascular toxicity resulting from effects on tyramine are a concern in human research (Bloom and Kupfer, 1995).

A number of drugs inhibit the neuronal re-uptake of serotonin. The tricyclic antidepressants (e.g. amitriptyline and imipramine) inhibit both norepinephrine and serotonin re-uptake by pre-synaptic terminals. There are also a number of serotonin specific re-uptake inhibitors (SSRIs), widely used clinically for a range of disorders. The relatively safe side effect profile of the available drugs and differences in specificity, again make them potentially useful tools for investigating relationships between 5-HT function and cognition.

Much of the work in classifying 5-HT receptors has been based on ligand binding studies and increasing numbers of receptor subtype specific ligands have been identified, including agonists, antagonists and partial agonists.

3.5 Serotonin receptors

There has been a rapid increase in the understanding of the receptors associated with serotonergic function both in the periphery and the CNS. At least 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes have been shown to mediate the effects of serotonin (Barnes and Sharp, 1999). For some of these receptor subtypes the neurochemical response to receptor activation, both 5-HT and other neurochemical systems, has been characterised. Two types of 5-HT receptors have been determined by molecular cloning: G-protein-coupled 5-HT receptors and ligand-gated ion channels. Both types of 5-HT receptors fall into distinct supergene families of receptors which are related to each other and which are defined by their structure and function.

5-HT_3 receptors are members of the ion-gated family of receptors. The model protein for this family is the nicotinic acetylcholine receptor, and this family includes cation channels (the nAChR, 5-HT_3 receptor) and anion channels (GABAa receptor, glutamate receptor). This family of receptors is involved in fast chemical synaptic transmission.

All other known 5-HT receptors except for 5-HT_3 are members of the G-protein-coupled (GPR) family of signal transducing receptors. This family of receptors can in addition to fast chemical synaptic transmission, have slower, longer-lasting and more diverse post-synaptic actions. This involves the neurotransmitter molecules binding to receptor proteins, which activate small proteins called G-proteins. These are free to move across the intracellular face of the postsynaptic membrane and activate effector proteins, including second messengers, which influence the nature of the postsynaptic action (Bear et al, 2001).

Table 1: 5-HT receptor subtypes

Receptor sub-type (previous nomenclature)	Neurochemical response to receptor activation	Agonists	Antagonists
5-HT_{1A}	5-HT release (-) Noradrenaline release (+) Acetylcholine release (-) Glutamate release (-)	8-OH-DPAT, busiprone, flesinoxan	WAY100135, WAY100635, SRA-333
5-HT_{1B} (5-$HT_{1D\beta}$ 5-$HT_{1B\ rat}$ 5-$HT_{1d\ human}$)	5-HT release (-) Acetylcholine release (-)	sumatriptan	SB-216641, SB-224289
5-HT_{1D} (5-$HT_{1D\alpha}$)	Not known	L694247, zolmitriptan	GR127935, GR125743, BRL 15572
5-HT_{1E}	Not known		
5-HT_{1F}	Not known	LY344864, LY334370	
5-HT_{2A} (5-HT_2)	Noradrenaline release (-)	DOI	MDL100907, ketanserin, ritanserin
5-HT_{2B} (5-HT_F)	Not known	mCPP, DOI, TFMPP, quipazine	RS127445, SB-206553, SB-204741
5-HT_{2C} (5-HT_{1C})	Noradrenaline release (-) Dopamine release (-)	mCPP, DOI, TFMPP,	SER082, mesugerline, SB-200646
5-HT_3	5-HT release (+) Acetylcholine release (-) GABA release (+) CCK release (+) Dopamine release (+)	m-cholor-phenyl-biguanide, 2-methyl-5-HT	granisetron, ondansetron, tropisetron
5-HT_4	5-HT release (+) Acetylcholine release (+) Dopamine release (+)	BIMU1, BIMU8	GR113808, SB-204070
5-HT_{5A}	Not known		
5-HT_{5B}	Not known		
5-HT_6	Acetylcholine release (-) Glutamate release (-)	2-methyl-5-HT	SB271046, Ro04-6790
5-HT_7	Not known		SB269970

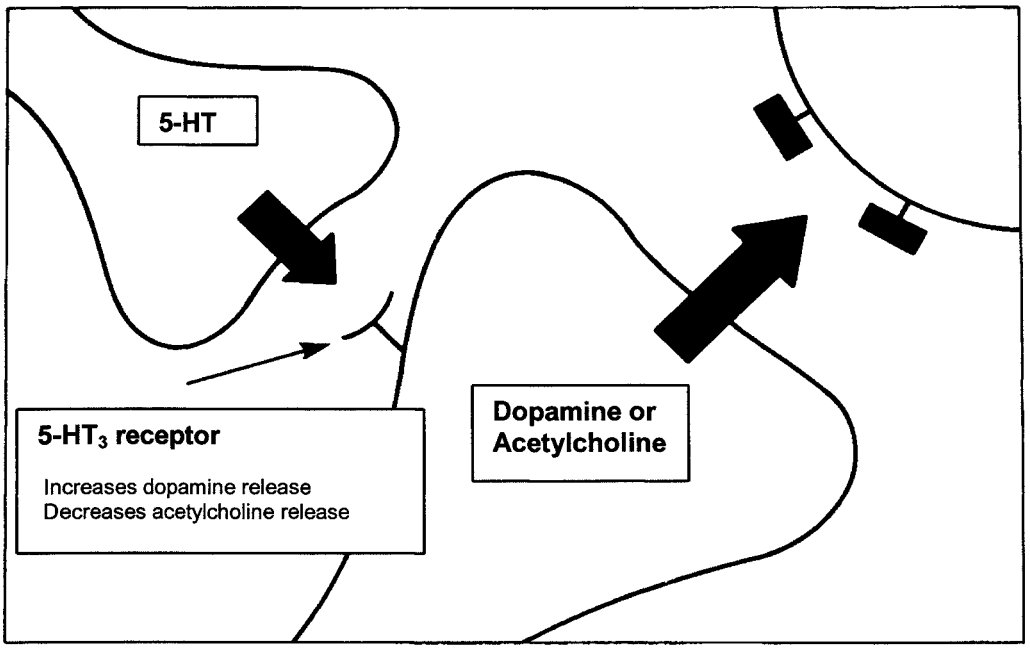
After Barnes and Sharp, 1999

3.6 Serotonergic regulation of other neurotransmitters

5-HT release is closely linked with behavioural arousal and/or activation. Both the discharge activity of 5-HT neurons and the subsequent release of 5-HT are dependent on behavioural state. Extracellular 5-HT levels in the hippocampus are lowest during sleep/rest and highest during the alert waking stage (Reuter et al., 1997). However, it is not the case that increased 5-HT function enhances arousal/activation. In fact, it is thought that 5-HT inhibits behavioural activation. Jacobs and Fornal (1999) propose that the primary function of serotonin is to facilitate motor output, whilst simultaneously suppressing sensory information processing to inhibit input which might disrupt motor behaviour. In contrast, when attending to sensory stimuli, 5-HT can be suppressed, sharpening sensory function and disfacilitating motor output.

Serotonin has been shown to be involved in the presynaptic regulation of the release of other neurotransmitters. For example 5-HT_3 receptors inhibit the release of acetylcholine in the cortex but increase the release of dopamine in the striatal and mesolimbic systems (e.g. Ugedo et al., 1989). These types of effects may be critical in understanding the behavioural effects of serotonergic drugs. For example the improved cognitive function and anti-psychotic action of 5-HT_3 antagonists (odansetron, granisetron) in animal models. The neurochemical response to receptor activation has been characterised in part for several of the receptor subtypes (Table 1). This is critical to the understanding of the behavioural effects of receptor activation and blockade, and detailed examples will be outlined in later sections.

Figure 4: Possible presynaptic 5-HT_3 modulation of neurotransmitter release



Redrawn from Feigner and Boyer, 1991

4 Cognition

4.1 Introduction

Cognitive neuropsychopharmacology attempts to investigate the neurochemical bases of human and animal cognition through the use various experimental techniques, including drug administration. Identification and classification of the neurons in the brain, in parallel with advances in the understanding of the processes, which underpin everyday behaviour, has led to a multidisciplinary approach. This approach combines the use of drugs as experimental probes with the use of cognitive behavioural techniques to study influences on cognition to further elucidate the relationships between neurotransmitters and behaviour. This has led to an understanding of the importance of particular neurotransmitter systems in various aspects of cognition including learning and memory, attention, and executive processes. As a more detailed understanding of these relationships begins to emerge, more and more targeted approaches to the treatment of cognitive dysfunction in various disease states can be taken, and also a more detailed understanding of the nature and source of adverse cognitive side-effects of drug treatments is constructed.

4.2 Neurotransmitter systems and attention

A unitary concept of arousal has been proposed by a number of researchers in the fields of psychology and behavioural neuroscience. For example Yerkes and Dodson (1908) commented on the relationship between arousal and processes such as drive and motivation. However, more recent theories have looked at more complex concepts, without this unitary nature. Allport (1992) argues for a fractionation of attentional processes based on brain chemical differentiation, such that there is no single locus of attention/arousal, and no single mechanism or computational resource. Rather, there are diverse neuropsychological control mechanisms. This latter type of concept appears to find much greater empirical support. Robbins (1997) in a detailed review of arousal systems and attentional processes focuses on research into the influences of the ascending monoaminergic (noradrenaline (NA), dopamine (DA), and serotonin (5-HT)) and cholinergic (acetylcholine (ACh)) pathways. It is concluded that the coeruleo-cortical NA system appears to have a role in maintenance of alertness to salient external stimuli; the mesolimbic and mesostriatal DA systems in activation of output (both cognitive and motor); and the cholinergic systems in enhancing stimulus processing at the cortical level (attentional selection, discrimination learning and spatial working memory). In contrast the 5-HT systems may serve to dampen the action of each of these other systems by promoting behavioural inhibition and cortical de-arousal. This allows for

the existence of several different attentional processes and several different systems modulating attention, at the neurotransmitter level. The importance of each of these neurotransmitter systems, excluding 5-HT, in attentional function has been well characterised. It is known that DA is of major importance in the modulation of attentional functions (for a review see Nieoullon, 2002), whilst both DA and NA have important influences on the working memory and attentional functions of the prefrontal cortex (Arnsten, 1997). Sarter et al., (2005) contend that the integrity of the cortical cholinergic system is necessary for attentional performance, and activity of cortical cholinergic inputs is selectively enhanced during attentional performance, and further that the system generally acts to optimise the processing of signals in attention-demanding contexts. However, research into the effects of 5-HT on attention has been less prominent.

4.3 Serotonin and cognition

The late 1970s and early 1980s, research into the relationship between serotonergic function and cognition was initially focussed on learning and memory, and was conducted in parallel with research into cholinergic involvement in these aspects of cognition (see next section). In the 1990s, a role for the serotonergic system in attention-deficit / hyperactivity disorder (ADHD) emerged. For example Comings (1993) found that blood serotonin and tryptophan levels were low in ADHD compared to healthy controls; whilst 5-HT function was shown to be associated with aggression / conduct disorder (Halperin et al., 1997) and impulsivity. However, these studies were more focussed on mood and other behaviours e.g. aggression and conduct disorder, rather than cognition or specifically attention. In addition during the 1980s, a role in the aetiology of schizophrenia was identified and here its importance was related to inhibition of the dopaminergic system. For example Ugedo et al. (1989) found a stimulatory effect of ritanserin (a 5-HT_2 antagonist) on midbrain dopaminergic systems. Though again, no role in cognitive deficits associated with the disease was studied. It had though become apparent that the serotonergic system was important in terms of cognitive function, specifically learning and memory; was involved in the aetiology of certain disease states associated with cognition dysfunction; and had an important modulatory effect of other neurotransmitters important in cognition. This led to a number of general theories of a modulatory role for serotonin in the regulation of other neurotransmitter systems, with researchers such as Jacobs, Tork and Azmitia proposing different but closely related inhibitory theories (see chapter 3 above). Furthermore, serotonergic dysfunction through normal ageing had been identified as well as more severe dysfunction in Alzheimer's disease, and this was linked to cognitive dysfunction in the disorder with respect to memory (e.g. Porter et al., 2003).

In 1997, Robbins et al. brought together a number of studies to address the roles certain neurotransmitter systems play in modulating particular

neuropsychological processes critical to attentional task performance under the title "arousal systems and attentional processes". Serotonin was identified as acting to inhibit other neurotransmitter systems involved in attention/cognition namely DA, NA, and ACh. Thus a role for serotonin in attention was established, which was mediated through its inhibitory influence on other key neurotransmitter systems.

4.3.1 Serotonin and Memory

In the late 1970s early 1980s a role for serotonin in memory consolidation was identified (e.g. Archer et al., 1981). P-chloroamphetamine (a 5-HT releasing compound) was shown to have a counter intuitive retrograde amnestic effect in rats. However, this was prevented by the serotonin reuptake inhibitor zimelidine. Furthermore, Weingartner et al. (1983) found that zimelidine reversed ethanol induced impairment of memory. Whilst Altman & Normile (1986) found that the serotonergic antagonists pirenperone, ketanserin, mianserin, methysergide and metergoline produced an increase in the latency to retrieval of previously learned drinking behaviour in mice. By the end of the 1990s, the general conclusion was that the serotonin system played an important role in processes underlying learning and memory and reviews of this body of work were conducted. In a review of the 5-HT system and cognition, Meneses (1999) outlined evidence from several areas in support of a role for 5-HT in learning and memory. In terms of brain distribution, the 5-HT pathways, reuptake site/transporter complex and receptors show regional distribution in brain areas implicated in learning and memory. In addition, the stimulation or blockade of presynaptic 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/2C}$ and 5-HT_3 receptors; postsynaptic 5-$HT_{2B/2C}$ and 5-HT_4 receptors; and 5-HT uptake/transporter sites modulate learning and memory. This evidence strongly suggested that the 5-HT system is important in normal learning and memory function, and the treatment and / or pathogenesis of cognitive disorders. Meneses suggested that selective agonists, neutral antagonists, agonists or inverse agonist properties for the various 5-HT receptor subtypes could constitute a new therapeutic opportunity for modulating learning and memory. Terry (2004) in a review of cognition enhancing drugs that target serotonergic receptors concludes that there is a clear role for serotonin in memory function; with the possibility that 5-HT selective ligands at receptor subtypes could serve as therapeutic agents to enhance cognition. It is notable that both reviews focus almost exclusively on learning and memory with no description of effects on attentional measures. However, it was notable that in the Terry review, whilst the contribution of 5-HT_2 receptors in the prefrontal cortex to attention and working memory is noted, no studies of attention were outlined, whilst in the Meneses review the text focused solely on learning and memory, suggesting a paucity of research in to attention.

4.4 Serotonin and attention

Studies which provide insight into the importance of the serotonergic system for attentional function come from a number of sources, with the most common in the literature being lesioning studies, tryptophan depletion, selective serotonin reuptake inhibition, receptor sub-type specific ligands and studies of clinical populations with altered serotonergic function. However, attention has not usually been a specific target of the investigations, with most studies investigating attention as part of wider investigation of cognition generally, or learning and memory specifically. The most notable exceptions to this are those studies by Schmitt and colleagues at Maastricht University considering the effects of acute tryptophan depletion and subchronic SSRI administration on focussed and vigilant attentional task performance. However, no systematic review of data or its import as related to attentional function has been conducted.

4.4.1 Tryptophan depletion

Reviewing sixteen published studies reporting data on attentional tasks over the period 1994 to 2005, the majority of studies (12) reported no effects of tryptophan depletion on attention. However, in four studies possible effects on attentional measures were seen. These studies report that tryptophan depletion may improve some aspects of attention including simple motor speed / attention and 'higher' attentional function measures such as Trails A and the Stroop test (Booij et al., 2005; Gallagher et al., 2003; Schmitt et al., 2000). These studies do not report consistent effects, with Gallagher et al. (2003) showing no effects on the Stroop test, whilst effects were seen in the other three studies. Therefore, it is not clear if these effects are a reliable and consistent effect of tryptophan depletion, or have occurred as chance findings in studies using several measures of cognition. Discussion of these data does suggest possible sources for this inconsistency, with Riedel and Schmitt arguing that enhancement of some aspects of attention would be predicted following tryptophan depletion given the influence of serotonin in inhibiting the activity of other neurotransmitters involved in attention such as NA, DA and ACh. Possible sources of inconsistent findings may include the subject population, the success of the depletion, and the measures employed. Population may influence the effects seen in several ways. Most focus has been given to patient populations, which may already have some underlying serotonergic dysfunction, particularly depressed patients and those with vulnerability to mood disorders, but also the sex of subjects may be relevant. Existing serotonergic dysfunction might potentially exacerbate the effects of tryptophan depletion (e.g. Porter et al., 2003). Also, studies have reported sex differences in serotonin metabolism, which might also influence findings in studies of tryptophan depletion, with lower rates of synthesis in females resulting in greater susceptibility to effects of tryptophan depletion and higher incidence of affective disorders (Nishizawa et al., 1997).

4.4.2 SSRIs / SNRIs etc

Selective serotonin reuptake inhibitors (SSRIs) and selective serotonin / norepinephrine reuptake inhibitors may influence cognition both acutely and following sub-chronic and / or chronic dosing. It is commonly reported that the SSRIs become effective in treating mood disorders after around two weeks of dosing and it is thought that the mechanism for this is down regulation of the post-synaptic 5-HT_{1A} receptors. Le Poul et al. (1995) found the potency of the 5-HT_{1A} agonist 8-OH-DPAT to be reduced flowing >3 days treatment with paroxetine and fluoxetine, concluding that 5-HT_{1A} autoreceptor desensitisation facilitates serotonergic neurotransmission, which contributes to antidepressant activity. In this respect the effects are comparable to those of the anti-depressant / anxiolytic 5-HT1A agonists e.g. buspirone, which are discussed in the following chapter. There is some evidence that the SSRIs and SNRIs may impair vigilant attention with sub-chronic / chronic dosing. However, different compounds have been seen to have unique effects.

The SSRI fluoxetine has been shown to impair vigilant attention with sub-chronic dosing (Ramaekers et al., 1995), whilst venlafaxine, a 5-HT reuptake inhibitor, which also inhibits reuptake of NA (an SNRI), has also been shown to impair vigilance performance. It is proposed that the inhibitory action of 5-HT on neurotransmitters modulating attention/arousal (DA, ACh, NA) is the mechanism behind this reduction in vigilance (O'Hanlon et al., 1998). Furthermore, sertraline, in contrast to citalopram, fluoxetine, paroxetine and venlafaxine, does not impair vigilance performance, which has been suggested as further evidence for sertraline exerting an additional, mild, central dopaminergic agonism (Schmitt et al., 2002).

It is known that dopamine is of major importance in the modulation of attentional functions (Nieoullon, 2002). Research has supported theories that serotonin may mediate the dopaminergic system and that this may be a key system through which serotonin influences attention (Jacobs and Fornal 1999). Reduction of central serotonergic activity by means of acute tryptophan depletion (ATD) has been associated with improved focussed attention, as measured by the Stroop Test (Booij et al. 2003; Schmitt et al. 2000). This is again consistent with an inhibitory action of 5-HT on neurotransmitters modulating attention/arousal (DA, ACh, NA).

Therefore, the evidence indicates that compounds acting to inhibit reuptake of serotonin may impair vigilant attention with sub-chronic dosing, possibly through the same mechanism of action as that which is efficacious in treating mood disorders i.e. down regulation of the post-synaptic 5-HT_{1A} receptor.

4.4.3 Receptor subtype specific compounds

A number of serotonin receptor sub-type specific compounds have been studied for their effects on cognition in both animal and human subjects. As discussed previously in the section on SSRIs these compounds include 5-HT_{1A} agonists, particularly the anxiolytic buspirone, but also compounds such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), ipsapirone and psilocybin. Buspirone has shown sedative / attention impairing effects in some studies (e.g. Bond and Lader, 1981; Holland et al., 1994), using 20 and 30 mg doses respectively, whilst studies with the more typical therapeutic dose level of 15 mg have not shown impaired cognition (e.g. Hart et al., 1991). Carter et al. (2005) studied the effects of the 5-$HT_{1A/2A}$ agonist psilocybin with and without the 5-HT_{2A} antagonist ketanserin for effects on a multiple object tracking task in healthy subjects. The data provided support for the theory that attentional effects are primarily mediated by the 5-HT_{1A} receptor, with pre-treatment with ketanserin failing to attenuate the attentional impairment produced by psilocybin. These data have indicated that 5-HT_{1A} antagonism is a possible target for therapeutic enhancement of cognition, and Schechter et al. (2005) have demonstrated that the 5-HT_{1A} antagonist lecozotan stimulates acetylcholine and glutamate release in rats and enhances learning and memory. The compound is now being developed by Wyeth research as a cognition enhancer for the treatment of AD. Whilst animal models of attention were not studied in the preclinical development of lecozotan, Nakamura et al. (2000) studied two-level choice reaction time task performance in rats using the 5-HT_{1A} agonist 8-OH-DPAT, the 5-$HT_{2A/2C}$ agonist DOI, the 5-HT_{1A} antagonist (-)-Alprenolol and the 5-HT_{2A} antagonist ritanserin. The data indicated both 5-HT_{1A} and $_{2A/2C}$ agonism are capable of impairing attention/vigilance in the rat, with antagonism of the respective receptors attenuating these effects. In contrast, flesinoxan a 5-HT_{1A} agonist has been shown to enhance cognition (attention and memory) in the elderly (van Harten et al., 1996), using the CDR system. However, given that this single study was in conflict with the majority of evidence, and the low group size and lack of dose dependency, it is likely this was a chance finding and should not be seen as clear contradictory evidence.

More recently studied serotonergic receptor subtypes have also been implicated in the regulation of cognition. For example 5-HT_6 receptor antagonists have shown effects in animal models of cognition (King et al., 2004), which may be through regulation of glutamatergic and cholinergic neuronal activity (Woolley et al., 2004). Furthermore, benefits to a model of attentional set-shifting have also been seen in the rat following treatment with a 5-HT_6 antagonist (Hatcher et al., 2005). However, these effects have yet to be replicated in human studies of cognition.

All these studies give support to the theory that serotonin acts to inhibit other neurotransmitter systems, which may then impair attentional performance; serotonergic agonists typically producing impaired attentional task performance, which may then be mediated by serotonergic antagonists. However, there may be

differences between the receptor subtypes, for example there is good support for an influence of the 5-HT_{1A} receptor, but data suggesting that 5-HT_{2A} receptor may not mediate attentional performance. Furthermore, antagonism itself has not been shown to directly enhance attentional task performance, only to mediate agonist effects.

4.4.4 MDMA

Acute administration of (+/-)-MDMA (3, 4-methylenedioxymethamphetamine), whilst known to enhance the release serotonin, has also been shown to have a wide range of additional pharmacological effects. Fischer et al. (2000) report that in addition to studies showing enhanced release of serotonin and dopamine, and interactions with a range of different receptors including 5-HT_2, M1 and M2 muscarinic acetylcholine (ACh), and histamine H1 receptors, perfusion with MDMA resulted in increased spontaneous ACh release, suggesting an even broader pharmacological action, which remains to be fully characterised. Each of these receptor targets may influence attention / attentional task performance with cholinergic function having direct influences on attention and histamine involved in alertness / arousal. Therefore, as a serotonergic antagonist, MDMA is not specific enough to make studies of acute effects on attention useful in investigating the relationships between the two. However, chronic effects may be more serotonin specific.

Chronic effects of MDMA on cognition have been investigated both in animal species and human recreational users. Parrott (2000) provides a summary of cognitive deficits, identified in drug free MDMA users, divided into three categories: memory, higher executive ability, impulsiveness and other cognitive functions. Whilst memory has been found to be impaired on a range of tasks, and there is consistent evidence for MDMA users to be more impulsive, studies assessing executive function have been less consistent. In respect of more basic information processing tasks of an attentional nature, little evidence for impairments has been noted. Therefore, the weight of evidence suggests that chronic effects of recreational use of MDMA, does not have a clear effect on attentional function in humans.

An area which has received less attention is the 'sub-acute' period of MDMA use following the acute period, whilst the 'hangover' effects of the drug are still evident – most notably the short-term depletion of levels of serotonin in the vescicle. There is a well documented sub-acute affective consequence of MDMA use the so called "mid-week blues". However, this has not been well characterised in respect of cognition. One study by Parrott and Lasky (1998) measured cognitive task performance at 2 and 7 days post recreational use, but did not report impairment. However, Huxter et al. (2006) reported subjective cognitive impairment

in the sub-acute phase (9-day period), but this became non-significant after controlling for alcohol use and sleep effects.

Therefore, both sub-acute and chronic effects of MDMA in recreational users may provide evidence to help investigate the relationship between serotonin and cognition, through temporary depletion of serotonin levels in the vescicles and possible serotonergic neurotoxicity respectively.

4.5 Conclusions

It is clear from the literature that there is evidence for an influence of serotonergic manipulations on attention in both animal and human studies. Limited support has been seen for an effect of tryptophan depletion on attention, and it may be that more tightly controlled methodology is required to produce consistent effects. There is stronger evidence for an effect of SSRIs and serotonergic agonists and antagonists, which points in particular to a role for certain receptor subtypes including the 5-HT_{1A} receptor. However, research is at a early stage for many of the receptor sub-types and it is clear that attention has not typically been a key focus of the studies conducted thus far. Therefore, there is a need to investigate serotonergic influences on attention in order both to confirm and extend previous findings.

5 Thesis

5.1 Purpose of thesis

The purpose of the present thesis is to further investigate the relationship between serotonergic function and aspects of cognition, primarily attention, in humans. The serotonergic system is diverse, with wide ranging influences on behaviour, and complex functional interactions with other neurotransmitter systems. Whilst there is clear evidence for a role for serotonergic function in learning and memory, influences on human attention have not been researched as extensively as other aspects of human cognition. However, the modulatory influence of the serotonergic system on other neurotransmitter systems directly involved in attention suggests a role for serotonin in human attention. Given the current interest in serotonergic drugs as potential cognition enhancers and the importance of attentional dysfunction in several disease states, it is important to investigate the nature of serotonergic influences on attention, in addition to learning and memory. Therefore, the present thesis will attempt to investigate the possible effects of serotonergic manipulations on attentional performance and cognition, and also to evaluate the importance of serotonin as a modulator of human attentional function.

5.2 Planned studies and hypotheses

In attempting to investigate the relationships between 5-HT function and attention in humans, particular paradigms provide more methodologically sound and more practical opportunities.

In the present thesis, four experimental paradigms have been used to influence 5-HT function and thus investigate the relationship between serotonin and attention. These are acute tryptophan depletion (ATD), 5-HT reuptake inhibition by use of SSRIs, a selective 5-HT_6 receptor antagonist, and potential serotonergic neurotoxicity in recreational MDMA users. One mechanism via which serotonin may influence attention/arousal is via an inhibitory influence on the dopaminergic system. Therefore, to investigate this inhibitory mechanism the effects of serotonergic manipulations have been compared and contrasted with similar dopaminergic manipulations. Initially, the sensitivity of the test battery was validated using studies of amphetamine a DA reuptake inhibitor. In the study of monoamine depletion, acute tryptophan depletion to reduce serotonin synthesis was compared to both acute tyrosine / phenylalanine depletion (ATyrD) to reduce DA and combined depletion. In the sub-chronic dosing SSRI study, a highly selective SSRI was compared with an SSRI with additional DA reuptake inhibition. The pilot study of recreational MDMA use was informed by a similar study

investigating effects of crack cocaine use in order to look at the possibility of comparing possible serotonergic neurotoxicity in primary MDMA users with possible dopaminergic neurotoxicity in primary cocaine users. The acute SSRI, alcohol interaction study allowed for study of possible acute effects on attention and their implications for safety.

The techniques of monoamine depletion to reduce neurotransmitter synthesis, and monoamine loading to increase synthesis, have received much interest recently as ways of safely investigating the relationships between levels of brain neurotransmitters (in particular 5-HT and DA) and various cognitive and affective parameters. ATD temporarily reduces the synthesis of 5-HT by reducing the availability of its precursor tryptophan (TRP). ATyrD reduces the synthesis of DA by reducing the availability of its precursors tyrosine (TYR) and phenylalanine (PHE). There is strong evidence to suggest that the manipulations do reduce 5-HT (Young and Leyton 2002) and DA (McTavish, Cowen et al. 1999; Harmer, McTavish et al. 2001) activity in each case. This makes acute monoamine depletion a safe, well-tolerated and practical technique for investigating the influences of 5-HT function in human subjects.

SSRIs are the most widely used antidepressants. The indications in which these compounds are used have rapidly expanded to the treatment of anxiety, phobias, panic, obsessive-compulsive disorder, eating disorders, premenstrual dysphoria, and premature ejaculation. The principal mode of action is inhibition of the reuptake of 5-HT into the presynaptic neuron, extending the time for which 5-HT affects post-synaptic receptors and therefore stimulating 5-HT neurotransmission throughout the brain (Stahl, 1998). The effects outlined above, the lack of extra pyramidal effects, and differences between the SSRIs in specificity and modes of action, again make this a useful technique for investigating the effects of 5-HT function in human subjects. The SSRIs are widely used clinically for a range of disorders and the relatively safe side effect profile of the available drugs and differences in specificity again make them potentially useful tools for investigating relationships between 5-HT function and cognition.

Animal research demonstrates that 5-HT_6 receptor function appears to involve the regulation or suppression of several neurotransmitter systems important to cognitive function (King et al., 2003), including central cholinergic (ACh) neurotransmission (Shirazi-Southall et al., 2002), and glutamate release (Dawson et al., 2001). The compound studied (a 5-HT_6 receptor antagonist) has shown efficacy in various animal models of Alzheimer's disease and was well tolerated in animals. Moreover, the animal data indicated a large margin between the doses of a 5-HT_6 receptor antagonist required to produce therapeutic effects and those inducing side effects (unpublished). Therefore, this compound may be efficacious in attenuating the cognitive impairment produced by ACh blockade in the scopolamine challenge model of cognitive impairment. There are a number of receptor subtype specific

drugs currently under investigation in a range of therapeutic areas including impaired cognition.

A number of studies in animals demonstrate that MDMA produces toxic effects on 5-HT neurons (e.g. Stone et al., 1986 and 1987), which may be directly relevant to long-term behavioural effects in human recreational users. Whilst there is little evidence for impairments on more basic information processing tasks of an attentional nature in MDMA users (Parrott, 2000), effects on these tasks are evident in chronic cocaine users (for a review of attentional findings see Horner, 1999). Therefore, studies of both drug-using groups may provide insights into the relative profiles of impairment, and theories on the aetiology of these impairments. MDMA (ecstasy) has received much research interest, with studies into both acute effects of the drug in clinical trials and effects of longer-term recreational use. Whilst studies of acute MDMA effects are difficult and expensive, recreational users provide a potentially large population to study effects of 5-HT release / and putative neurotoxicity.

Study 1 (Chapter 6): Vigilance task validation: A computerised Mackworth Clock task and a brief vigilance task

Aims:

- To validate a new computerised implementation of the Mackworth Clock task and an existing brief vigilance task, new Mackworth Clock task data were compared to Digit Vigilance task data, and intra-task performance on both 'vigilance' tasks was compared

Hypotheses:

- Mackworth Clock task data would be similar to data collected using other versions of the task
- Both Mackworth Clock task data and Digit Vigilance Task data would display a 'vigilance decrement' over the task duration

Study 2 (Chapter 7): Sensitivity of the attentional task battery of the cognitive drug research computerised assessment system to the effects of an amphetamine analogue

Aims:

- To investigate the sensitivity of the attentional task battery of the cognitive drug research computerised assessment system to the effects of enhanced DA function using an amphetamine analogue

Hypotheses:

- The attentional task battery of the cognitive drug research computerised assessment system would show improvements in performance from enhanced DA function

Study 3 (Chapter 8): Identification of the functional interactions between serotonin and dopamine on human attention

Aims:

- To investigate the effects of acute tryptophan depletion (ATD), acute tyrosine/phenylalanine depletion (ATyrD) and combined depletion ATD+ATyrD, in comparison to a balanced control condition

Hypotheses:

- An acute reduction of serotonergic neurotransmission by acute tryptophan (TRP) depletion would improve selective attention and sustained attention
- An acute reduction of dopamine neurotransmission by acute tyrosine (TYR) and phenylalanine (PHE) depletion would impair selective attention and sustained attention
- The performance effects of reduced serotonergic neurotransmission would be attenuated by concomitant lowering of dopamine neurotransmission
- No manipulation would affect mood or alertness

Study 4 (Chapter 9): A Comparison of the Effect of Escitalopram versus Sertraline on Focussed and Vigilant Attention in Healthy Young Subjects

Aims:

- To assess whether s-citalopram has an adverse effect on focused and vigilant attention performance, in comparison to the cognitive profile seen with sertraline, to further support the theory that effects of the SSRIs on vigilance are primarily mediated by 5-HT inhibition of DA

Hypotheses:

- S-citalopram in comparison to sertraline would show a poorer comparative profile of effects on vigilant attention

Study 5 (Chapter 10): Dapoxetine has no Cognitive Interactions with Ethanol in Healthy Male Volunteers

Aims:

- To assess whether dapoxetine, a novel SSRI for the treatment of premature ejaculation, has an adverse effect on focused and vigilant attention, and cognition in combination with alcohol (ethanol)

Hypotheses:

- Dapoxetine will be cognitively safe and well tolerated when dosed acutely, both with and without concomitant administration of ethanol

Study 6 (Chapter 11): Effectiveness of a 5-HT_6 Receptor Antagonist in Reversing Scopolamine-Induced Cognitive Impairment

Aims:

- Validation of the experimental paradigm by studying the ability of physostigmine (a prototypical anti-cholinesterase) in attenuating scopolamine induced impairment. Studying the effectiveness of a 5-HT_6 receptor antagonist in reversing scopolamine-induced cognitive impairment

Hypotheses:

- A 5-HT_6 receptor antagonist would be able to attenuate/reverse scopolamine-induced cognitive impairment via modulatory actions on cholinergic neurotransmission and glutamate release

Study 7 (Chapters 12 and 13): Cognitive performance in humans during a smoked cocaine binge-abstinence cycle AND Sleep and cognition during recreational 'ecstasy' use: An acute use pilot study of cognitive function assessment, and actigraphy, temperature and saliva analyses.

Aims:

- To investigate the effects of smoked crack cocaine on cognition and sleep over a binge abstinence cycle, and to pilot an experimental paradigm to assess cognition and sleep across a single (binge) instance of recreational ecstasy use

Hypotheses:

- Use of MDMA will produce a specific profile of cognitive impairment distinct from that seen in crack cocaine users, and related to the neurotransmitter systems affected by these drugs of abuse

5.3 Measures and methods

A criticism of many traditional tests of cognition/attention, for example the Digit Symbol Substitution Test (DSST), has been that they confound a range of functions and that they are not able to rule out change in response style (speed/accuracy trade-off). The consequence of the latter problem is that subjects are not penalised for trading accuracy off against speed. A change in the accuracy of performance as assessed by a test such as the DSST is not a definitive measure of change in cognitive function, as it might represent a change in the strategy with which the task was performed. Other tasks do not measure cognitive function in the first place; for example Critical Flicker Fusion frequency is simply a psychophysical threshold.

This disillusionment with traditional techniques has led many researchers to automate tasks known to assess cognitive function in a more specific manner. The principle motivation for automation was to enable speed of performance to be assessed at the same time as accuracy, in order that speed/accuracy trade-off could be identified. Tasks were selected on the basis of their ability to reflect activity in particular cognitive domains such as attention or verbal recognition. An advantage of assessing speed, which soon became obvious was that it was often more sensitive than accuracy.

The CDR system has its roots in the automation of tests in the 1970's (Wesnes, 1977; Wesnes and Warburton, 1978) using the early Laboratory Mini-Computers. The full utility of the system was soon realised in the prototypes, which were installed on the early microcomputers. The system was installed onto the IBM PC in the mid-1980's, where it still remains; though it has since been moved from DOS to the windows environment. The system has a range of core tasks, which may be supplemented with a wide range of additional procedures. It also has the ability to facilitate the administration of traditional tasks. The core tasks of the system are described below (Table 2). The keyboard is not used in any task. Most tasks involve responses made via a customised response module containing a 'YES' and a 'NO' button (Figure 5). There are over 50 parallel forms of the tasks, which are available in most languages and are all brief (1-3 minutes). Different versions have been developed and validated for volunteer (young and elderly) and various patient populations (e.g. Simpson et al., 1991, 1989). The utility, reliability and validity of the system have all been exhaustively demonstrated and discussed (Wesnes et al., 1999; Wesnes et al., 1987; Wesnes and Pincock, 2002).

Therefore, the CDR System provides an ideal tool with which to investigate attentional performance. The tasks are well validated, easy to use, brief and with multiple parallel forms. The tasks are domain specific to allow for assessment of speed/accuracy trade-off and make use of sensitive reaction time recordings. In addition, the core CDR System battery enables assessment of other cognitive domains, including working memory, and episodic secondary memory.

Figure 5: Current laptop based CDR System

Table 2: CDR system tasks

COGNITIVE DOMAIN	TASK
ATTENTION	Simple Reaction Time
	Choice Reaction Time
	Digit Vigilance
EXECUTIVE FUNCTION AND WORKING MEMORY	Rapid Visual Information Processing
	Semantic Reasoning
	Logical Reasoning
	Numeric Working Memory
	Spatial Working Memory
EPISODIC SECONDARY MEMORY	Word Recall
	Word Recognition
	Picture Recognition
	Face Recognition
MOTOR CONTROL	Joystick Tracking task
	Tapping Task
	Postural stability task
PSYCHOPHYSICAL THRESHOLDS	Critical Flicker Fusion

Importantly, the CDR System has shown previous sensitivity to drugs acting on a variety of neurotransmitter systems and disease states with differing aetiologies.

The attentional tasks (Simple Reaction Time, Choice Reaction Time and Digit Vigilance) have shown sensitivity to disruption of dopaminergic (DA) neurotransmission. This includes sensitivity both to cognitive deficits in conditions in which dopaminergic function is compromised e.g. Parkinson's Disease (PD), Parkinson's Disease Dementia (PDD), and Dementia with Lewy Bodies (DLB) (e.g. Ballard et al., 2001, 2002), and the effects of DA antagonists such as haloperidol (e.g. Beuzen et al., 1999; Legangneux et al., 2000).

The CDR System core tasks have also been extensively validated in patients with Alzheimer's disease (AD) (Simpson et al., 1991; Nicholl et al., 1995; Holland et al., 1997; Wesnes, 2000), which has a marked cholinergic component. The tasks have shown high sensitivity to the cognitive deficits associated with AD, and have been able to differentiate AD from other forms of dementia. The CDR system has shown sensitivity to cholinesterase inhibitors, which increase the amount of acetylcholine (ACh) at the neuronal synaptic cleft by inhibiting acetylcholinesterase (AChE). Benefits of these treatments have been seen both in AD (e.g. velnacrine Goa and Fitton, 1994; galantamine Wesnes et al., 1994a; Wesnes et al., 1998) and in DLB (e.g. rivastigmine McKeith et al., 2000; Wesnes et al., 2002). Furthermore, sensitivity to cognitive deficits produced by scopolamine, a

muscarinic cholinergic receptor antagonist, which produces impairment to cognition has been well established. This research has been conducted in both young (Wesnes and Simpson 1988; Wesnes et al., 1988; Ebert et al., 1998) and elderly (Barker et al., 1995; Jones et al., 1991) subjects. In addition, these scopolamine-induced deficits have been shown to closely match the cognitive profile of deficits seen in AD compared to controls, as predicted from the cholinergic dysfunction apparent in AD (Simpson et al., 1991; Walker et al., 2000; Ballard et al., 2002).

The CDR system (Wesnes et al., 1987) has also been widely used to detect effects of drugs with a variety of effects on monoaminergic function. This includes the monoamine oxidase inhibitor moclobemide (Anand et al., 1990), the monoamine reuptake inhibitors sibutramine, reboxetine and paroxetine (Wesnes et al., 2000; Ferguson et al., 2003), and experimental multiple potentiators of monoaminergic systems including NS2330 (Wesnes et al., 2001) and NS2359 (Bosworth et al., 1999). In addition the CDR Digit Vigilance task has previously shown impairments to both accuracy and reaction time following combined monoamine depletion (TRP, TYR, PHE deficient mixture), in comparison to a balanced control mixture (Matrenza et al., 2004).

Therefore, in addition to the CDR Systems general properties and extensive validation, the widespread use of the system in cognitive research with compounds known to influence various aspects of neurotransmitter function provides an unrivalled literature with which to characterise the outcomes of investigative research.

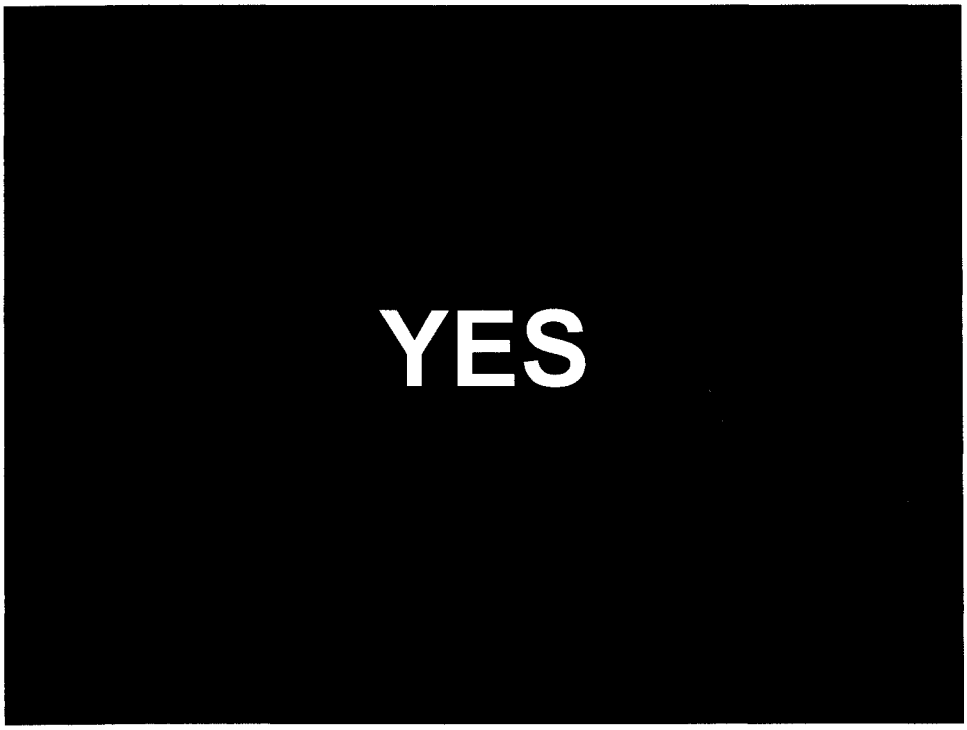
5.4 Detailed attentional task descriptions

5.4.1 Simple Reaction Time

Task operation

The volunteer is instructed to press the 'YES' response button as quickly as possible every time the word 'YES' is presented on the screen. A fixed number of stimuli are presented with varying inter-stimulus intervals (Figure 6).

Figure 6: Simple Reaction Time Stimulus



Measures

Task	Name of SAS variable	Unit / format	Derivation
Simple Reaction Time	SRT	msec	Mean of individual correct responses
Simple Reaction Time - Outliers	SRTL	#	Number of calculated outliers
Simple Reaction Time - Standard Deviation	SRTSD	msec	Standard Deviation of individual correct responses
Simple Reaction Time – Median	SRTM	msec	Median of individual correct responses

Scientific Rationale

Simple reaction time is defined as "the interval between the onset of the stimulus and the response under the condition that the subject has been instructed to respond as rapidly as possible" (Teichner, 1954). The reaction time to each stimulus consists of three components:

- the latent period during reception,
- the relay and transmission of sensory impulses to higher centres (perception time PT), and

- the time interval during the preparation and execution of the motor response (motor time MT).

Therefore, reaction time RT = PT + MT

The Simple Reaction Time task is part of the attentional sub-battery of the CDR System. The measures derived are designed to assess attention. Several mechanisms believed to contribute to attentional function, may influence perception time and motor time for task stimuli, and therefore influence the recorded measures. Increases in any of the derived measures may be generally interpreted as reflecting poorer attention, though the measures do not identify the specific underlying mechanisms, which may contribute to any increase or decrease.

Rationale for stimulus files

The stimulus files are designed to present the stimuli with a varying inter-stimulus interval, within a fixed time window. This is in order that the stimulus onset is unpredictable (to ensure that the subject must continually attend to the stimuli and cannot develop a rhythmical response pattern) and to ensure that the intervals between stimuli are neither too long nor too short. In order to achieve this a minimum and maximum gap may be specified in the 'Configuration Settings' file. Prior to the execution of the task, the software generates a series of inter-stimulus intervals on a pseudo-random basis, ranged between the defined minimum and maximum values, using the CDR number and visit numbers as the seed value.

The stimuli themselves are presented as white, local language (e.g. 'YES' for English speakers), characters on a black background. These stimuli are presented in the centre of the laptop screen or high-resolution monitor, as a fixed proportion of screen size. This is to try and standardise, to some extent, size and intensity of the stimuli, both of which may have an effect on perceptual processing (e.g. Mansfield, 1973). In addition, procedures are in place to ensure hardware within individual studies is standardised, to keep these variables constant. The stimuli remain on screen until a response is made.

Task variations

The number of stimuli presented may be varied. In healthy subjects 50 stimuli are usually presented. In unimpaired patient populations 30 stimuli may be presented. In cognitively impaired populations 20 stimuli are usually presented. This decision is usually based on concerns over fatigue and task difficulty in the various populations, or study constraints. Studies have yet to be conducted to assess the effect of number of stimuli, on the measures derived from the task.

The minimum and maximum gap between stimuli may be varied. In healthy subjects the minimum gap is usually 1000 msec and the maximum gap 3500 msec. In impaired populations the minimum gap is usually 1000 msec and the maximum

gap is 2500 msec. This is to ensure that the individual stimuli are not presented too close together, or too far apart. Studies have yet to be conducted to assess the effect of stimulus interval, on the measures derived from the task.

The minimum and maximum response time allowed may also be specified in the 'Configuration Settings' file. This is typically between 100 msec and 30,000 msec. Responses outside this window result in the stimulus being represented. This is to prevent recording of pre-emptive responses, where there was not enough elapsed time for the stimulus to have been fully perceived by the subject, and to prevent recording of responses when the subject was not attempting to attend to the task. Studies have yet to be conducted to assess the effect of the response window, on the measures derived from the task.

Data Collected

The task records the stimulus presented, the button pressed, and the response time, for each individual stimulus.

Validation

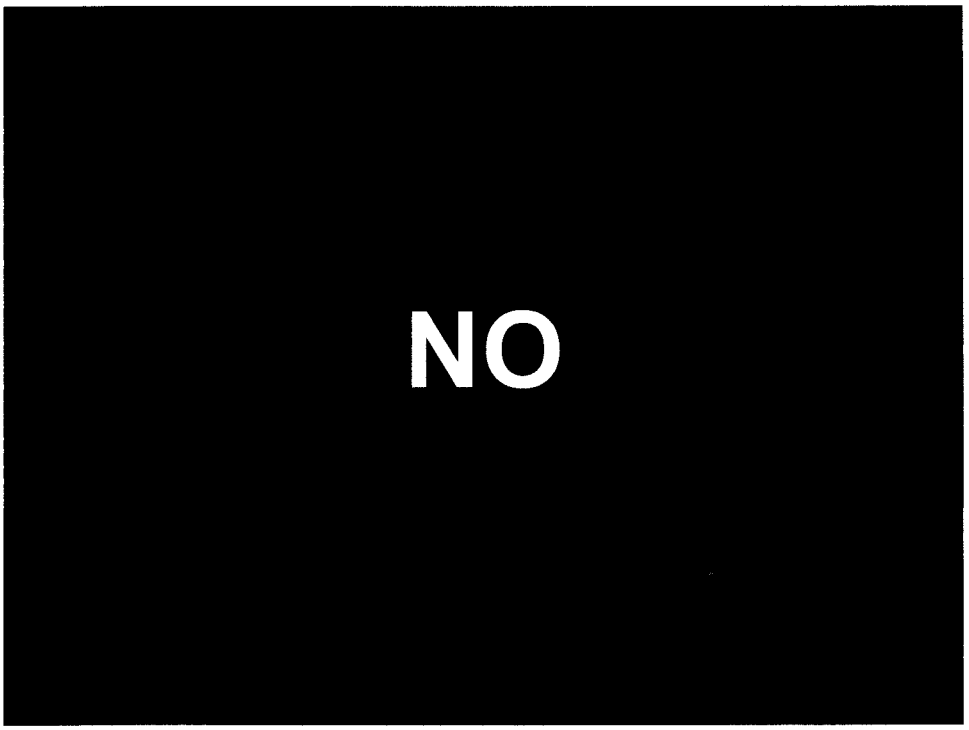
Principle components analysis has established the construct validity of the attentional task measures in young healthy subjects, middle aged healthy subjects, elderly healthy subjects and Alzheimer's patients (Wesnes et al, 1994b; Wesnes et al, 2000). The speed measures from the Simple Reaction Time, Choice Reaction Time and Digit Vigilance tasks reliably load on a single factor, whilst the accuracy measures from the CRT and Vigilance tasks tend to load on a separate factor.

5.4.2 Choice Reaction Time

Task operation

Either the word 'NO' or the word 'YES' is presented on the screen. The volunteer is instructed to press the corresponding button as quickly as possible. There are a fixed number of trials for which each stimulus word is chosen randomly with equal probability, with a varying inter-stimulus interval (Figure 7).

Figure 7: Choice Reaction Time Stimulus



Measures

Task	Name of SAS variable	Unit / format	Derivation
Choice Reaction Time	CRT	msec	Mean of individual correct responses
Choice Reaction Time - Outliers	CRTL	#	Number of calculated outliers
Choice Reaction Time - Standard Deviation	CRTSD	msec	Standard Deviation of individual correct responses
Choice Reaction Time - Median	CRTM	msec	Median of individual correct responses
Choice Reaction Time Accuracy	CRTACC	%	Percentage of stimuli responded to correctly

Scientific Rationale

In the Choice Reaction Time (CRT) task the volunteer has to make a decision on the basis of which stimulus is presented. Therefore, additional processing and neuropsychological mechanisms are hypothesised in addition to those in the Simple Reaction Time (SRT) task.

If SRT = Perception time (PT) + Motor time (MT)

Then CRT = PT + MT + CP (Choice Processing)

The Hick-Hyman Law describes a linear function: reaction times increase in proportion to the number of choices available. By subtracting simple reaction time from choice reaction time
'cognitive decision time' (CogRT) can be calculated.

The Choice Reaction Time task is part of the attentional sub-battery of the CDR System. The measures derived are designed to assess attention. Several mechanisms believed to contribute to 'attentional function', as a general concept, may influence the recorded measures. Reductions in accuracy or increases in the other derived measures may be generally interpreted as reflecting poorer attention, though the measures do not identify the specific underlying mechanisms, which may contribute to any increase or decrease. The derivation of both accuracy and reaction time measures allows for assessment of 'speed-accuracy trade-off'.

Rationale for stimulus files

The stimulus files are designed in the same manner as those for the Simple Reaction Time task. In addition, 'YES' and 'NO' stimuli are presented on a pseudo-random basis, but with equal probability. Therefore, out of 50 stimuli presented 25 will be 'YES' and 25 will be 'NO'. This ensures that any difference in response between 'yes' and 'no' stimuli, or the right and left hand, is evenly balanced within the task.

Task variations

The task variations match those for the Simple Reaction Time task.

Data Collected

The task records the stimulus presented, the button pressed, and the response time, for each individual stimulus.

Validation

Connection of the CDR System to a Neuromapper (McClelland et al, 1995) to record evoked potential (EP) to each stimuli, identified a clear P300 in the CRT task, but no reliable P300 in the SRT task. The P300 component of the EP is believed to represent an index of cognitive evaluation of stimuli. A significant correlation was also identified between speed on the CRT task and amplitude and latency of the P300. This provides clear support for the proposed additional 'choice processing' in the CRT task.

As with the Simple Reaction time task principle components analysis has established the construct validity of the attentional sub-battery measures in young

healthy subjects, middle aged healthy subjects, elderly healthy subjects and Alzheimer's patients (Wesnes et al, 1994b; Wesnes et al, 2000).

5.4.3 Digit Vigilance

Task operation

A target digit is randomly selected and constantly displayed on the right of the screen. A series of digits are then presented in the centre of the screen at a fixed rate, and the volunteer is required to press the 'YES' button as quickly as possible every time the digit in the series matches the target digit. A fixed number of digits are presented, which determines the number of targets and, along with the presentation rate, the duration of the task (Figure 8, Figure 9).

Figure 8: Digit Vigilance Task Target Stimulus

Figure 9: Digit Vigilance Task Target Stimulus plus Digit Series

Measures

Task	Name of SAS variable	Unit / format	Derivation
Digit Vigilance - Targets Detected	VIGACC	%	Percentage of targets responded to within time window
Digit Vigilance - Speed	VIGRT	msec	Mean of individual responses to targets within time window
Digit Vigilance - False Alarms	VIGFA	#	Number of responses falling outside of specified time window
Digit Vigilance – Standard Deviation	VIGSD	msec	Standard Deviation of individual responses to targets within time window

Scientific Rationale

Digit Vigilance is a task based on early continuous performance tasks such as the Mackworth Clock task (e.g. Mackworth, 1961). Early vigilance research was based on wartime studies of radar operators, who had to maintain their attention on a radar screen for long periods of time. The Mackworth Clock task was used to investigate the 'vigilance decrement' (a reduction in numbers of targets detected and an increase in reaction times to those targets over time) and factors influencing

the decrement and general attentional performance. The Mackworth clock task typically lasted for 45 minutes, with very few stimuli, simulating the conditions when monitoring radar screens. The Digit Vigilance task is designed to allow for a sensitive assessment of vigilance in a brief period of time (two to three minutes), by presenting many stimuli in rapid succession, ensuring the brevity and sensitivity required for use in a clinical trial.

The Digit Vigilance task is part of the attentional sub-battery of the CDR System. The measures derived are designed to assess vigilance or sustained attention. Several mechanisms believed to contribute to 'attentional function', as a general concept, may influence speed and accuracy of target detection. Reductions in accuracy (or sensitivity), or increases in the other derived measures may be generally interpreted as reflecting poorer attention, though the measures do not identify the specific underlying mechanisms, which may contribute to any increase or decrease.

Rationale for stimulus files

The stimulus files are designed to present the stimuli continuously in rapid succession, with target digits interspersed among them. This is in order that the stimulus onset is unpredictable (to ensure that the subject must continually attend to the stimuli and cannot develop a rhythmical response pattern). In order to achieve this, stimulus files of 450 digits (digits are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9) are used. Each digit appears 45 times, with 0 as the first digit in each file and then a pseudo random series of digits following that, with the digits spaced throughout the file, so any individual digit is not repeated without at least two intervening digits. By presenting 0 first, and not using this as a target digit, it can be ensured that the volunteer does not have to respond to the very first stimulus of the task. The digits are organised into blocks of 50, in which each digit appears equally often. By having a gap of at least two digits the volunteer will usually have either 1200 msec (young healthy) between onset of one target and the next (greater than the 1000 msec window – see below), or 2250 msec (greater than the 1500 msec window – see below). Currently, there are 76 different stimulus files. The target digit is selected by the software, prior to the execution of the task, using the CDR number and visit numbers as the seed value.

Task variations

The number of stimuli presented may be varied. In healthy subjects 450 stimuli are usually presented, at a rate of 150 per minute, with 45 targets, meaning the task runs continuously for 3 minutes. In impaired patient populations 160 stimuli may be presented, at a rate of 80 per minute, with 15 targets, meaning the task runs for approximately 2 minutes. This decision is usually based on concerns over fatigue and task difficulty in the various populations.

Duration of stimulus presentation (SP) and inter-stimulus interval (ISI) may be specified. This is typically 350 msec SP and 50 msec ISI for young healthy subjects (150 stimuli per minute), and 700 msec SP and 50 msec ISI for impaired subjects (80 stimuli per minute).

The timing of the individual responses runs from onset of the first target stimulus, and is reset upon presentation of each subsequent target stimulus.

The allowed response window may also be specified in the 'Configuration Settings' file. This is typically between 350 msec and 1000 msec for healthy subjects and between 350 msec and 1500 msec for impaired patient populations. Responses within the window result in a 'correct detection', contributing the accuracy and speed measures from the task. Responses outside this window result in a 'false alarm'. Studies have yet to be conducted to assess the effect of the response window, on the measures derived from the task. The 350 msec lower boundary is currently in place due to problems with 'button bounce', in which more that one response was being recorded for a button press. This has replaced a previous 50 msec value.

Data Collected

The task records the stimulus presented, the button pressed, and the response time, for each individual response.

Validation

The criterion validity of the task has been assessed in comparison to letter cancellation during smoking and deprivation in regular smokers (Parrott and Garnham, 1995). Letter cancellation is a pencil and paper test of attention, which has been used in psychological research for more than 80 years. The test involves scanning a sheet of printed letters, and deleting each instance of the target letter. Both tasks were sensitive to lowered rates of detection and slowed response times under deprivation, demonstrating the criterion validity of the Digit Vigilance task.

Principle components analysis has established the construct validity of the attentional sub-battery measures in young healthy subjects, middle aged healthy subjects, elderly healthy subjects and Alzheimer's patients (Wesnes et al, 1994b; Wesnes et al, 2000).

6 Vigilance task validation: A computerised Mackworth Clock task and a brief vigilance task

6.1 Abstract

The Mackworth Clock test was devised to measure concentration and was used to investigate the ability to sustain concentration / attention over periods of time. Various versions of the test have been employed to investigate the nature of sustained attention, following the identification of the 'vigilance decrement', a decline in performance on the test over its duration (typically between 45 minutes and 2 hours). It has since proven sensitive to the performance enhancing effects of amphetamine, and decrements induced by sleep loss, alcohol and selective serotonin reuptake inhibitors. The CDR Digit Vigilance task was designed to provide a far more brief assessment of vigilant attention (3 minutes), and has shown sensitivity to effects of ageing, sleep deprivation, fatigue and alcohol. However, the existence of a vigilance decrement over its duration has not been established.

This study was designed to validate a new computerised implementation of the 45-minute Mackworth Clock test on the CDR platform, and an existing brief vigilance task. Total Mackworth Clock test data were compared to data previously collected, and intra-task performance on both 'vigilance' tasks was compared.

Mackworth Clock test performance with the computerised task closely matched the data for another version of the test, indicating the different test implementations were comparable. Furthermore, both the 3-minute Digit Vigilance task and the 45-minute Mackworth Clock task showed significant increases in reaction times, and reductions in accuracy over the duration of the test/task. This supported the contention that the Digit Vigilance task was subject to a 'vigilance decrement' over the period of performance, and was a valid assessment of vigilant attention despite the short (3 minute) duration.

6.2 Introduction

The purpose of the present study was to investigate the psychometric properties of two assessments of vigilant attention: the CDR Digit Vigilance task; and a computerised version of the Mackworth Clock task, designed to sit alongside the existing CDR battery tasks, using the same platform and response box.

The Mackworth Clock test was originally designed and used to investigate vigilant attention and influences on performance (e.g. Mackworth, 1961). Studies using the task established the existence of a 'vigilance decrement' (a reduction in numbers of targets detected and an increase in reaction times to those targets over time), as well as identifying factors influencing the decrement and general attentional performance (e.g. Mackworth, 1965).

The Digit Vigilance task was based on early continuous performance tasks such as the Mackworth Clock test. The Digit Vigilance task was designed to allow for a sensitive assessment of vigilance in a brief period of time (two to three minutes), by presenting many stimuli in rapid succession, ensuring the brevity and sensitivity required for use in a clinical trial. However, the existence of a 'vigilance decrement' to performance within the task has not been established.

The new computerised version of the Mackworth Clock task was designed to be a direct replication of the task employed by Schmitt et al. (2002) see "Task Operation" below. The Mackworth clock test has been used to investigate vigilant attention in a range of studies, following the investigations in the 1960's into the vigilance decrement and the influence of amphetamines. Quilter et al. (1983) used the task to show a decline in performance with ageing, which was most marked at around 70 years of age, whilst Giambra and Quilter (1988) showed a longitudinal decline in performance, but no evidence for a change in the performance decrement within the test with increasing age. The test has also shown performance declines related to sleep loss (e.g. Brendel et al., 1990; Hoch et al., 1992) and alcohol (e.g. Williamson et al., 2001). A series of studies using a 45-minute version of the test have shown sensitivity to performance decrements induced by Venlafaxine (O'Hanlon et al., 1998), Paroxetine (Schmitt et al., 2000), and citalopram (Riedel et al., 2005). This indicates that this 45 minutes version of the test may be well suited to detecting vigilance effects associated with subchronic effects of selective serotonin reuptake inhibition and other monoaminergic effects, whilst also being shorter and so more practicable than the 1-2 hour versions employed in other studies.

The CDR Digit Vigilance task has shown sensitivity to effects of ageing (CDR normative database v3.0), sleep deprivation (Wesnes & Macher, 2004), and alcohol (e.g. Wesnes et al., 2000). However, the task has not previously shown sensitivity either to improvements following amphetamine or decrements associated with subchronic effects of selective serotonin reuptake inhibition and other monoaminergic effects. Furthermore, intra-task changes in performance have not been investigated.

The present study was planned to investigate intra-task changes in performance on the CDR Digit Vigilance task, to demonstrate the existence of a vigilance decrement and to validate and compare to a new computerised implementation of a 45-minute version of the Mackworth Clock test.

6.3 Hypotheses

- It was hypothesised that the new computerised implementation of the Mackworth Clock task based on previously published parameters would collect comparable data (mean +/- standard deviation), to previously published data in a separate population of young healthy subjects, supporting the validity of the new task implementation.

- It was hypothesised that a decline in performance would be evident over the duration of the Mackworth Clock task – a 'vigilance decrement'.
- It was hypothesised that a similar 'vigilance decrement' would be evident in a shorter more intense Digit Vigilance task.

6.4 Methods and Materials

6.4.1 Subjects

Thirty-two healthy subjects (16 male, 16 female), between 20 and 45 years of age (mean 30.6, standard deviation 7.8 years), were studied.

6.4.2 Ethics

The study was conducted at The Psychiatric Research Institute, Wichita, USA and received local Institutional Review Board approval.

6.4.3 Design

The study 'reprocessed' individual response data from the Mackworth Clock test and Digit Vigilance task to look at changes in performance over the duration of the tasks (the potential 'vigilance decrement'), as opposed to summary scores for the whole of the task duration. Data were reprocessed for baseline performance (pre-dose on day 1, post training) prior to entry into a longer duration treatment study. All subjects completed both the Mackworth Clock test and Digit Vigilance task.

6.4.4 Assessments

Computerised Mackworth Clock test

Task Operation

This task has been widely used in studies of human vigilance (Mackworth, 1961) and is sensitive to the effects of various drugs including SSRIs (Schmitt et al., 2002). Subjects viewed a computer screen displaying a circular arrangement of 60 white dots (the 'clock-face'). The dots were briefly illuminated by turning red in a clockwise rotation of one per 500 msec. Subjects were instructed that at rare intervals the illuminating sequence would skip one dot. Subjects were instructed to respond to this signal by pressing a button as quickly as possible. Responses made within 1500 msec of a signal were registered as correct detections. Thirty signal jumps occurred over a 45-minute period with intervals ranging between 10 and 230 seconds. Outcome measures were the number of correct detections and the corresponding reaction times, and the number of false alarms (responses outside the specified window) (Table 3).

Table 3: Mackworth Clock Measures

Task	Name of SAS variable	Unit / format	Derivation
Mackworth Clock - Accuracy	MCKACC	%	Percentage of targets responded to within time window
Mackworth Clock – Speed	MCKRT	msec	Mean of individual responses to targets within time window
Mackworth Clock – False Alarms	MCKFA	#	Number of responses falling outside of specified time window
Mackworth Clock – Standard Deviation	MCKSD	msec	Standard Deviation of individual responses to targets within time window

Rationale for stimulus files

The stimulus presentation was designed to replicate the previous implementations of the task. The light moves at a rate of one space every 500 msec. Thirty target jumps occur over a 45-minute period. The target jumps are organised into blocks of 10. There is a gap of between 20 moves (10 secs) and 561 moves (230.5 secs) between target jumps. The timing of the individual responses runs from onset of the first target stimulus, and is reset upon presentation of each subsequent target stimulus.

Data Collected

The task records the stimulus presented, the button press, and the response time, for each individual response.

Digit Vigilance task

The Cognitive Drug Research Digit Vigilance task was designed as a rapid assessment of vigilance. A target digit is randomly selected and constantly displayed on the right of the screen. A series of digits is then presented in the centre of the screen at a fixed rate, and the volunteer is required to press the 'YES' button as quickly as possible every time the digit in the series matches the target digit. The task lasted 3 minutes, with 45 target stimuli, and recorded the stimulus presented, the button press, and the response time, for each individual response.

6.4.5 Statistical Analysis

Reaction time data to the individual targets in each task were split into three 'blocks' of responses. For the Mackworth Clock task data were reanalysed as 3 blocks of 10 responses, with each block equal to 15 minutes of task performance. For the Digit Vigilance task data were reanalysed as 3 blocks of 15 responses, with each block equal to 1 minute of task performance. One-way ANOVA was performed on block, with comparisons conducted between each block.

6.5 Results

Average performance for the whole population at baseline (day 1 pre-dose) on the Mackworth Clock test showed correct detections at 79% (+/- 5% standard error), reaction times at 674 msec (+/- 32 msec standard error), and 2 false alarms.

Block-by-block analyses showed a statistically significant effect of block for both the Mackworth Clock task and the Digit Vigilance task for both reaction time and accuracy. Reaction times increased from block 1 to blocks 2 and 3 during the performance of the Mackworth Clock task (Table 4), whilst there was an increase from each block to the next in the Digit Vigilance task (Table 5). For both tasks a statistically significant block-by-block decline in accuracy was also evident.

Note: accuracy measures for both tasks represent cumulative performance i.e. block 2 includes errors from block 1 and additional errors in block 2, block 3 includes errors in blocks 1 and 2 and additional errors in block 3.

Table 4: Mackworth Clock test 15-minute Block Analysis

Main effect of block p=0.0001			
Reaction Time	Difference of LSmeans (reaction time)	Standard Error (reaction time)	P-value
Block 1 Vs 2	-76.37	18.11	**0.0001**
Block 1 Vs 3	-52.34	18.57	**0.0049**
Block 2 Vs 3	24.03	19.25	0.2123
Main effect of block p=0.0001			
Accuracy	Difference of LSmeans (accuracy)	Standard Error (accuracy)	P-value
Block 1 Vs 2	13.7	3.9	**0.0004**
Block 1 Vs 3	32.2	4.0	**0.0001**
Block 2 Vs 3	19.0	4.1	**0.0001**

Figure 10: Mackworth Clock Reaction Time by 15-minute Blocks

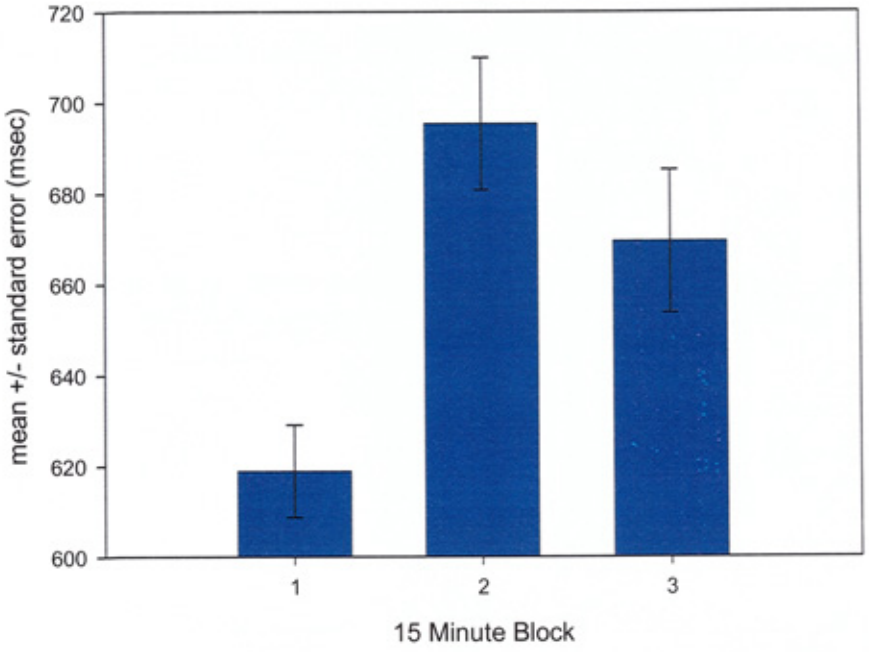


Figure 11: Mackworth Clock Accuracy by 15-minute Blocks

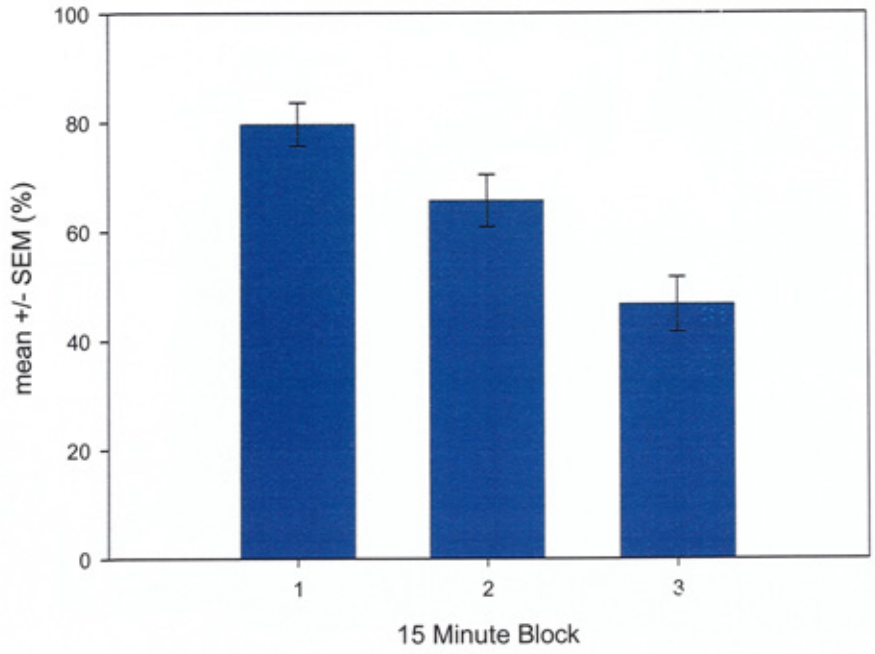


Table 5: Digit Vigilance task 3-minute Block Analysis

Main effect of block p=0.0001			
Reaction Time	Difference of LSmeans (reaction time)	Standard Error (reaction time)	P-value
Block 1 Vs 2	-12.26	3.04	**0.0001**
Block 1 Vs 3	-20.94	3.05	**0.0001**
Block 2 Vs 3	-8.68	3.05	**0.0045**
Main effect of block p=0.0001			
Accuracy	Difference of LSmeans (accuracy)	Standard Error (accuracy)	P-value
Block 1 Vs 2	8.8	1.5	**0.0001**
Block 1 Vs 3	19.9	1.5	**0.0001**
Block 2 Vs 3	11.1	1.5	**0.0001**

Figure 12: Digit Vigilance Task Reaction Time by 3-minute Blocks

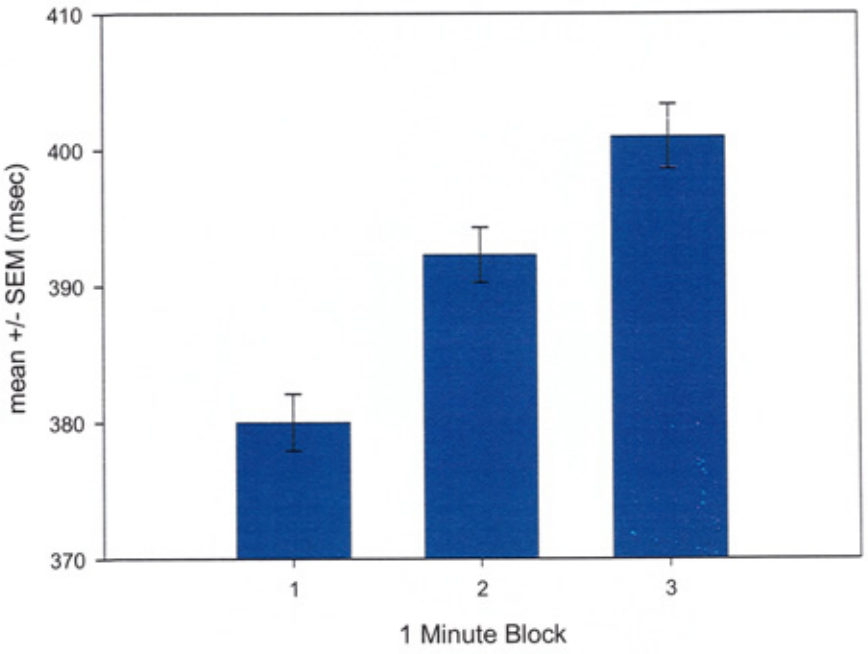
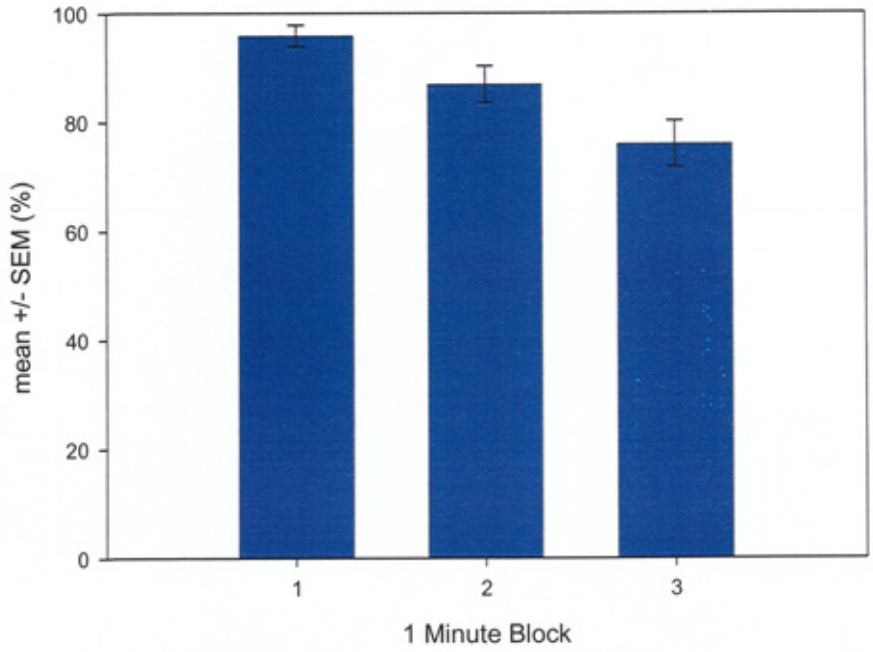


Figure 13: Digit Vigilance Task Accuracy by 3-minute Blocks



6.6 Discussion

Mackworth Clock task performance at day 0 in the Schmitt et al. (2002) study (approximate data for group means averaged over the three study treatments) showed correct detections at approximately 80% (+/- 3% standard error), reaction times at 750 msec (+/- 25 msec standard error), and 1.5 false alarms. Taking into account the different study populations and designs, this similarity in task performance indicated that the present task implementation closely matched the version used by Schmitt et al.

The data from the current computerised Mackworth Clock task showed a clear vigilance decrement in terms of significantly slowed response times in the second two blocks compared to the first block (Figure 10). For accuracy, a block on block decline was seen (Figure 11). This clearly validated the task implementation in terms of identifying a vigilance decrement, demonstrating that response times to targets were slower in the final two 15-minute (10 target) blocks, and less targets were detected in each successive block.

The Digit Vigilance task showed a slightly different pattern of vigilance decrement (Figure 12). Response times in each 1-minute (15 target) block were progressively slower over the task. Block 2 being significantly slower than block 1 and block 3 significantly slower than both block 1 and 2. Again the same was true of the accuracy data, which showed a decline in performance with each successive block.

Therefore the data supported the theory that a more intense task (rapid more frequent stimulus presentation) would replicate vigilance task conditions, over a shorter assessment period.

The identification of comparable scores on the Mackworth Clock task in the present population in comparison to the version used by Schmitt et al., (2002) suggest the new version is a close replication and likely to have similar properties. Therefore, it can be assumed that data gathered using this task could be compared to similar versions of the Mackworth Clock task in order to make general statements regarding effects on vigilant and sustained attention. Furthermore, the existence of a vigilance decrement in both tasks in terms of reaction times supports the contention that a shorter more intense task may be used to assess aspects of vigilant attention.

Data from Mackworth (1961) using a 2-hour version of the Mackworth Clock test identified a vigilance decrement in terms of increased errors an increased response times over ½ hour blocks. The specific pattern for the placebo and no tablet conditions was for successive declines from blocks 1 to 2 to 3, but then a partial recovery of the decline in block 4 (Table 6). Amphetamine improved performance on both measures in all blocks, and did not show such a clear pattern of vigilance decrement.

Data from Mackworth (1965) using a 1 hour version of the Mackworth Clock test identified a vigilance decrement in terms of d' (combined accuracy and false alarms – reaction time data not reported). The size of the decline in each successive block showed some fluctuation. However, the difference between block 1 and block 6 always indicated a decline in performance in each study period (Table 7 - only study period 1 data are shown below). The effect of amphetamine was to both improve mean d' and to reduce the difference between blocks 1 and 6.

The current 45 minute Mackworth Clock test showed successive declines in accuracy comparable to the earlier 60 minute version, with the earlier 2 hour version only showing attenuation of the decline in the final ½ hour block, which suggested this may only occur following more than 1- 1.5 hours test duration. However, reaction times in the current 45-minute test did show some attenuation in the final block, which was not statistically significantly different to the second block. This suggested that reaction times may not follow the same pattern as accuracy, as though the 2 hour version also showed attenuation of reaction time decline in the final ½ hour block, reaction time data were not reported for the 1 hour task, so it is unclear what pattern may be apparent over blocks of shorter duration and a pattern different from successive decline may be seen.

The CDR Digit Vigilance task showed a pattern of successive decline in accuracy block on block as seen for the current 45 minute Mackworth Clock test, the earlier 60 minute version, and the first 3 blocks of the earlier 2 hour version. Reaction times also showed successive decline. Whilst the tasks are greatly different in terms of the test duration, the numbers of stimuli presented are broadly comparable, and the existence of a similar pattern of vigilance decrement indicates that the Digit Vigilance task is a valid measure of sustained attention.

In conclusion, data for both the current 45 minute Mackworth Clock test and the CDR Digit Vigilance task, indicated both were valid measures of sustained / vigilant attention.

Table 6: Two-hour Mackworth Clock test Data by 30-minute Blocks

Group	First ½ hour	Second ½ hour	Third ½ hour	Fourth ½ hour
Mean percentage of missed signals				
Amphetamine ('Benzedrine' tablets 10 mg)	5.6	7.7	6.2	7.4
Placebo (Dummy tablets)	7.4	14.5	18.5	19.6
No tablets	7.4	18.1	21.9	19.4
Mean response time (msec)				
Amphetamine ('Benzedrine' tablets 10 mg)	1000	1050	1050	1100
Placebo (Dummy tablets)	1060	1280	1320	1240
No tablets	1200	1250	1300	1280

Mackworth NH (1961)

Table 7: One-hour Mackworth Clock test Data by 10-minute Blocks

		Group 1			Group 2			Group 3		
10 Minute Block	Study Period	% seen	% false alarm	d'	% seen	% false alarm	d'	% seen	% false alarm	d'
		Amphetamine			Placebo			Placebo		
1	1	65	0.05	3.65	77	0.12	3.77	60	0.12	3.29
2		59	0.09	3.30	68	0.15	3.44	59	0.12	3.26
3		55	0.07	3.30	57	0.11	3.23	43	0.14	2.82
4		56	0.07	3.32	53	0.12	3.11	44	0.13	2.87
5		55	0.10	3.20	50	0.12	3.03	45	0.13	2.89
6		53	0.11	3.13	42	0.13	2.81	40	0.16	2.69
Mean		57	0.08	3.32	58	0.12	3.23	48	0.13	2.97
1-6		**12**	**-0.06**	**0.52**	**35**	**-0.01**	**0.96**	**20**	**-0.04**	**0.60**

Mackworth JF (1965)

7 Sensitivity of the attentional task battery of the cognitive drug research computerised assessment system in normal volunteers to the effects of an amphetamine analogue

7.1 Abstract

D-amphetamine has been shown to enhance selective and vigilant attention through activation of the dopaminergic system. In addition, measures of attention are compromised in disorders with underlying dopaminergic pathology, and during treatment with drugs that act as antagonists at dopaminergic neurons, such as the antipsychotics. The Cognitive Drug Research attentional task battery has shown sensitivity to impaired attention in disorders with dopaminergic pathology. This supports the use of the battery as an appropriate tool for investigating human attention and dopaminergic function. However, previous studies have not been conducted to demonstrate sensitivity to benefits from enhanced dopaminergic function in normal volunteers. The present study investigated attentional task performance following administration of an amphetamine analogue.

Eight subjects (4 male, 4 female; mean age 51.8, standard deviation 11.0) completed a 5-way cross-over study design of three doses of an amphetamine analogue, with 2 placebo periods. Assessment of attention was conducted at pre-dose and at 1.5 and 2.5 hours post-dose in each period.

A clear pattern of dose dependent improvements to reaction times was identified with increasing dose. In addition, there was evidence indicating that amphetamine primarily acted by reducing the decline seen to reaction times with repeated assessment over the study day. The data demonstrated the sensitivity of the attentional task battery to improvements in performance following administration of a dopaminergic agonist, supporting it's use in studies of factors influencing attention via the dopaminergic system.

7.2 Introduction

Early studies of amphetamine and human performance formed part of the development of theories of vigilance and attention. These identified that d-amphetamine was able to prevent or protect against the typical vigilance decrement in detections and reaction time in the classic Mackworth Clock task (e.g. Mackworth, 1961; Mackworth 1965). Studies in animals were able to demonstrate that amphetamines induce release of catecholamines from nerve terminals and also raise extracellular dopamine (DA) levels (e.g. Schmitz et al., 2001). Behavioural evidence suggests that the activating properties of these drugs actually depend on activation of the DA system, resulting in enhanced locomotor stimulation and behavioural activation (Willner and Scheel-Kruger eds, 1991). DA is now widely considered to play a key role in the regulation of cognition and attention (e.g. Nieoullon, 2002). Furthermore, dysfunction of the DA system may underlie cognitive deficits in various forms of dementia, Schizophrenia, and Attention Deficit Hyperactivity Disorder (ADHD) (e.g. Court et al., 2000; Nieoullon, 2002; Seiver et al., 2002), all of which have shown ameliorative action of DA agonists or antagonists.

In Parkinson's disease (PD) the function of DA pathways is severely compromised and DA pathology is the primary neurological hallmark of the disease. The cognitive changes most associated with PD are those of executive dysfunction and memory / visuospatial impairment. Executive function may be broadly characterised as the ability to plan and organise goal directed behaviours, and attention is considered to be a central element of this. PD patients demonstrate deficits on several tasks believed to contain aspects of attention, such as the Stroop colour-word test and the trail-making task. DA function has been clearly linked to these deficits (e.g. Kaasinen et al., 2002).

The Cognitive Drug Research (CDR) computerised assessment system contains three core tasks designed to assess attention: Simple Reaction Time, Choice Reaction Time and Digit Vigilance. Each of these tasks involves focusing on visual stimuli in order to make appropriate speeded responses, in common with classic tests of attention. These tasks have demonstrated sensitivity to cognitive deficits in conditions in which dopaminergic function is compromised (e.g. Ballard et al., 2001, 2002), and the effects of DA antagonists such as haloperidol (e.g. Beuzen et al., 1999; Legangneux et al., 2000). In addition, factor analysis supports the construct validity of the 'attentional battery' by demonstrating that the speed measures from the three tasks consistently load on a single factor, strengthening the theory that each one is assessing aspects of a unified attentional mechanism (e.g. Wesnes et al., 2000; Wesnes et al., 1994b). However, the sensitivity of these tasks to enhancement via DA activation has yet to be demonstrated.

The compound studied here, in common with d-amphetamine is a sympathomimetic amine with central nervous system (CNS) stimulant activity. It is

a relatively weak CNS stimulant with very much less euphoric effect and addictive potential when compared to d-amphetamine. The compound has a significantly lower incidence off unwanted side-effects such as CNS stimulation, locomotor abnormalities, and sleep disturbances when compared to d-amphetamine (unpublished research).

As part of a study to further define the optimal pharmacokinetic (PK) profile of the compound for improvement of memory, the attentional task battery of the CDR System was administered. This provided an opportunity to investigate the sensitivity of the three attentional tasks to effects of DA activation, and also characterise dose dependent effects of the compound on the task measures.

7.3 Hypothesis

- It was hypothesised that the compound studied would improve attentional task performance via its actions on DA

7.4 Methods and Materials

7.4.1 Subjects

Eight healthy male and female subjects (4 male, 4 female) between 40 and 80 years of age (mean 51.8, standard deviation 11.0) were studied in two groups of four.

7.4.2 Ethics

The study was conducted at a commercial phase I unit and supported by a sponsor pharmaceutical company. Ethical approval was obtained from the appropriate regulatory bodies.

7.4.3 Design

This was a phase I, randomised, double-blind, placebo controlled, non-ascending oral dose study. Training on the cognitive test battery took place prior to the first day of dosing of the trial in order to ensure an optimal level of performance for the baseline assessment on the first study day. Each volunteer completed four training sessions, 2 sessions being conducted at screening and 2 sessions on the day before the first study period. On each study day, the task battery was performed prior to the intake of the study drug and at 1.5 and 2.5 hours post-dose, on each of the study periods.

7.4.4 Treatments

Dose levels of (0, 5, 15 and 30 mg) were administered in a cross over design with 5 periods, with each subject receiving every active dose and two placebo administrations. Doses were taken orally as whole capsules.

7.4.5 Assessments

An attentional test battery from the CDR computerised cognitive assessment system was administered as part of the cognitive assessment in the study. All tasks were computer-controlled, the information being presented on high resolution screens, and the responses recorded via a response module containing two buttons, one marked 'NO' and the other 'YES'. The tasks were administered in the following order:

Simple Reaction Time

The word 'yes' was presented on the monitor. The volunteer was required to press the corresponding button as quickly as possible. There were 50 trials, with randomly varying inter-stimulus interval between 1 and 3.5 seconds. The task measured reaction time (msec).

Choice Reaction Time

Either the word 'no' or the word 'yes' was presented on the monitor. The volunteer was required to press the corresponding button as quickly as possible. There were 50 trials, in which the stimulus word was chosen at random with equal probability, randomly varying inter-stimulus interval between 1 and 3.5 seconds. The task measured accuracy (%) and reaction time (msec).

Digit Vigilance

A target digit was randomly selected and constantly displayed to the right of the screen. A series of digits was then presented in the centre of the screen at the rate of 150 per minute and the volunteer was required to press the 'YES' button as quickly as possible every time the digit in the series matched the target digit. There were 45 targets in the series. The task lasted for 3 minutes. A 1000 msec window for responses to target stimuli was used and the task measured accuracy (%) and reaction time (msec) to responses within the target window, and false alarms (#) (responses outside the target window).

Power of Attention

Power of Attention (msec) is a composite score derived by summing the reaction times from the three attentional tasks (Simple Reaction Time, Choice Reaction Time, and Digit Vigilance). This provides a measure of overall 'speed of attention'.

Continuity of Attention

Continuity of Attention (#) is a composite score derived by summing the number of targets detected out of 50 in the Choice Reaction Time tasks and out of 45 in the Digit Vigilance task, then subtracting the number of false alarms made in the Digit Vigilance task. This provides a measure of overall 'accuracy of attention'.

Response Variability

Response Variability (%) is a composite score derived by calculating the mean of the coefficients of variance of the reaction times from the three attentional tasks. This provides a measure of overall 'fluctuations in attention'.

Cognitive Reaction Time

The Cognitive Reaction Time measure (msec) is derived by subtracting Simple Reaction Time from Choice Reaction Time, to approximate the 'cognitive' or choice portion of the response time.

7.4.6 Statistical Analysis

Analysis of Variance (ANOVA) was conducted on each measure at each time point, using the unadjusted data. Fixed terms were fitted to the model for dose and period, with a random effect of subjects. Contrasts were then conducted to fit linear and quadratic trends to the data for dose level. A pooled placebo dose group was used in the analyses, combining data from both placebo periods.

7.5 Results

Table 8: ANOVA analyses F-ratios and p-values for the main effect of dose and the linear and quadratic trends, performed at each study timepoint

Measure	Time	Effect of Dose	Trend
Simple Reaction Time	Pre-dose	F=0.33 p=0.81	-
	1.5 hours	F=1.34 p=0.29	-
	2.5 hours	F=3.27 p=0.04	Linear p=0.01
Choice Reaction Time	Pre-dose	F=0.28 p=0.84	-
	1.5 hours	F=2.01 p=0.14	Linear p=0.03
	2.5 hours	F=0.60 p=0.62	-
Digit Vigilance Speed	Pre-dose	F=1.08 p=0.38	-
	1.5 hours	F=2.68 p=0.07	Linear p=0.02
	2.5 hours	F=5.04 p=0.01	Linear p<0.01
Digit Vigilance Targets Detected	Pre-dose	F=1.08 p=0.37	-
	1.5 hours	F=0.56 p=0.64	-
	2.5 hours	F=1.08 p=0.38	-
Power of Attention	Pre-dose	F=0.54 p=0.66	-
	1.5 hours	F=3.53 p=0.03	Linear p<0.01
	2.5 hours	F=4.17 p=0.02	Linear p<0.01
Response Variability	Pre-dose	F=0.84 p=0.49	-
	1.5 hours	F=2.45 p=0.09	Quadratic p=0.09
	2.5 hours	F=0.88 p=0.47	-
Continuity of Attention	Pre-dose	F=0.42 p=0.74	-
	1.5 hours	F=1.02 p=0.40	-
	2.5 hours	F=0.20 p=0.90	-
Cognitive Reaction Time	Pre-dose	F=0.37 p=0.78	-
	1.5 hours	F=0.50 p=0.69	-
	2.5 hours	F=0.48 p=0.70	-

The data for Simple Reaction Time showed a significant effect of dose at 2.5 hours, with a significant linear trend identified. The data showed a general pattern for faster reaction times with increasing dose, although the 0.5 mg dose showed a slight decline against placebo.

The data for Choice Reaction Time did not show any significant effects of dose at either 1.5 or 2.5 hours. However, a signal for an effect of dose was seen at 1.5 hours and a significant linear trend identified. The data showed a general pattern for faster reaction times with increasing dose.

The data for Digit Vigilance Speed showed significant effects of dose and significant linear trends at both 1.5 and 2.5 hours. The data showed a general pattern for faster reaction times with increasing dose, although the 0.5 mg dose again showed a slight decline against placebo.

The data for Power of Attention reflected that seen for the individual reaction time measures, in particular Digit Vigilance Speed. Significant effects of dose and significant linear trends were identified at both 1.5 and 2.5 hours, with the pattern of data supporting a general benefit to reaction times with increased dose (Figure 20).

For Simple Reaction Time, Choice Reaction Time and Digit Vigilance (and the composite score Power of Attention), there was a general pattern for a decline in performance (increased reaction times) with successive assessments. The clearest pattern to emerge was for an effect of the 15 and 30 mg doses to prevent this decline, primarily at 2.5 hours, rather than improve performance above pre-dose level. For Choice reaction Time, the pattern was different and indicated a dose dependent pattern for improved performance with increasing dose, above levels at pre-dose, primarily at 1.5 hours. This decline with successive assessments over the day was not apparent for the other measures.

The data for the accuracy measures, response variability, and Cognitive Reaction Time showed no support for linear effects of dose, either in the analyses or the general pattern of the data. A statistical signal was seen for a quadratic trend for response variability at 1.5 hours. The data indicated that this was largely due to a reduction in variability with 0.5 mg. However, these data did not suggest a clear dose dependent pattern, and with weak statistical support did not support a genuine effect.

Figure 14: Effect of Amphetamine Analogue on Simple Reaction Time (mean)

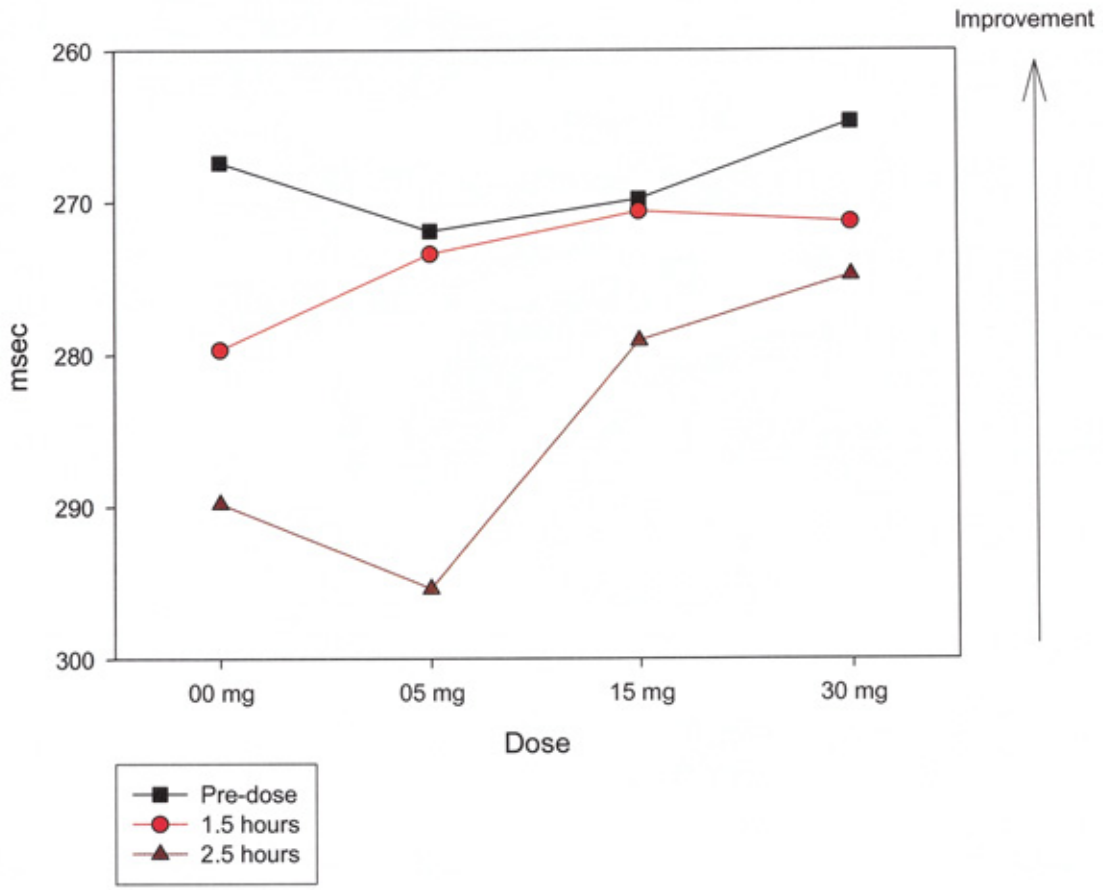


Figure 15: Effect of Amphetamine Analogue on Choice Reaction Time (mean)

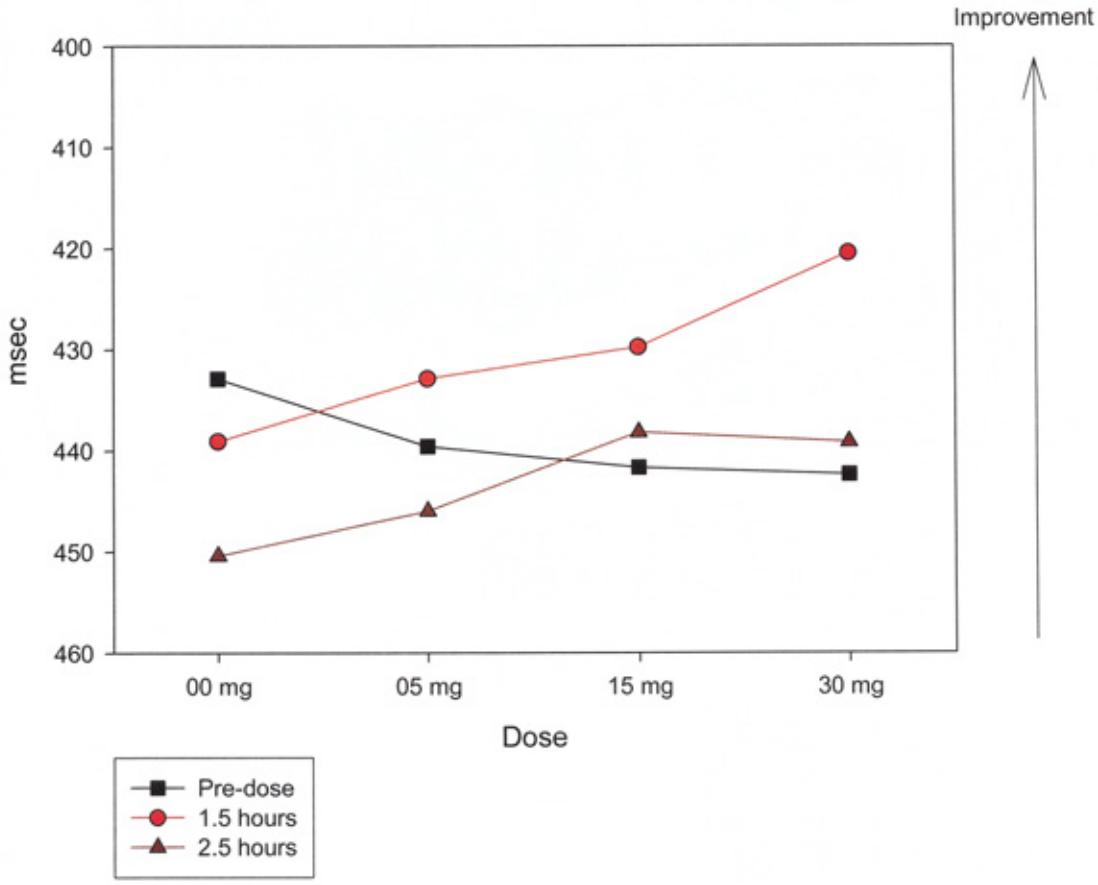


Figure 16: Effect of Amphetamine Analogue on Choice Reaction Time Accuracy (mean)

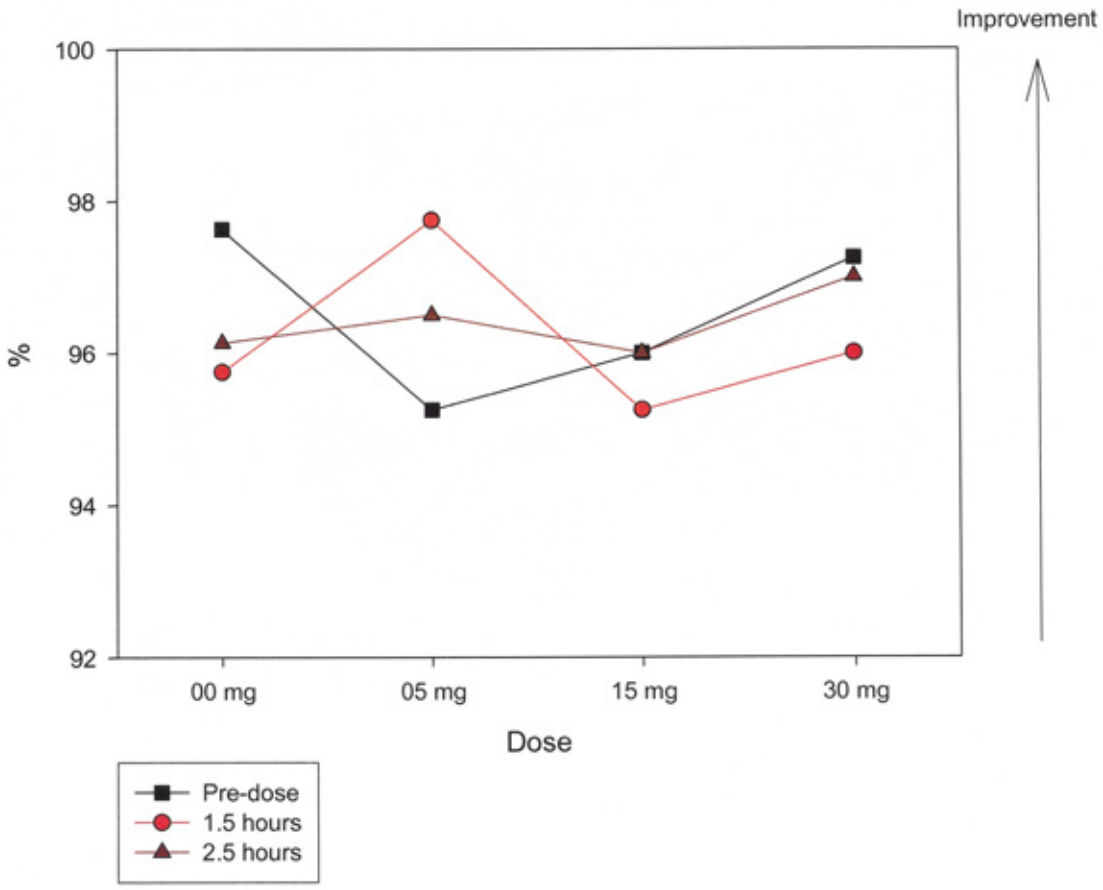


Figure 17: Effect of Amphetamine Analogue on Digit Vigilance Speed (mean)

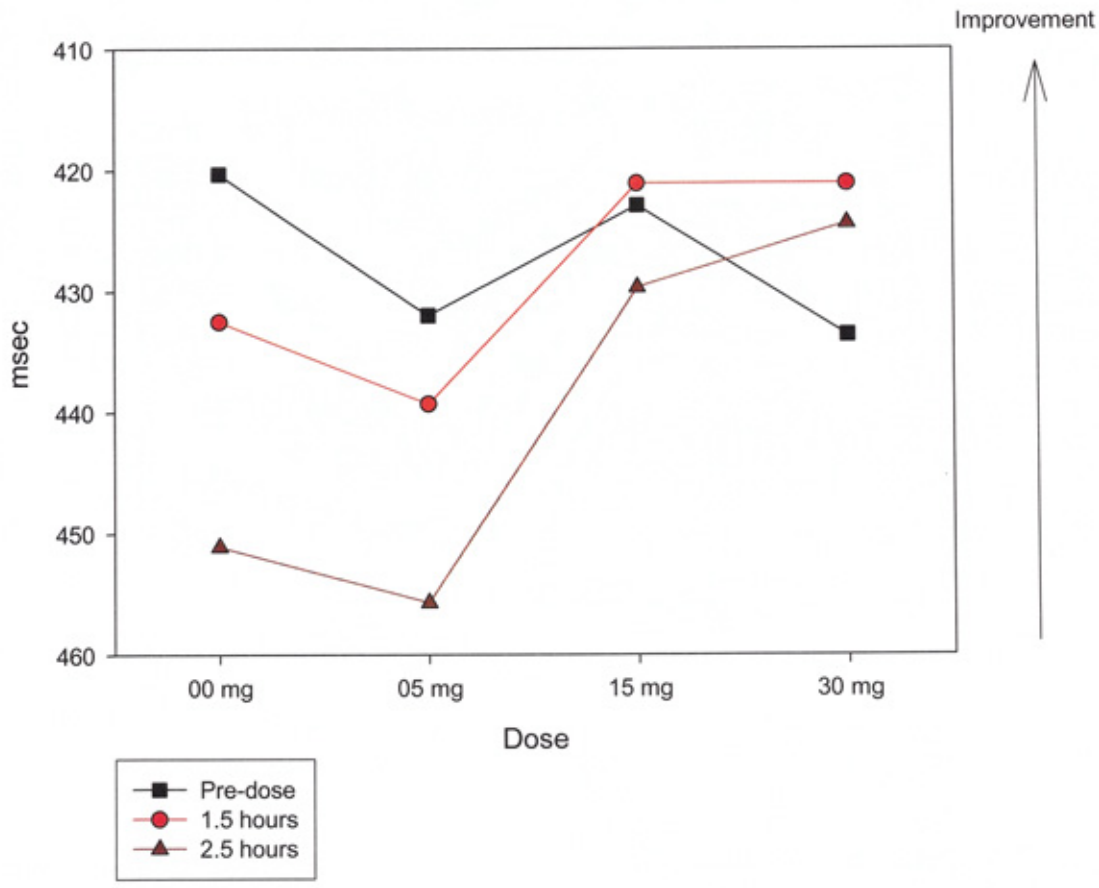


Figure 18: Effect of Amphetamine Analogue on Digit Vigilance Targets Detected (mean)

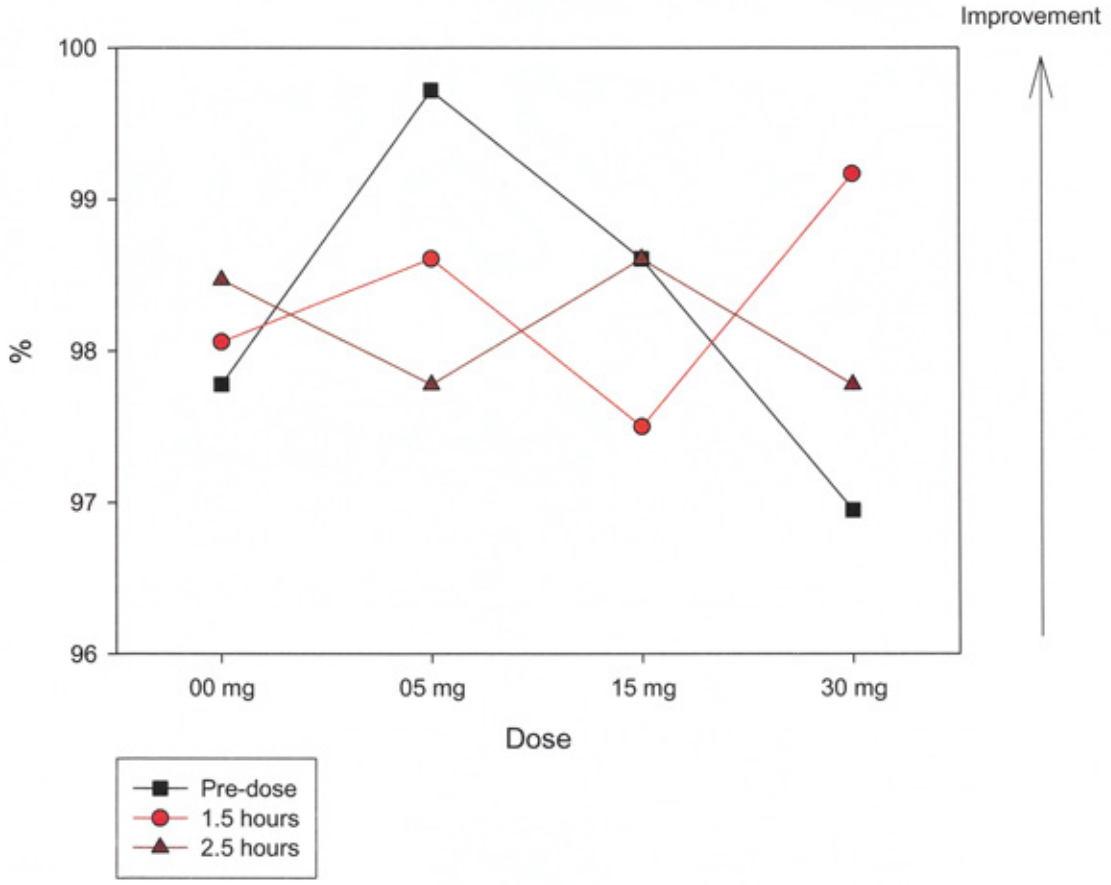


Figure 19: Effect of Amphetamine Analogue on Cognitive Reaction Time (mean)

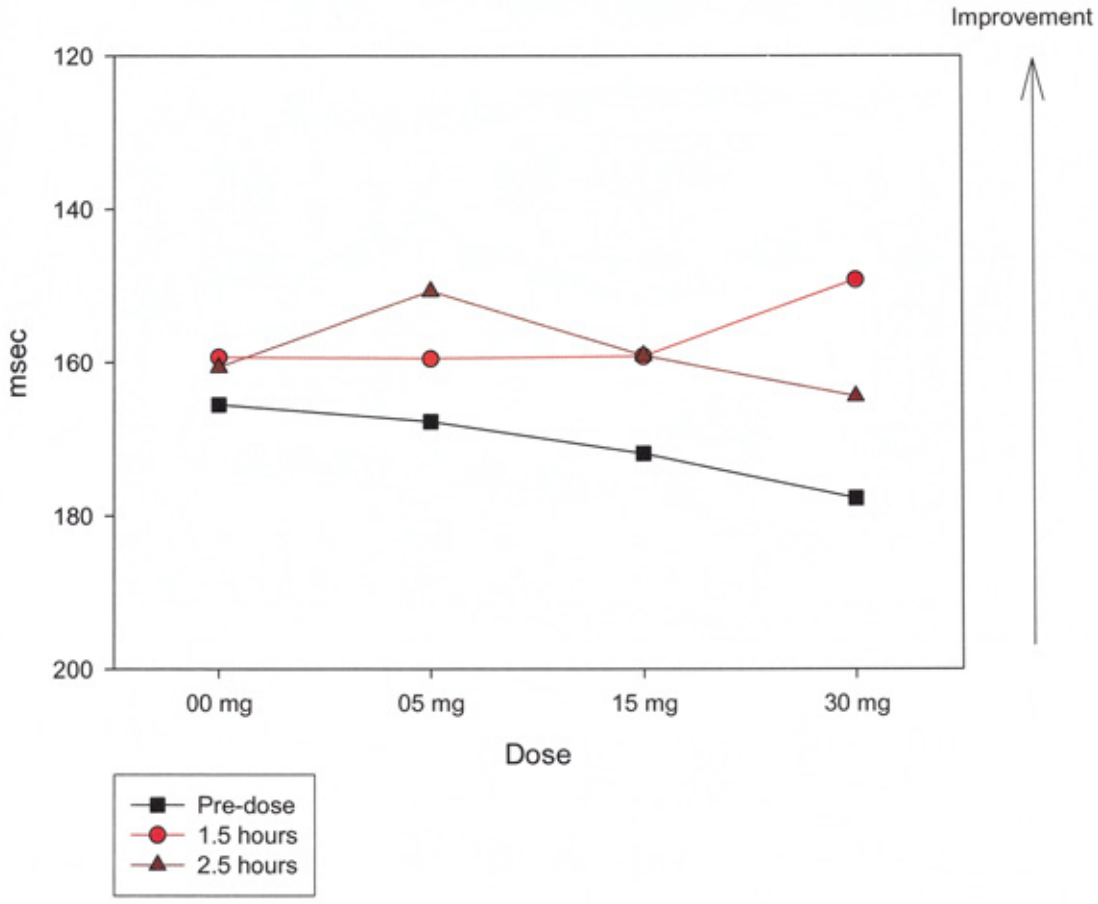
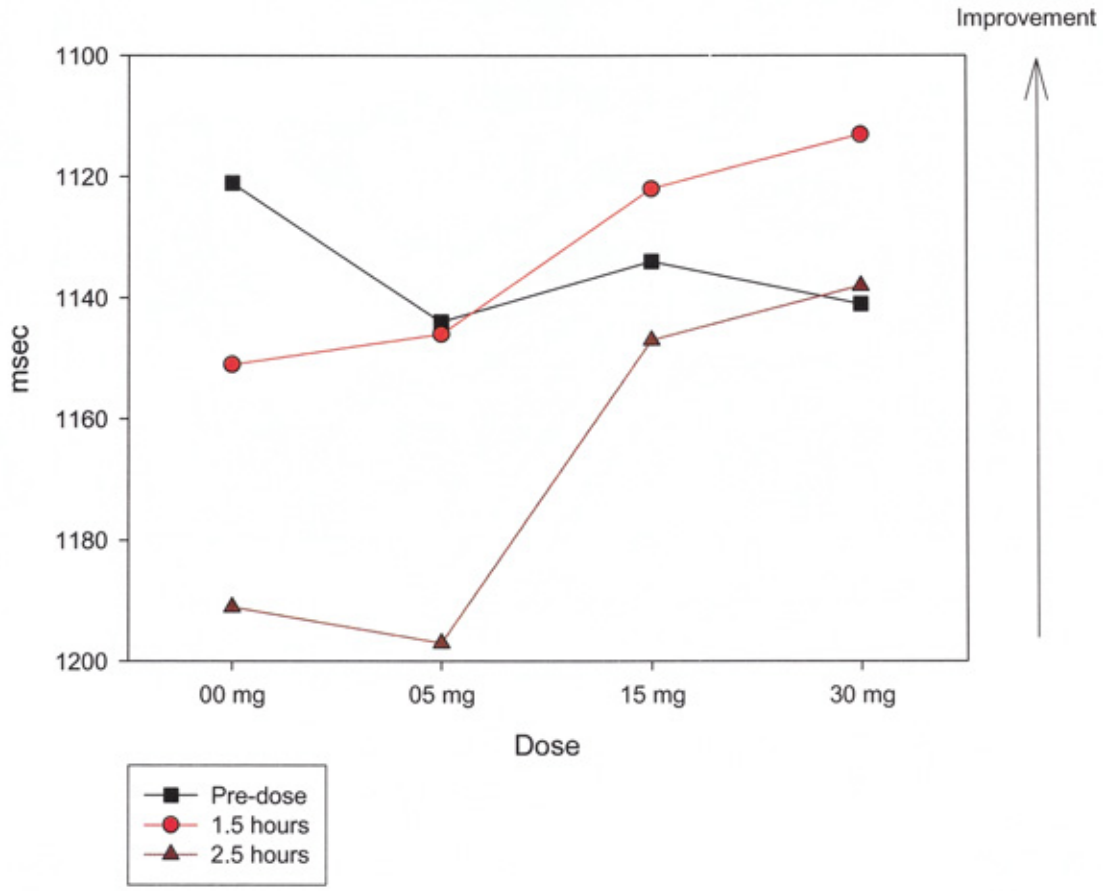


Figure 20: Effect of Amphetamine Analogue on Power of Attention (mean)



7.6 Discussion

The results demonstrated a benefit of the compound to speed of response on the Simple Reaction Time, Choice Reaction Time and Digit Vigilance tasks. Linear trends were identified for all three tasks, with additional support for benefits from the composite reaction time measure Power of Attention (Figure 20). The trends supported faster, responses with increased doses of the compound. The ANOVAs and trend analyses performed on the pre-dose data indicated that this was not the result of any baseline differences between the dose groups e.g. due to change in performance over the successive periods. Considering the data at pre-dose, two different patterns emerged. The first was for a decline in performance (increased response times) with repeated assessment. The tasks showed declines with placebo and the 5.0 mg dose from pre-dose to 1.5 hours and then to 2.5 hours. This suggested that some effect of repeated assessment or the study environment resulted in a decrement to performance e.g. increasing fatigue or reduced motivation. The effect of the compound on Simple Reaction Time and Digit Vigilance Speed was to prevent this decrement in a dose dependent fashion primarily for the 15 and 30 mg doses at 2.5 hours post-dose, when the greatest decrement was evident. No decrement was seen to performance with the other measures, and this may in part account for why no effects of the compound were evident here, as it may primarily act to prevent decrements to performance. However, the Choice Reaction Time and Digit Vigilance Speed measures showed a linear trend at 1.5 hours, which indicated some ability to improve performance above pre-dose levels. This suggests several different factors may have been interacting to influence performance. Early effects of the compound (1.5 hours) may be able to produce improvements above pre-dose performance on measures particularly sensitive to these effects (Choice Reaction Time, Digit Vigilance Speed). However, later effects (2.5 hours) may be most apparent on measures more sensitive to a decline in performance with repeated assessment (Simple Reaction Tie and Digit Vigilance).

The observed benefits are open to two possible interpretations. The compound may affect some aspect of attention, resulting in improved speed of response in these tasks, which have a strong attentional component, or it may affect motor control. Improvements to the accuracy or the Cognitive Reaction Time measures would have provided the clearest support for a benefit to attention over motor control, however this was not observed. If improvements to accuracy were identified, this would demonstrate some benefit to information processing in the tasks, strengthening an interpretation of improved attention. Similarly the Cognitive Reaction Time measure assesses information processing, approximating the 'choice' portion of the response time in the Choice Reaction Time task. However, effects on these measures were not identified. Therefore, an interpretation of enhanced motor control is still possible.

In the present study the pattern of findings may have been due to several factors. There may be ceiling effects on the accuracy measures, which prevent the identification of benefits in healthy subjects, or the compound itself may not be potent enough or given at a high enough dose to demonstrate these benefits. These benefits may then only become apparent when accuracy is impaired in some way. Therefore, the data may be interpreted as demonstrating the sensitivity of the reaction time measures to either attentional or motor effects of DA activation. It is notable that here, the benefit in comparison to placebo with the 15 and 30 mg doses on the Power of Attention measure was greatest at 2.5 hours, where reaction times were longer generally. This might suggest that a greater effect of the compound could emerge under conditions of mild impairment, through some effect of the repeated assessment or time of assessment. Early studies using the Mackworth Clock test (Mackworth 1961 and 1965) indicated that amphetamine was able both to improve performance on measures of accuracy and reaction time, and attenuate the size of the vigilance decrement, consistent with the effects seen here. And it is possible that the effect of repeated assessment in the present study was to produce a kind of vigilance decrement from trial to trial, though this was not within a single task.

A potential criticism of the study design was the relatively low number of subjects (N=8). This was intended to reduce the risk of potential adverse events to the lowest number of experimental subjects, basing the power on pharmacokinetic measures. However, the study used a cross-over design, and therefore made comparisons within subjects, which is more powerful than a parallel group design. In addition, the 2 placebo periods were pooled providing a group of N=16. Consideration of the data showed both statistically significant effects on reaction time data, and little evidence for dose related patterns in data on the other measures, which may not have reached statistical significance due to the small sample size. Therefore, it is unlikely that the results were influenced to any great extent by a lack of power.

Both Schizophrenia and Parkinson's disease provide examples of disorders where DA pathology may be the underlying cause of cognitive dysfunction. Schizophrenia has been modelled as a state of hyper DA function. In acute schizophrenia it has been proposed that attentional dysfunction may be the result of impaired latent inhibition (causing superior learning about pre-exposed cues), which results in a deficit to selective attention, through heightened distractibility. This is supported by disruption of latent inhibition in the rat following d-amphetamine administration, and exaggerated latent inhibition in humans following DA antagonist administration (Robbins, 1991). In Parkinson's disease it has been recognised that the deficiency in DA is uneven through the brain, and that DA replacement therapy may lead to differential cognitive responses, with some aspects of function are enhanced, whilst others are impaired (Kaisinen et al., 2002). D-amphetamine administration in healthy subjects has been shown to produce differential responses

on various task measures. D-amphetamine has been shown to improve response speed and accuracy, but only in task conditions requiring selective attention (Servan-Schreiber et al., 1998). It has been demonstrated that processing may be speeded only in hard response conditions, whilst distraction time is reduced only in easy response conditions (Halliday et al., 1990). The effects of the DA agonist pergolide were found to be dependent on additional aspects such as task difficulty, and pre-existing ability (Kimberg et al., 2003).

In conclusion, the reaction time measures were undoubtedly sensitive to the effects of the compound, and this effect most likely occurred through some cognitive effect of DA activation (e.g. Halliday et al., 1990; Servan-Schreiber et al., 1998; Nieoullon 2002). However, it was not possible to demonstrate sensitivity of the accuracy and Cognitive Reaction Time measures. Despite this, the CDR measures are clearly sensitive to both attentional impairments resulting from DA dysfunction in disease states and from DA antagonists, and the effects of DA activation. Therefore, the study supports the validity of the tasks as a tool for investigating human attention, particularly in relation to modulation of the DA system.

8 Identification of the functional interactions between serotonin and dopamine on human attention

8.1 Abstract

This study outlines the outcome of the first four-way, cross-over study, investigating the effects of acute tryptophan depletion (ATD), acute tyrosine / phenylalanine depletion (ATyrD) and combined depletion (ATD+AtyrD), in comparison to a balanced control condition.

The effects of lowering serotonergic neurotransmission (acute tryptophan depletion), lowering dopamine neurotransmission (acute tyrosine / phenylalanine depletion), and a combination of these manipulations on attention, were assessed in 16 healthy young male and female subjects using a double-blind, placebo controlled, four-way cross-over design. On each test day assessments were completed at baseline and 5 hours post-drink. Assessments included a 45-minute Mackworth Clock Test and a Digit Vigilance task to measure sustained attention, and simple and choice reaction time tests, and a Stroop Colour Word Test for selective attention. In addition, assessments of mood, plasma amino acid levels, and prolactin levels were made.

It was proposed that; i) an acute reduction of serotonergic neurotransmission by acute tryptophan (TRP) depletion would improve selective attention and sustained attention, ii) an acute reduction of dopamine neurotransmission by acute tyrosine (TYR) and phenylalanine (PHE) depletion would impair selective attention and sustained attention, iii) the performance effects of reduced serotonergic neurotransmission would be attenuated by concomitant lowering of dopamine neurotransmission, iv) neither manipulation would affect mood or alertness.

Two-way analysis of variance was conducted with factors for drink-condition and time. Significant drink-condition*time interactions were identified for ratios of tryptophan, tyrosine, and phenylalanine, with reductions in the predicted directions ($p<0.001$). However, no effects of drink condition were identified on measures of attention, mood or plasma prolactin. Plasma prolactin levels declined from pre to post-drink in all conditions, which was inconsistent with the reduction in ratios of tyrosine and phenylalanine against the other large neutral amino acids (LNAA) in the acute tyrosine / phenylalanine depletion and combined depletion conditions.

Robust acute tryptophan depletion and acute tyrosine / phenylalanine depletion was identified in terms of both plasma concentrations and ratios, in the predicted directions. However, effects on prolactin did not support the predicted effects on dopamine neurotransmission. Furthermore, no cognitive effects were identified. The results provide further evidence that acute tryptophan depletion and

acute tyrosine / phenylalanine depletion techniques do not produce reliable effects on attention and working memory.

8.2 Introduction

The neurotransmitter serotonin has been implicated in a wide range of behavioural functions, such as mood regulation, impulsivity, and aggression. Serotonergic disturbances are linked to a great variety of psychopathological states, including depression, bipolar disorder, obsessive compulsive disorders, post-traumatic stress disorder, and ADHD.

In the past decade, it has become clear that serotonin may also play an important role in normal and disturbed cognitive functioning (Meneses 1999; Buhot, Martin et al. 2000). Serotonin is implicated in long-term memory function and cognitive flexibility (Rogers, Blackshaw et al. 1999; Riedel, Klaassen et al. 2002). Experimental studies have also identified various attention functions that are modulated by serotonin. For example, a reduction of central serotonergic activity by means of acute tryptophan depletion was associated with improved focussed attention, as measured by the Stroop Test (Schmitt, Jorissen et al. 2000; Booij, van der Does et al. 2003), dichotic listening (Schmitt, Jorissen et al. 2000) and EEG measures (Ahveninen, Jaaskelainen et al. 2003). Further, several studies of selective serotonin reuptake inhibitors (SSRIs) have consistently shown that enhancement of serotonergic neurotransmission induces decrements in sustained attention (Ramaekers, Muntjewerff et al. 1995; O'Hanlon, Robbe et al. 1998; Schmitt 2002; Schmitt, Ramaekers et al. 2002).

It is known that dopamine is of major importance in the modulation of attentional functions (for a review see (Nieoullon 2002). Enhancement of DA activity can improve both selective attention (Servan-Schreiber, Bruno et al. 1998) and sustained attention (Koelega 1993), whereas impaired performance is seen following dopamine inhibition (Nicholson and Pascoe 1990; Koelega 1993; Kahkonen, Ahveninen et al. 2001; Kahkonen, Ahveninen et al. 2002; Nieoullon 2002). Recent research has supported theories that serotonin may mediate the dopaminergic system and that this may be a key system through which serotonin influences attention. It is known that serotonin exerts significant inhibitory influences over dopamine networks via post-synaptic 5-HT2A/2C receptors located at the substantia nigra and ventral segmental area, as well as on DA afferents in striatal, limbic, and cortical areas (Kapur and Remington 1996; Millan, LeJeune et al. 2000; Daw, Kakade et al. 2002). Changes in frontocortical and mesolimbic DA activity due to altered 5-HT modulation were proposed to underlie the observed attentional changes following 5-HT manipulation (Ahveninen, Kahkonen et al. 2002; Schmitt 2002; Schmitt, Ramaekers et al. 2002), but this has never been systematically investigated.

The current study was designed to further investigate the functional interactions between serotonin and dopamine neurotransmitter systems with regard to human attention functions. The effects of a lowering of serotonergic neurotransmission (by means of acute tryptophan depletion), lowering of dopamine neurotransmission (by means of acute tyrosine depletion), and a combination of these manipulations on various attentional parameters was assessed (see below). The combined condition allowed an insight in the role of serotonergic/dopaminergic interactions on the cognitive parameters assessed. Specifically, based on above considerations, it was expected that the cognitive effects of low serotonin levels would be attenuated by concomitant dopamine inhibition. This study will provide the first evidence of functional serotonergic-dopaminergic interactions with regard to human attention and working memory functions.

The Cognitive Drug Research (CDR) Ltd. computerised assessment system has been utilised in a large number of clinical trials and includes three primary tests of attention; Simple Reaction Time, Choice Reaction Time and Digit Vigilance. Sponsored commercial trials have indicated the sensitivity of the CDR measures to drug induced enhancements and decrements. However, the sensitivity of these measures in acute tryptophan depletion has not been clearly demonstrated, or compared to tasks of focussed attention (e.g. Stroop) and longer vigilance tests (e.g. 45 minutes Mackworth Clock Task) versus the 3-minute Digit Vigilance task. A further aim of the research was to validate the CDR tests within the acute tryptophan depletion paradigm.

The CDR system (Wesnes et al., 1987) has been widely used to detect drug effects on human cognitive performance, including the effects of the monoamine oxidase inhibitor moclobemide (Anand et al., 1990), and the monoamine reuptake inhibitors sibutramine, reboxetine and paroxetine (Wesnes et al., 2000; Ferguson et al., 2003). In addition the CDR Digit Vigilance task has previously shown impairments to both accuracy and reaction time following a combined monoamine depletion (tryptophan, tyrosine, phenylalanine deficient), in comparison to a balanced control (Matrenza et al., 2004).

To control for possible confounding by mood changes and global sedation or activation, visual analogue scales assessing mood and activation were incorporated in the test battery. Furthermore, plasma prolactin levels were assessed as a neuroendocrine marker of central dopamine and serotonin activity.

8.3 Hypotheses

- An acute reduction of serotonergic neurotransmission by acute tryptophan depletion would improve selective attention and sustained attention in young healthy subjects.
- An acute reduction of dopamine neurotransmission by acute tyrosine and phenylalanine depletion will impair selective attention and sustained attention in young healthy subjects.

- The performance effects of reduced serotonergic neurotransmission will be attenuated by concomitant lowering of dopamine neurotransmission.
- Neither manipulation will affect mood or alertness.

8.4 Methods and materials

8.4.1 Subjects

The study was planned to recruit a total of twenty male and female subjects. All subjects underwent a screening protocol including pre-screening and a standard medical questionnaire on physical and mental health. Previous studies have shown that with a sample population of 12 to 24 subjects, the effects of tryptophan and tyrosine depletion can be detected, with the assessment methods used in the current study (Riedel, Klaassen et al. 1999; Schmitt, Jorissen et al. 2000; Harmer, McTavish et al. 2001; Nathan, Harrison et al. 2002; Booij, van der Does et al. 2003; Harrison, Olver et al. 2003; Matrenza, Hughes et al. 2003; Nathan, Hughes et al. 2003).

Exclusion criteria

Subjects suffering from or with a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness were excluded. Other exclusion criteria were excessive drinking (>20 glasses of alcohol containing beverages a week), pregnancy or lactation, use of medication other than oral contraceptives, use of illicit drugs, and any sensory or motor deficits, which could reasonably be expected to affect test performance. Those subjects who had a first-degree relative with a psychiatric disorder or a history of a psychiatric disorder were also excluded.

Inclusion criteria

Inclusion criteria were: 21 to 35 years of age, healthy (i.e. absence of all exclusion criteria), normal static binocular acuity (corrected or uncorrected), body mass index between 18.5 and 30, and willingness to sign informed consent.

8.4.2 Ethics

The study was run at Maastricht University, NL. Before the start of the study approval of the study protocol by the Medical Ethics Committee of Maastricht University and the Maastricht Academic Hospital's Board of Directors was obtained. The study was supported by a research grant from Euron, Marie-Curie.

8.4.3 Design

The study was conducted as a double-blind, placebo controlled, four-way crossover design. Twenty-one, young (18-45), healthy male and female subjects were recruited to attend a medical examination, a practice session during which subjects were familiarised with the cognitive tests used in the study, as well as four test days, each separated by a minimum five-day washout period. On completion of the study seventeen subjects had completed all four test days (mean age 23.2 years, standard deviation 4.2; 15 male, 2 female), whilst at least one test day had been completed by all twenty-one subjects.

Individual assignment to order of treatment condition was randomised using a Latin-square design, with each participant undergoing each treatment. These treatments consisted of 1] acute tryptophan depletion 2] acute tyrosine and phenylalanine depletion 3] combined tryptophan, tyrosine and phenylalanine depletion 4] placebo.

8.4.4 Treatments

A) Acute Tryptophan depletion

Acute tryptophan depletion (ATD) causes a temporary global reduction of 5-HT synthesis in the brain by decreasing the availability of the serotonin precursor L-tryptophan in the brain (Young et al., 1985). Oral administration of tryptophan-free amino acid mixture stimulates protein synthesis, thereby clearing tryptophan from the blood. In addition, it reduces the transport of plasma tryptophan into the brain by increasing the amount of Large Neutral Amino Acids (LNAA), which compete, with tryptophan for the active transport sites across the blood brain barrier. Administration of 100 g of a tryptophan-free amino acid mixture has been shown to cause a significant lowering of 5-HT synthesis throughout the brain after 5 hours in healthy human subjects (Nishizawa, Benkelfat et al. 1997). Although there is no direct evidence in humans, serotonin release and thus serotonergic neurotransmission is presumably reduced as well (Young and Leyton 2002). This method has been known for more than 25 years (Biggio, Fadda et al. 1974) and over the last decade it has become increasingly popular as a methodological tool for serotonergic studies in humans (Reilly, McTavish et al. 1997; Bell, Abrams et al. 2001).

The acute tryptophan depletion amino acid mixture (100 g) consisted of fifteen amino acids in the following composition: 5.5 g L-alanine, 3.2 g glycine, 3.2 g L-histidine, 8.9 g L-lysine, 12.2 g L-proline, 6.9 g L-serine, 6.5 g L-threonine, 4.9 g L-arginine, 2.7 g L-cysteine, and 3.0 g L-methionine; LNAA's: 8.0 g L-isoleucine, 13.5 g L-leucine, 5.7 g L-phenylalanine, 6.9 g L-tyrosine, 8.9 g L-valine.

B) Acute Tyrosine and Phenylalanine depletion (ATPD)

The syntheses of dopamine and noradrenaline in the brain is dependent on the availability of their precursor amino acid tyrosine from plasma. Acute administration of an amino acid mixture that selectively lacks both tyrosine, and its precursor phenylalanine, results in a marked lowering of dopamine synthesis. Evidence suggests that noradrenergic function is not significantly affected (McTavish, Cowen et al. 1999; Harmer, McTavish et al. 2001).

The ATPD mixture consisted of the aforementioned acute tryptophan depletion mixture minus 6.9 g tyrosine and 5.7 g phenylalanine, plus 2.3 g tryptophan (Hughes, Matrenza et al. 2003; Matrenza, Hughes et al. 2003; Nathan, Hughes et al. 2003).

C) Combined acute tryptophan depletion and ATPD

For the combined inhibition of serotonin and dopamine the aforementioned acute tryptophan depletion mixture minus 6.9 g tyrosine and 5.7 g phenylalanine was

used (Hughes, Matrenza et al. 2003; Matrenza, Hughes et al. 2003; Nathan, Hughes et al. 2003).

D) Placebo
The placebo mixture consists of the acute tryptophan depletion mixture with 2.3 g added tryptophan (Schmitt, Jorissen et al. 2000).

All mixtures were suspended in 200 ml tap water prior to administration and flavoured with black-current in order to disguise the unpalatable taste.

8.4.5 Assessments

On each testing day subjects were assessed twice: at baseline and 5 hours after administration of the amino acid drink. The assessments consisted of several cognitive tasks, mood scales and other questionnaires and the collection of a blood sample. The complete battery took around 2 hours to complete.

COGNITIVE ASSESSMENTS

All tasks were computer-controlled, the information being presented on high resolution screens, and the responses recorded via a response module containing two buttons, one marked 'NO' and the other 'YES' (with the exception of the Stroop task, which was presented as printed cards and required verbal responses). The cognitive tests were administered in the following order:

Stroop Colour Word Test (Stroop 1935)
First a card with 100 colour names is read as quickly as possible by the volunteer, followed by a card in which the same number of coloured patches must be named. On the third card colour names are printed in incongruous coloured ink. The colour of the ink must be named and not the word itself. The outcome parameters are completion time for each card and the interference measure: (time card III / (time card I + time card II)/2)*100.

CDR Attention Tests
Simple Reaction Time
Digit Vigilance
Choice Reaction Time

Computerised Mackworth Clock task
The computerised 'Mackworth Clock' task displayed a simulated 'clock dial' on the screen as a circle consisting of 60 lights. The lit point moved around the 'clock dial'

one point at a time in a clockwise direction. Occasionally the light jumped two points at a time. The volunteer was instructed to continuously view the 'clock dial' and when they noticed this jump press a button marked 'YES' as quickly as possible. The task lasted 45 minutes, with 30 target jumps. The stimulus presented, the button press, and the response time for each individual response were recorded.

MOOD SCALES AND OTHER SUBJECTIVE MEASURES

Profile of Mood States (POMS) (McNair, Lorr et al. 1971)

The POMS is a self-evaluation scale for short, alternating states. The POMS consists of 72 adjectives comprising six bipolar mood factors (Energetic-Tired, Elated-Depressed, Agreeable-Hostile, Confident-Unsure, Composed-Anxious and Clearheaded-Confused). Next to each adjective is a five-point scale. In this way, the respondent can indicate in what amount these items are appropriate to his mood.

Bond and Lader Visual Analogue Scales (Bond and Lader 1974)

The Bond and Lader VAS is an often-used self-evaluation mood rating scale. In total, 16 dimensions of mood are given. The subject is asked to mark, on a 100 mm line to what extent the described state is appropriate to him/her at that moment in time. The Bond and Lader VAS distinguishes three affective dimensions; alertness, contentment and calmness.

Vegetative side effects

A list (5-point scales) of 10 vegetative side effects was completed. The list contains the following items: headache, feeling cold, feeling hot, dizziness, transpiration, blurred vision, nausea, palpitations, dry mouth and abdominal complaints.

BLOOD ANALYSES

Amino Acid concentrations

Blood samples (10 ml) were collected by venipuncture in sodium heparin tubes. Following collection, blood samples were placed on ice immediately after collection, and centrifuged at 4°C (5 min. at 5000 rpm) within 30 minutes. Subsequently, 100 µl plasma was mixed with 4 mg sulfasalicyl acid, frozen in liquid nitrogen and stored at -80°C until quantitative amino acid analysis by high-performance liquid chromatography (van Eijk, Huinck et al. 1994). The total plasma concentration of long chain amino acids (LNAAs: tryptophan, tyrosine, valine, phenylalanine, leucine and isoleucine), and the ratio between plasma levels of individual LNAAs and other LNAAs was determined.

Prolactin

Following collection, blood samples were placed on ice immediately after collection, and centrifuged at 4°C (5 min. at 5000 rpm) within 30 minutes. Plasma samples were stored at –30°C until analysis using a standard immuno-radiometric assay.

PROCEDURE

After enrolment in the study, subjects underwent a training session. During this session all cognitive tests were practised to familiarise the subjects with the study procedures and minimise procedural learning effects (Wesnes and Pincock, 2002).

On the day prior to each test day the use of alcohol or drugs was prohibited. Subjects were instructed to arrive at the laboratory well-rested (following a normal night's sleep), after an overnight fast (except water) starting at 2200. One cup of coffee or tea (without milk or sugar) was allowed on the morning of the test day to prevent possible caffeine withdrawal effects. Female subjects were tested in the follicular phase of the menstrual cycle.

Upon arrival (0800) subjects completed the mood and sleep quality questionnaires, and subsequently performed the first set of assessments (mood, cognition, blood sample / total 2 hours). Then, they received the amino acid drink (1000), which was to be consumed within 15 minutes. During the ensuing four hours subjects remained in the laboratory. At 1300 hours subjects received a low-tryptophan, low-protein lunch. Starting at 1500 the second assessments (mood, cognition, blood sample) were conducted. The test day ended at 1700.

8.4.6 Statistical Analysis

Analysis was carried out using SAS v6.12. Plasma prolactin, amino acid levels, cognitive, mood and sleep measures were analysed using repeated measures analysis of variance (ANOVA) with drink, time and period as within-subject factors.

8.5 Results

Data were obtained at baseline and 5 hours from a total of 17 subjects completing all four study periods. A number of subjects dropped out of the study following nausea during the first study or second period. Results are reported for the 17 subjects completing all four periods unless otherwise stated.

Biochemical measures

Table 1 shows the results of blood samples for the 17 subjects completing all four periods.

Plasma tryptophan decreased by 65.7% in the tryptophan depleted condition and by 35.1% in the tyrosine and typtophan depleted condition. In the balanced condition plasma tryptophan declined by 1.8%, whilst in the tyrosine depleted condition it increased by 17.2%.

Plasma tyrosine decreased by 68.5% in the tyrosine depleted condition and by 62.9% in the tyrosine and typtophan depleted condition. In the balanced condition plasma tyrosine increased by 163%, whilst in the tryptophan depleted condition it increased by 144.5%.

Plasma phenylalanine decreased by 48.6% in the tyrosine depleted condition and by 43.8% in the tyrosine and typtophan depleted condition. In the balanced condition plasma phenylalanine increased by 12.7%, whilst in the tryptophan depleted condition it increased by 21.2%.

Analysis of plasma levels indicated significant drink by time interactions for tryptophan [$F(3,102) = 92.93$, $p < 0.001$], tyrosine [$F(3,102) = 160.83$, $p < 0.001$], phenylalanine [$F(3,102) = 62.65$, $p < 0.001$], and tyrosine+phenylalanine [$F(3,102) = 170.16$, $p < 0.001$].

Analysis of the ratio of plasma levels to the LNAA indicated significant drink by time interactions for tryptophan:LNAA [$F(3,102) = 47.09$, $p < 0.001$], tyrosine:LNAA [$F(3,102) = 135.13$, $p < 0.001$], phenylalanine:LNAA [$F(3,102) = 25.08$, $p < 0.001$], and tyrosine+phenylalanine:LNAA [$F(3,102) = 120.04$, $p < 0.001$].

Table 9 shows that both the balanced and tryptophan depletion conditions increased tyrosine+phenylalanine:LNAA ratio, while this was reduced in the tyrosine and combined conditions, consistent with the predicted effects. Tryptophan:LNAA ratio was reduced in all conditions, though the magnitude of the change in each condition was again consistent with the predicted effects.

Analysis of plasma prolactin levels (U/l) showed no significant drink by time interaction. However a significant effect of time was seen [$F(3,95) = 22.52$, $p < 0.001$], with a reduction in prolactin levels evident in each condition. No prolactin data were available for subject 12. Therefore, only 15 subjects contributed data to this analysis.

Table 9: Plasma levels (nm/ml) and ratio to other large neutral amino acids (LNAA) for tryptophan, tyrosine, phenylalanine and tyrosine and phenylalanine combined, plasma levels of prolactin (U/l), at baseline and 4 hours post-drink ingestion for each drink condition

	Balanced		Tryptophan-depleted	
	Baseline	Post-drink	Baseline	Post-drink
tryptophan (nm/ml)	36.69 ± 6.22	36.02 ± 6.34	38.91 ± 6.96	13.33 ± 5.73 **
tryptophan : LNAA (ratio)	7.20 ± 1.08	4.48 ± 1.26 **	7.69 ± 1.29	1.64 ± 0.92 **
tyrosine (nm/ml)	49.44 ± 12.68	130.01 ± 30.42 **	50.79 ± 13.03	124.16 ± 29.00 **
tyrosine : LNAA (ratio)	9.92 ± 2.28	17.98 ± 4.64 **	10.11 ± 1.60	16.59 ± 2.80 **
phenylalanine (nm/ml)	52.93 ± 7.30	59.64 ± 13.63 *	53.80 ± 8.09	65.18 ± 8.37 **
phenylalanine : LNAA (ratio)	10.72 ± 1.12	7.58 ± 1.87 **	10.92 ± 1.17	8.25 ± 1.31 **
tyrosine + phenylalanine (nm/ml)	102.37 ± 17.44	189.65 ± 37.04 **	104.59 ± 19.95	189.34 ± 34.38 **
tyrosine + phenylalanine : LNAA (ratio)	32.15 ± 4.72	40.81 ± 8.86 **	32.85 ± 4.11	38.50 ± 5.26 **
Prolactin (U/l)	0.207 ± 0.052	0.187 ± 0.065	0.211 ± 0.058	0.180 ± 0.047

Continued on following page

	Tyrosine-depleted		Tyrosine/Tryptophan-depleted	
	Baseline	Post-drink	Baseline	Post-drink
tryptophan (nm/ml)	39.02 ± 6.45	45.74 ± 6.27 **	38.06 ± 6.64	24.71 ± 6.75 **
tryptophan : LNAA (ratio)	7.48 ± 0.80	6.06 ± 1.05 **	7.40 ± 1.12	3.72 ± 1.40 **
Tyrosine (nm/ml)	50.78 ± 14.55	16.02 ± 5.36 **	50.06 ± 13.92	18.55 ± 10.22 **
tyrosine : LNAA (ratio)	9.81 ± 2.00	2.07 ± 0.79 **	9.88 ± 2.23	2.81 ± 1.88 **
phenylalanine (nm/ml)	53.22 ± 9.39	27.38 ± 7.74 **	52.78 ± 7.88	29.64 ± 9.28 **
phenylalanine : LNAA (ratio)	10.54 ± 0.84	4.53 ± 1.89 **	10.47 ± 1.10	3.63 ± 1.35 **
tyrosine + phenylalanine (nm/ml)	104.00 ± 23.14	43.40 ± 11.91 **	102.84 ± 20.20	48.19 ± 18.92 **
tyrosine + phenylalanine : LNAA (ratio)	31.33 ± 4.61	8.12 ± 2.74 **	31.77 ± 4.32	10.69 ± 5.68 **
Prolactin (U/l)	0.233 ± 0.057	0.178 ± 0.074 *	0.223 ± 0.066	0.162 ± 0.045 **

Data are mean ± SD. Statistical significance relates to the t-tests (Baseline Vs Post-drink) from the ANOVA model * $p < 0.05$, ** $p < 0.001$.

Figure 21: Change in the Ratio of Tyrosine (TYR) and Tryptophan (TRP) to other Large Neutral Amino Acids for each Drink Condition (mean +/- sem)

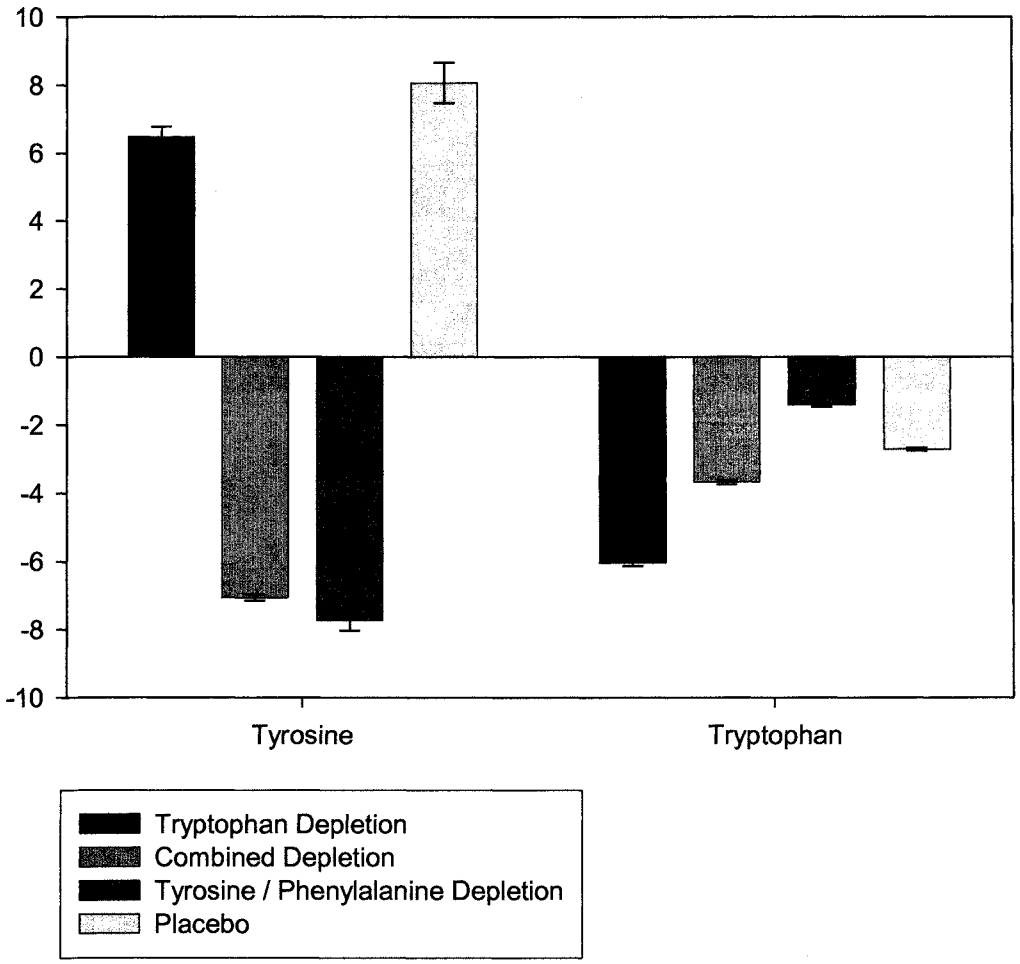
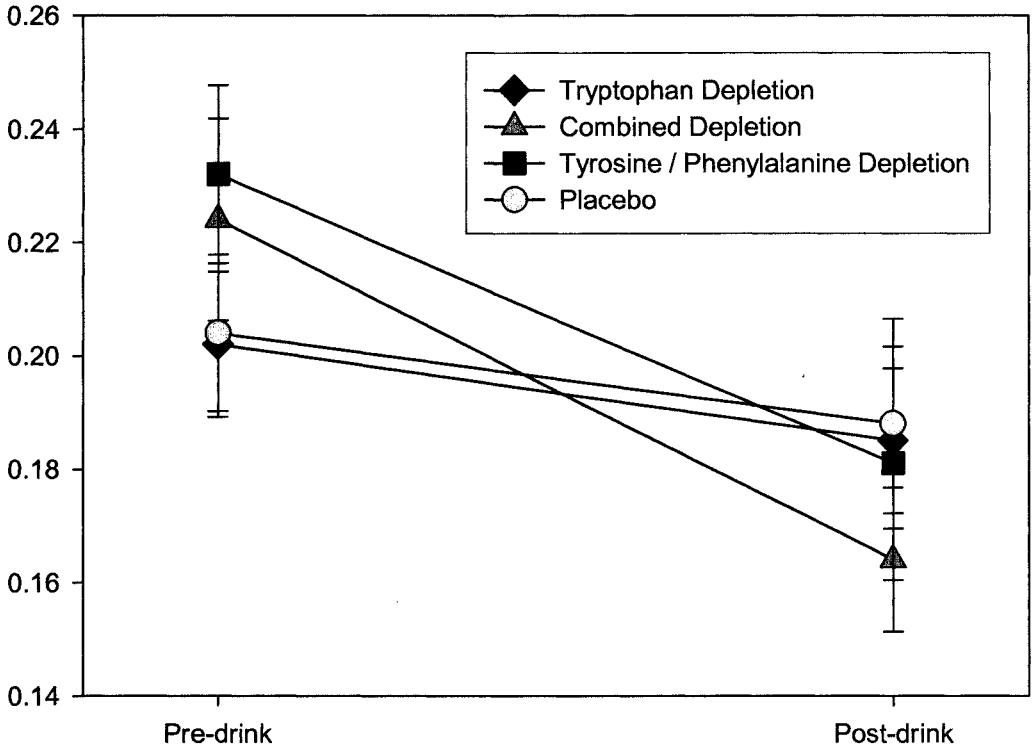


Figure 22: Change in Prolactin (U/L) for each Drink Condition (mean +/- sem)



Attention Assessments

Table 2 shows the results of attention assessment for the 17 subjects completing all four periods. For the Mackworth Clock task subject 15 detected no targets correctly post-drink in period 2 therefore had no reaction time ('speed') data, and subject 18 did not perform the task during period 2.

Analysis of the data showed no significant drink by time interactions.

Table 10: ANOVA analysis of attention assessments

Assessment of Attention	F value	P Value
Simple Reaction Time	F(3,102)=0.40	0.7556
Choice Reaction Time	F(3,102)=0.56	0.6444
Digit Vigilance – Targets Detected	F(3,102)=0.22	0.8851
Digit Vigilance – Speed	F(3,102)=1.04	0.3791
Stroop Interference	F(3,102)=0.52	0.6705
Mackworth Clock – Targets Detected	F(3,100)=0.92	0.4324
Mackworth Clock – Speed	F(3,99)=0.27	0.8465
Mackworth Clock – False Alarms	F(3,100)=0.23	0.8732
Power of Attention	F(3,102)=0.17	0.9134
Continuity of Attention	F(3,102)=0.67	0.5732
Response Variability	F(3,102)=1.02	0.3858

Mood Assessments

Analysis of the mood assessments (POMS and Bond-Lader VAS) for the 17 subjects completing all four periods showed no significant drink by time interactions. No mood data were available for subject 19. Therefore only 16 subjects contributed data to this analysis.

8.6 Discussion

Table 11: Comparative efficacy of depletion treatments

	Balanced	Tryptophan-depleted	Tyrosine-depleted	Tyrosine/Tryptophan-depleted
tryptophan (nm/ml)	-1.8% (+154.2%)	-65.7% (-97.4%)	+17.2% (+113.6%)	-35.1% (-45.8%)
tryptophan : LNAA (ratio)	-37.8% (-34.8%)	-78.7%	-19.0%	-49.7% (-86.4%)
tyrosine (nm/ml)	+163.0% (+184.4%)	+144.5% (+168.1%)	-68.5% (-63.8%)	-62.9% (-73.75%)
tyrosine : LNAA (ratio)	+81.3% (-28.2%)	+64.1%	-78.9%	-71.6% (-93.8%)
phenylalanine (nm/ml)	+12.7% (+184.2%)	+21.2% (+132.9%)	-48.6% (-80.6%)	-43.8% (-78.1%)
phenylalanine : LNAA (ratio)	-29.3% (-30.84%)	-24.5%	-57.0%	-65.3% (-94.5%)

Balanced and combined depletion values in brackets from Matrenza et al (2004). Tryptophan and Tyrosine/Phenylalanine depletion values in brackets from Harrison et al (2004).

Comparative efficacy of depletion treatments

Data from Matrenza et al (2004) and Harrison et al (2004) highlight two important issues in the monoamine depletion methodology (Table 11). Firstly, it is clear that on occasions absolute % change does not bear a close relationship to change in ratio. As control of synthesis is immediately controlled by competition between tryptophan, tyrosine, phenylalanine, valine, leucine and isoleucine, which share the same mechanism of uptake, it is critical ratios are reported in addition to actual values, as this will give the clearest guide to effects on synthesis. Secondly, when considering the effects of any one treatment on a single monoamine, the concomitant changes in the other monoamines cannot be ignored (Badawy, 2005). Badawy argues that this second point may explain why effects of monoamine depletion are not consistently identified, due to simultaneous increase in synthesis of one transmitter whilst synthesis of another is reduced. In the present study, it

would be expected from the hypotheses that the increases for example in tyrosine:LNAA ratio, whilst tryptophan:LNAA ratio reduced in the acute tryptophan depletion treatment, would have resulted in an even greater behavioural effect than if only tryptophan:LNAA had been affected. In fact, what may be more critical to evaluate in terms of the paradigm used is why such large effects of the balanced condition should be apparent, and why the effects of the control would differ so greatly between this study and that of Matrenza et al (2004). However, this does suggest that the effects of acute tryptophan depletion previously identified may not be solely due to a reduction in serotonin synthesis, but rather a concomitant increase in dopamine synthesis due to changes in competition between the LNAAs (Figure 21).

Comparison to previous studies of monoamine depletion

Schmitt et al (2000) identified improved attention compared to a control condition following acute tryptophan depletion in healthy male and female subjects, while Matrenza et al (2004) found that simultaneous acute tryptophan depletion and acute tyrosine / phenylalanine depletion (combined depletion) in healthy female subjects impaired attention. In the Matrenza et al (2004) study, the same Digit Vigilance task as employed in the present study was administered, with results in line with the experimental hypotheses investigated here. However, in a study of selective effects of acute tryptophan depletion and acute tyrosine / phenylalanine depletion compared to a control condition, in healthy female subjects again employing the Digit Vigilance task, no effects on attention were identified (Harrison et al., 2004). In these two studies (Matrenza et al., 2004; Harrison et al., 2004) all of the conditions employed in the present study have been investigated using the same task battery, but the effects on attention have not been consistent. Furthermore, cognitive effects of monoamine depletion have not been identified consistently in all studies. Recent studies still question the ability of acute dietary tyrosine depletion to affect neuropsychological function (Lythe et al., 2005). Though in the present study, the lack of effects in the predicted direction on prolactin may indicate a failure of the experimental manipulations in influence DA function, rather than question the manipulation more generally. Additionally, acute tryptophan depletion has also recently failed to show effects on attention in healthy male subjects (Hughes et al., 2003).

It is notable that a previous tryptophan and tyrosine/phenylalanine depletion study (Harrison et al, 2004), failed to find effects on the attention tests from the CDR battery (Simple and Choice Reaction Time and Digit Vigilance). This is in contrast to studies which have shown tryptophan depletion to improve focussed attention (Stroop Test: (Schmitt, Jorissen et al. 2000; Booij, van der Does et al. 2003) and SSRIs to induce decrements to sustained attention (Ramaekers, Muntjewerff et al. 1995; O'Hanlon, Robbe et al. 1998; Schmitt 2002; Schmitt, Ramaekers et al. 2002). Therefore, potentially it may be argued that the CDR

system measures were not sensitive to the manipulations. However, this possibility is argued against by the lack of sensitivity on the Stroop and Mackworth Clock tasks in the present study, and the sensitivity of the Digit Vigilance measure to combined depletion (tryptophan, tyrosine, phenylalanine deficient) in Matrenza et al (2004). The study has not therefore provided any validation of the CDR tests in the present paradigm.

Conclusions

The role of the 5-HT system in attention has yet to be fully characterised, although there are data from a number of sources, which implicate the 5-HT system in inhibitory processes that can influence attention. The role of the DA system in attention is strongly supported, with consistent evidence to support a role in modulating attentional performance. The lack of the predicted attentional effects in the present study may be attributed to a number of possible factors. Evidence of the neuropsychological effects of monoamine depletion is inconsistent, and this may reflect an inability of dietary manipulation to influence behaviour in a measurable way, or more specifically an inability of dietary manipulation to influence attention. Another factor to draw out is the relative change in ratios produced by the experimental manipulations. The effects of the control conditions can be seen to vary quite widely between studies, and the success of the experimental manipulation (fasting and monoamine drink) and the resultant difference between control/balanced drinks, which have an effect on ratios themselves and the experimental (depleted) treatments may be important.

The number of depletion studies, which have now been conducted, may allow for meta-analyses of data to approach the question of relative depletion ratio. In particular, the investigation of the effects of balanced control conditions, which typically produce changes in ratios of LNAAs themselves may help to define threshold levels for behavioural effects.

9 A Comparison of the Effect of Escitalopram versus Sertraline on Focussed and Vigilant Attention in Healthy Young Subjects

9.1 Abstract

Sertraline in contrast to citalopram, fluoxetine and paroxetine does not impair vigilance performance using the Mackworth clock task, and this has been suggested as evidence for sertraline exerting mild central dopamine agonism. The present study compared relative side effect profiles of sertraline and escitalopram treatment by assessment of cognitive and driving performance, and CYP-2D6 activity. The current reports outlines the results from the cognitive function assessments only.

The study was a single-site, randomised, double-blind parallel group comparison of the effects of escitalopram (20mg/day) versus sertraline (100mg/day) in young (18–45 years of age) healthy subjects (n=32) on cognitive and driving performance, and CYP 2D6, as assessed by changes in the clearance of metoprolol. Cognitive performance was assessed using the Cognitive Drug Research (CDR) battery (repeated assessments on each day) and included a computerised version of the Mackworth Clock test for vigilance (single assessment on each day). Tests were administered on day 1 and on day 16 of the assessment period. Data were analysed using SAS® PROC MIXED, ANOVA models.

Clear differentiation was not seen between escitalopram and sertraline on measures of attention. However, on the Mackworth clock test, there was some evidence for a differential treatment effect dependent on time of day.

The data did not support impaired performance with escitalopram compared to sertraline. This finding was unexpected. These findings provide further support for the benign effect of these two SSRIs on higher cognitive function related to memory, attention, reaction time, and vigilance. Given the widespread use of these medications, these findings are reassuring as to their safety from these important perspectives.

9.2 Introduction

Behavioral neuropsychopharmacological studies have provided evidence that particular attentional functions are mediated by cholinergic, dopaminergic, noradrenergic, and serotonergic neurochemical modulation. The monoamines dopamine (DA) and acetylcholine (ACh) are thought to be primarily associated with internally driven, slow changing 'energetic state', rather than externally mediated attention related functions, whilst noradrenaline (NA) is believed to be primarily involved with externally driven, rapid and transient changes in response to stimuli. In contrast, the neurotransmitter serotonin (5-HT) is thought to inhibit the activity of these other neurotransmitter systems (Robbins, 1997).

Serotonin selective reuptake inhibitors (SSRIs) are the most widely used antidepressants. There are six marketed SSRIs: citalopram, escitalopram (S-citalopram), fluoxetine, fluvoxamine, paroxetine, and sertraline. While many of the adverse effects of SSRIs are similar, differential effects have been seen on vigilance performance. Fluoxetine has been shown to impair performance on the 45 minute Mackworth Clock task (Ramaekers et al., 1995). Venlafaxine, a 5-HT reuptake inhibitor, which also inhibits reuptake of NA, has also been shown to impair vigilance performance (O'Hanlon et al., 1998). O'Hanlon et al., propose that the inhibitory action of 5-HT on neurotransmitters modulating attention/arousal (DA, ACh, NA) is the mechanism behind this reduction in vigilance. Further, it has been proposed that inhibition of DA, rather than NA, is critical to this effect on vigilance performance. Early studies identified that d-amphetamine, which increases extracellular DA, was able to prevent or protect against the typical vigilance decrement in detections and reaction time in the classic Mackworth Clock task (e.g. Mackworth, 1961; Mackworth 1965). In contrast, NA does not appear to be critical to vigilance performance as impairment was identified with Venlafaxine, which inhibits NA reuptake in addition to 5-HT (O'Hanlon et al., 1998).

Sertraline is an SSRI that, in addition to its inhibition of 5-HT reuptake, possesses a relatively high affinity for the human DA transporter and may also facilitate DA neurotransmission (Tatsumi et al., 1997). Sertraline has been demonstrated to inhibit DA reuptake in vitro with one-third the potency of D-amphetamine (Bolden-Watson et al., 1993) and does not appear to increase prolactin levels in humans (for a mini-review of SSRI effects on neuroendocrine function see Raap et al., 1999). Prolactin release is associated with increased serotonergic input, but is inhibited by dopaminergic input, suggesting a DA effect of sertraline antagonising the 5-HT effect on prolactin. Sertraline in contrast to citalopram, fluoxetine, paroxetine, and the serotonin-norepinephrine reuptake inhibitor venlafaxine does not impair vigilance performance using the Mackworth clock task and this has been suggested as further evidence for sertraline exerting mild central dopamine agonism (Schmitt et al., 2002). Schmitt et al. contend that enhanced serotonergic neurotransmission leads to amplified inhibition of DA

neurotransmission, which in turn results in reduced vigilance. However, with sertraline the effects of enhanced 5-HT inhibition of DA neurotransmission may be attenuated by simultaneous stimulation of DA neurotransmission.

S-citalopram is the most potent and selective of the SSRIs and represents the most useful compound for determining the effects of inhibition of the neuronal uptake pump for serotonin on focused and vigilant attention performance. The present study was designed to assess, amongst other parameters, whether s-citalopram has an adverse effect on focused and vigilant attention performance, in comparison to the cognitive profile seen with sertraline. This would further support the theory that effects of the SSRIs on vigilance are primarily mediated by 5-HT inhibition of DA.

9.3 Hypotheses

- It was hypothesised that s-citalopram would have an adverse effect on vigilant attention, which would not be apparent with sertraline, and task performance would therefore differentiate the two compounds.

9.4 Methods and Materials

9.4.1 Subjects

Thirty-two healthy subjects (16 male, 16 female), between 20 and 45 years of age (mean 30.6, standard deviation 6.4), were recruited. During screening, the mental and physical health of each volunteer was assessed by means of a health questionnaire, medical examination, routine electrocardiogram, blood haematology and chemistry and standard urine screening. A urine test for pregnancy was conducted for women. A urine test for drugs of abuse was conducted both at screening and prior to each cognitive assessment. Excluded were subjects with any history of or current cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness. Other exclusion criteria were; use of prescription or non-prescription medication within the past week, a requirement for any medication (including vitamins and herbals) during the course of the study (except as allowed by the study physician), personal or family history of long QT syndrome or any EKG abnormality considered by the study physician to be clinically significant, history of seizure disorder, donation of blood within the last 30 days, treatment with any investigational drug within the last 30 days, history of intolerance or allergy to citalopram, s-citalopram, metoprolol or sertraline, a usual caffeine intake greater than three caffeinated drinks per day.

9.4.2 Ethics

The study was conducted at The Psychiatric Research Institute, Whicita, USA and received local Institutional Review Board approval. The study was supported by an investigator initiated research grant from Pfizer Inc.

9.4.3 Design

The study was a single-site, randomised, double-blind, parallel group comparison of the effects of subchronic treatment (16 days) with s-citalopram (20 mg/day) versus sertraline (100 mg/day) in young (18–45 years of age) healthy subjects on cognitive performance.

9.4.4 Treatment

Treatment with s-citalopram and sertraline began at 10 and 50 mg/day, respectively on day 1 and was advanced to 20 and 100 mg/day on day 4 and then stayed constant throughout the remainder of the study. All doses were administered with approximately 240 mL of water. Doses were administered either in the morning (approximately 8 AM) or afternoon (approximately 2 PM), on each study day.

9.4.5 Assessments

Cognitive performance was assessed using tasks from the Cognitive Drug Research (CDR) battery, which also included a computerised version of the Mackworth Clock task for vigilance. The assessments were completed on day 1 and on day 16 of the administration of either s-citalopram or sertraline. Subjects were admitted to the unit on day 0 prior to dinner and were tested on day 1. After completion of the pre-dose tests, they received their first dose of either 10 mg of s-citalopram or 50 mg of sertraline and were then discharged from the unit. They were re-admitted to the unit on the evening of day 15 prior to dinner and the procedures repeated on day 16. The CDR battery was completed at pre-dose (7 AM or 1 PM), 2 hours post-dose (10 AM or 4 PM), and 4 hours post-dose (12 PM or 6 PM). The Mackworth Clock task was administered following the 2 hours post-dose CDR assessment only (10.30 AM or 4.30 PM).

CDR battery

The CDR computerised cognitive assessment system (Wesnes et al., 1987) has been widely used to detect drug effects on human cognitive performance, including the effects of the monoamine oxidase inhibitor moclobemide (Anand et al., 1990), and the monoamine reuptake inhibitors sibutramine, reboxetine and paroxetine (Wesnes et al., 2000; Ferguson et al., 2003). Parallel forms of the tasks were

administered at each assessment. Stimuli were presented on colour monitors, and all responses were made via response boxes with two buttons, one marked "YES" and one marked "NO". The tasks were completed in the following order:

- Immediate Word Recall
- Simple Reaction Time
- Digit Vigilance
- Choice Reaction Time
- Delayed Word Recall
- Word Recognition
- Picture Recognition

Task parameters and assessments procedures matched those for previous studies (e.g. Wesnes et al., 2000; Ferguson et al., 2003).

Computerised Mackworth Clock task

The computerised 'Mackworth Clock' task displayed a simulated 'clock dial' on the screen as a circle consisting of 60 lights. The lit point moved around the 'clock dial' one point at a time in a clockwise direction. Occasionally the light jumped two points at a time. The volunteer was instructed to continuously view the 'clock dial' and when they noticed this jump press a button marked 'YES' as quickly as possible. The task lasted 45 minutes, with 30 target jumps. The stimulus presented, the button press, and the response time for each individual response were recorded.

9.5 Statistical Analysis

CDR task variables

For each measure (excluding the Mackworth Clock task variables), repeated measures analysis of covariance (ANCOVA), was conducted on the difference from baseline data using SAS PROC MIXED. Fixed terms were fitted to the model for treatment (escitalopram, sertraline), day (Day 1, Day 16), visit (2 hours, 4 hours), time (AM, PM), and the treatment*time, treatment*day, treatment*visit, time*day, time*visit, day*visit, treatment*time*day, treatment*time*visit, treatment*day*visit, time*day*visit, and treatment*time*day*visit interactions. A random effect of subjects was fitted to the model. Pre-dose (baseline) scores by Day were used as a covariate. Significance of the interactions was tested at the 0.05 level. All testing was two-tailed. Stepwise removal of non-significant interactions was completed, removing higher order interactions first. If the interaction was found to be significant, appropriate comparisons were conducted between treatments.

Mackworth Clock task variables

ANCOVA was conducted on the difference from baseline data using SAS PROC MIXED. Fixed terms were fitted to the model for treatment, time, and the treatment*time interaction. A random effect of subjects was fitted to the model. Day 1 (baseline) scores were used as a covariate. Significance of the interaction was tested at the 0.05 level. All testing was two-tailed. If the interaction was found to be significant, appropriate comparisons were conducted between treatments.

Analyses were conducted on all available subject data.

9.6 Results

All subjects completed the protocol. S-citalopram and sertraline were both well tolerated and no unexpected or serious adverse effects occurred.

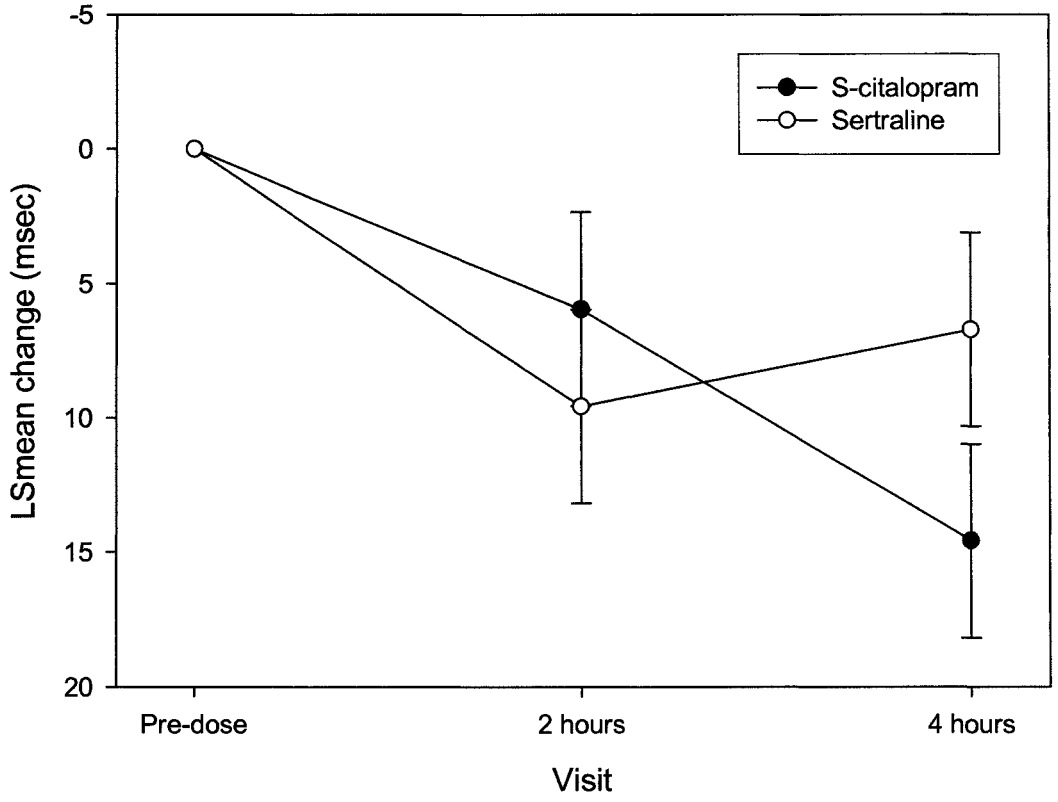
Primary Analysis (CDR task variables)

This analysis was designed to consider the effect of treatment at post-dose visits (2 and 4 hours), on Days 1 and 16, and both AM and PM dosing. The analysis included possible single (acute) and multiple (subchronic) dosing effects. From the ten ANCOVAs conducted, two significant treatment*visit interactions ($p \leq 0.05$) were identified, for Simple Reaction Time and Digit Vigilance Speed, and one significant treatment*day interaction for Delayed Word Recall.

Note: plots are LSmeans from the ANCOVA analyses conducted on the difference from baseline data. Therefore, only those factors which were significant in the model were plotted, as non-significant terms were removed.

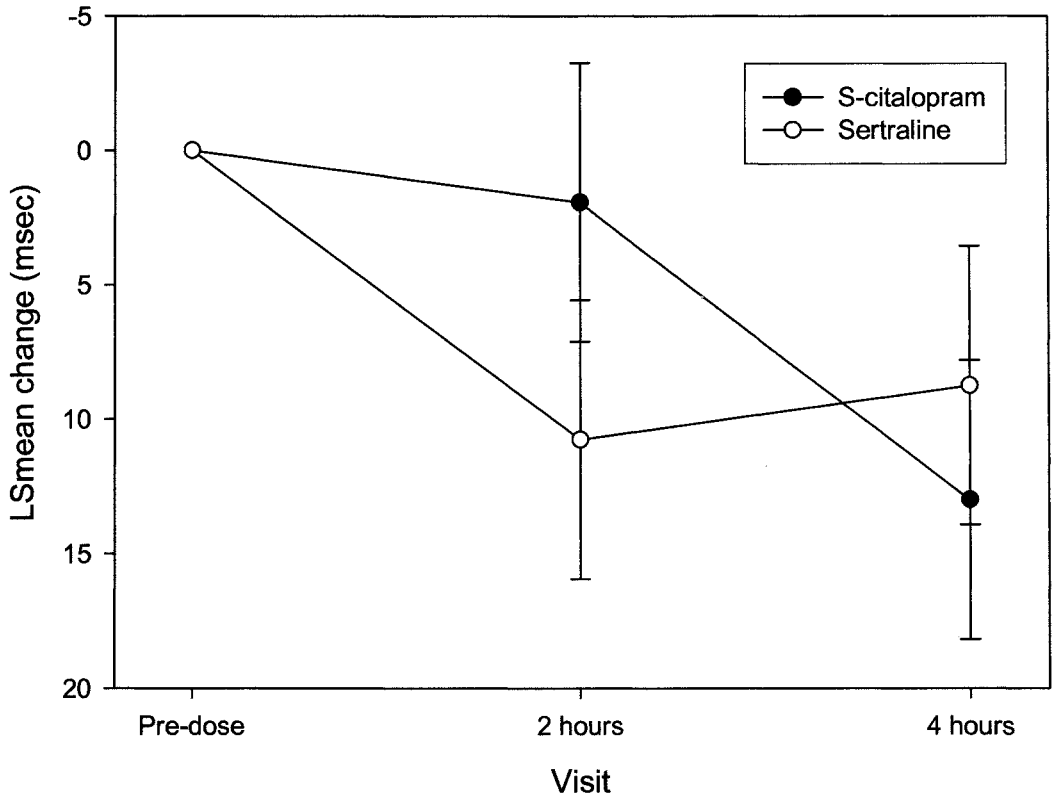
A significant treatment*visit interaction was identified from the ANCOVA for Simple Reaction Time [$F(1,91)=5.13$, $p=0.026$]. The data showed post-dosing declines for both treatments, with the interaction resulting primarily from a smaller 4 hour decline with sertraline. The comparisons did not support significant differences between the treatments at either visit (2 or 4 hours).

Figure 23: Effect of SSRIs on Simple Reaction Time



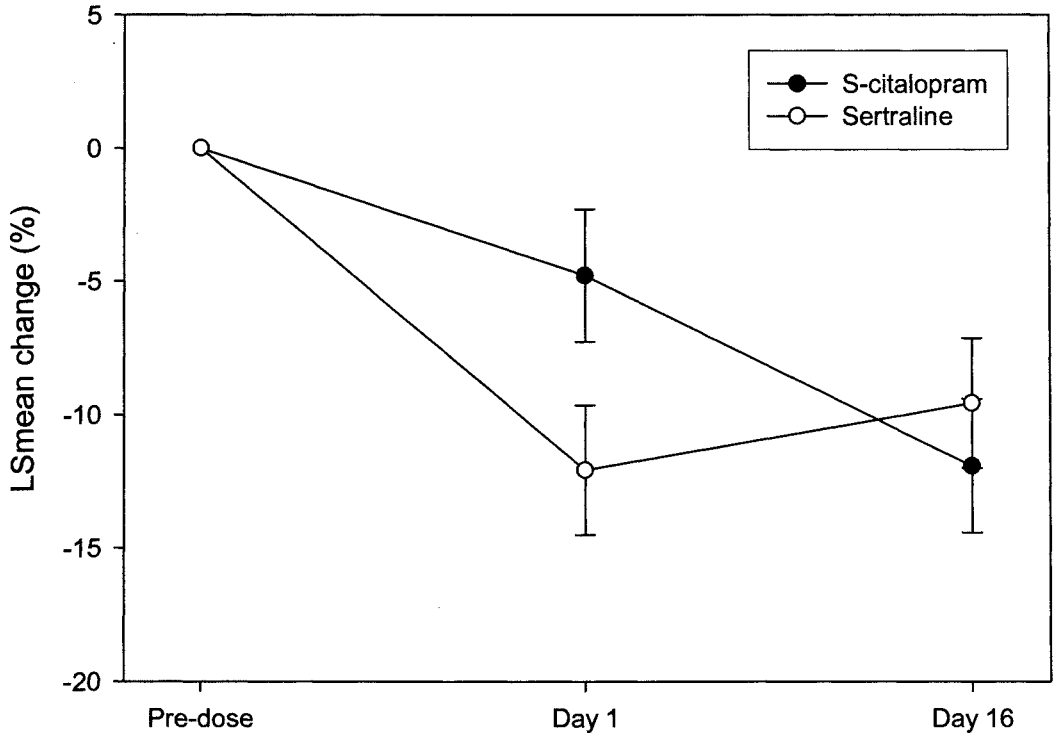
A significant treatment*visit interaction was identified for Digit Vigilance Speed [F(1,91)=4.46, p=0.037]. The data showed that the treatment*visit interaction primarily resulted from a slightly greater 2 hour decline with sertraline, then a reversal of this pattern at 4 hours. The comparisons did not support significant differences between the treatments at either visit (2 or 4 hours).

Figure 24: Effect of SSRIs on Digit Vigilance Speed



A significant treatment*visit interaction was identified for Delayed Word Recall Accuracy [F(1,91)=4.40, p=0.022]. The data showed that the interaction primarily resulted from a greater decline with sertraline on Day 1. The comparisons supported a significant benefit for s-citalopram on Day 1.

Figure 25: Effect of SSRIs on Delayed Word Recall Accuracy



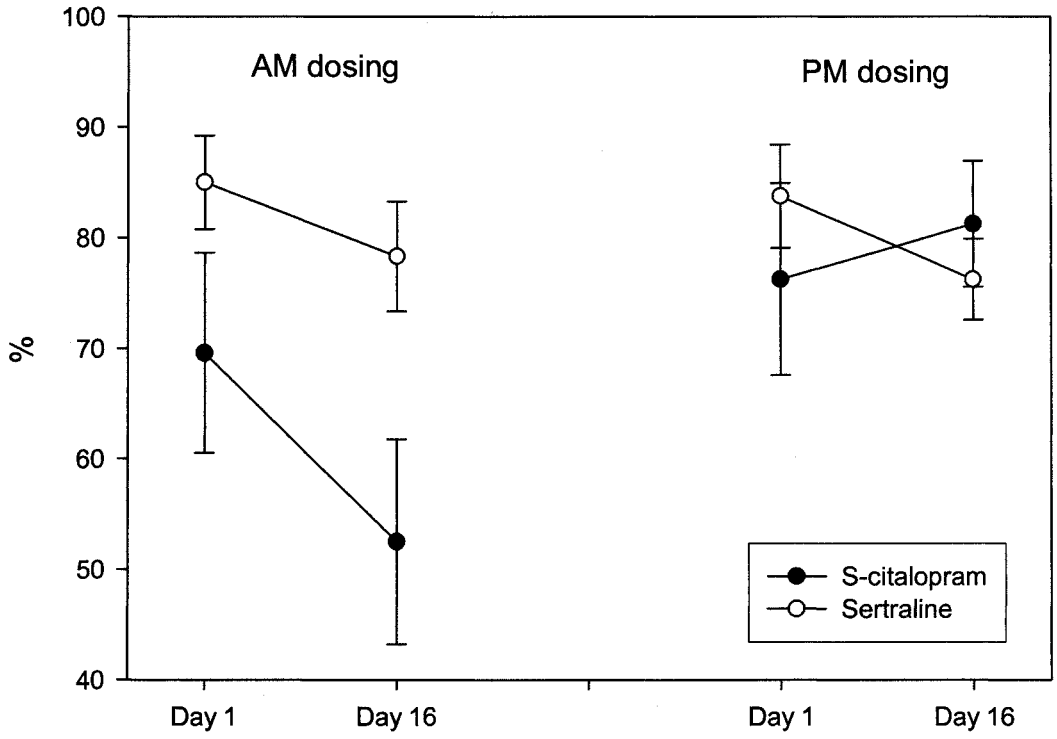
Primary Analysis (Mackworth Clock task variables)

This analysis was designed to look at the effect of multiple dose treatment at Day 16 (change from baseline Day 1), and the possible interaction with AM Vs PM dosing (time). The ANCOVAs conducted on the 3 Mackworth Clock task variables identified significant treatment*time interactions for Targets Detected and Speed ($p \leq 0.05$) with a further signal for a treatment*time interaction for False Alarms ($p \leq 0.1$).

Note: plots are unadjusted means from the descriptive statistics and Day 1 represents pre-dose baseline performance.

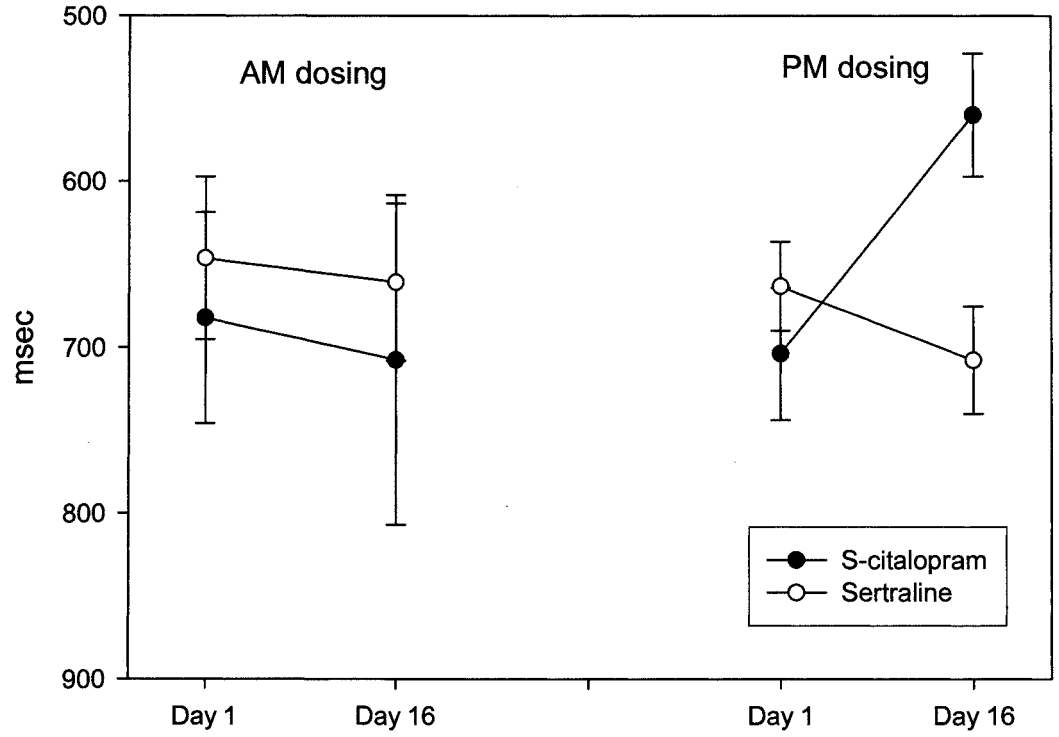
The data for Targets Detected showed a clear Day 16 decline for s-citalopram with AM dosing, with the a different pattern evident with PM dosing, though without clear separation between the treatments. A significant treatment*time interaction was identified from the ANCOVA [$F(1,27)=6.43$, $p \leq 0.05$]. The t-tests from the LSmeans statements showed a significant decrement for s-citalopram against sertraline on Day 16 with AM dosing only ($p \leq 0.05$).

Figure 26: Effect of SSRIs on Mackworth Clock Accuracy (mean +/- sem)



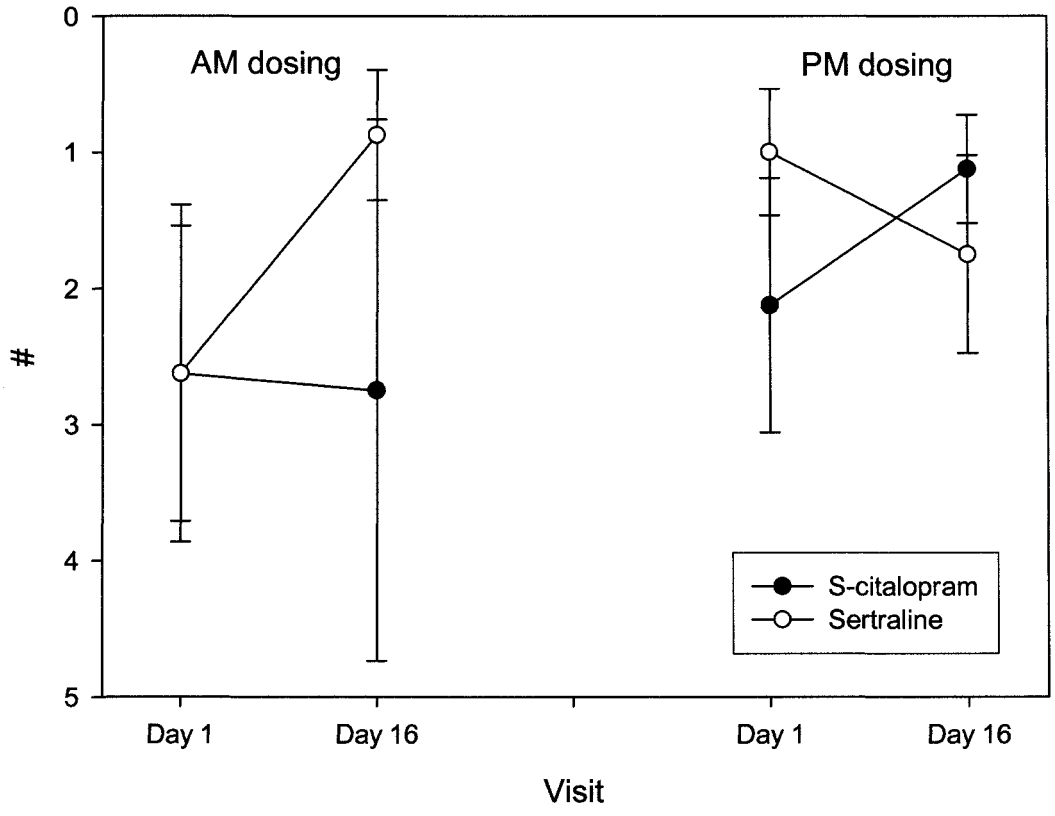
The data for Speed showed very little change in performance with AM dosing. However, with PM dosing, a large improvement was evident on Day 16 with s-citalopram. A significant treatment*time interaction was identified from the ANCOVA [$F(1,27)=6.51$, $p \leq 0.05$]. The t-tests from the LSmeans statements showed a significant benefit for s-citalopram against sertraline on Day 16 with PM dosing ($p \leq 0.05$).

Figure 27: Effect of SSRIs on Mackworth Clock Speed (mean +/- sem)



The data for False Alarms indicated a slight benefit for sertraline with AM dosing, with the reverse pattern evident with PM dosing. A signal for a treatment*time interaction was identified from the ANCOVA [$F(1,27)=3.46$, $p \leq 0.1$].

Figure 28: Effect of SSRIs on Mackworth Clock False Alarms (mean +/- sem)



9.7 Discussion

The data for the CDR task variables did not strongly support a clear pattern of treatment effects. The reaction time data from the tasks tended to show greater decline with sertraline at 2 hours and greater decline with s-citalopram at 4 hours overall (Day 1 acute and Day 16 subchronic). Whilst the accuracy measures tended to show greater decline with sertraline on Day 1 (acute) and greater decline with s-citalopram on Day 16 (subchronic). However, clear adverse effects of s-citalopram on attention following subchronic dosing (Day 16), were not apparent.
The data for the Mackworth Clock task indicated a differential pattern of treatment effects, which was dosing and assessment time dependent. For AM dosing there was limited support for a decrement for s-citalopram against sertraline on the Targets Detected measure (accuracy). This pattern was further supported by the Speed (reaction time) and False Alarms (incorrect responses) measures, which did not indicate any change in response strategy (speed/accuracy trade-off). For PM dosing a benefit for s-citalopram over sertraline was evident for the Speed measure, again this was supported by the pattern of data for the other task variables. However, again clear adverse effects of s-citalopram on vigilant attention following subchronic dosing (Day 16), were not apparent.

The study provided only limited support for predicted differential attentional effects between s-citalopram and sertraline. This was primarily indicated by a greater decline at 4 hours with s-citalopram on the Simple Reaction Time measure (focussed attention), with some limited support from the pattern of data with the Digit Vigilance Speed measure (vigilant attention). Effects of either treatment on focussed attention were not expected (Simple and Choice Reaction Time) as focussed attention has not previously been shown to be consistently adversely affected by SSRI treatment (Schmitt et al., 2002). Furthermore, adverse effects have not been seen on these tasks with acute administration of sibutramine a 5-HT and NA reuptake inhibitor, with no effects on DA (Wesnes et al., 2000), or subchronic treatment with sertraline (Williams et al., 1996). Contrary to the predicted effects, evidence of an adverse effect on vigilance was not seen for s-citalopram. With only a benefit to speed on the Mackworth Clock task emerging independently of time of assessment/dosing. This may have been due either to the cognitive effects of s-citalopram being dependent on time of day of assessment/dosing, or some unique effect of s-citalopram itself, which makes it devoid of adverse effects on vigilance, in contrast to citalopram, fluoxetine, paroxetine, and the serotonin-norepinephrine reuptake inhibitor venlafaxine.

Previous studies have not investigated effects of time of day of cognitive assessment with subchronic SSRI treatment. For example in the Schmitt et al. (2002) study, which identified vigilance impairing effects of paroxetine in comparison to sertraline, cognition was assessed between 12 and 6 PM, with no

AM assessment. Studies have shown patterns of declining alertness/performance with time awake, with a circadian effect superimposed (Horowitz et al., 2003). Therefore, it is theoretically possible that an effect of s-citalopram in the present study was time of assessment dependent, due to an interaction with circadian influences on cognition.

Two main criticisms of the study design are apparent. Firstly, the lack of a placebo may be a flaw in the methodology. Without a placebo it is not clear what effects s-citalopram and sertraline may be having against the bench mark of placebo, i.e. both may be resulting in a decrement or benefit relative to what would be seen with a placebo arm. However, the decision not to include this treatment group was based on a prior study (Schmitt et al., 2002), which demonstrated that paroxetine resulted in a statistically significant decrement to vigilant attention relative to sertraline, but neither treatment was statistically significantly different to the placebo arm which was included in the study. Based on these data, the study was primarily intended to show that sertraline would be similarly differentiated from s-citalopram, rather than to establish the difference from placebo. Furthermore, Reidel et al., (2005) have since shown that citalopram could be similarly differentiated from sertraline following sub-chronic dosing. Therefore, the lack of a placebo arm does not strongly influence conclusions based on the hypotheses of this study, though it does limit the ability to discuss the data in wider context. Secondly, the clearest effect of emerge from the study was for a difference in treatment effect on the Mackworth Clock test dependent on time of day of dosing and assessment. It must be recognised that the study was not designed to investigate this, and the AM / PM dosing and assessment were only included as an economic consideration, so that two groups could be run per day. Therefore, there is a confound between time of dosing and time of assessment and the relative effects of each cannot be differentiated in this study.

S-citalopram does have some unique properties in comparison to previously assessed SSRIs; a more efficient blockade of the serotonin transporter protein, and a lack of other actions such as the anticholinergic effect of paroxetine. Paroxetine has been shown to produce a vigilance decrement and also has a relatively high affinity for muscarinic ACh receptors. In-vitro affinity of paroxetine for muscarinic receptors is six times that of sertraline (Hyttel, 1994). In theory the anticholinergic effects of paroxetine may directly contribute to the effects on vigilance. Though it must also be noted that the SSRI fluoxetine (O'Hanlon et al., 1998) and the SNRI venlafaxine (Ramaekers et al., 1995), drugs without significant anticholinergic effects, have produced vigilance impairment. It was predicted therefore, that the more potent and specific effect of s-citalopram would support and confirm an adverse effect on attention/vigilance through 5-HT inhibition of DA.

The possibility of time of day dependent effects support a second weaker conclusion, of a genuine treatment effect dependent on time of day of assessment/dosing, primarily for the Mackworth Clock task. This differentiation

between the tasks is plausible, given that the CDR tasks Simple and Choice Reaction Time assessed focussed attention, whilst the Digit Vigilance task (which showed some possible treatment effects) and the Mackworth Clock task, both assessed vigilant attention. If this differentiation was genuine there are several possible causes. These could include an effect of time of day of dosing on pharmacokinetics of either drug, or pharmacodynamics (e.g. an interaction with circadian effects and/or sleep architecture). It is unlikely that the pharmacokinetics of either drug were altered by time of day of dosing, as these factors have been evaluated early in the drug development and are part of the investigator brochure information. Sleep effects on cognition are increasingly documented in the domains of vigilance, attention and psychomotor performance (e.g. Van Dongen et al., 2003; Jewett et al., 1999. The AM vs PM assessment gave rise to differential proximity to the circadian mid-afternoon decline in alertness (or "post-prandial dip"). In addition, serotonin is believed to be intimately involved in sleep regulation (Pace-Schott and Hobson, 2002). This potentially gives rise to a complex interaction between potential treatment effects, sleep, cognition and circadian rhythm. As sleep parameters were not assessed during the study, and these aspects can only be discussed in theoretical terms. However, there are known effects of SSRIs on sleep, which have the potential to influence vigilant attention. In healthy subjects using single dose and subchronic treatment, the SSRIs suppress rapid eye movement (REM) sleep, probably through an increase in serotonin in the brain stem (Ridout et al., 2003; Feige et al., 2002; Schlosser et al., 1997). In addition, they may also have other unique effects on sleep parameters related either to additional activity e.g. anticholinergic effects of paroxetine, or relative potency of 5-HT reuptake inhibition (Wilson et al., 2004). Whilst it is known that sleep disruption can impair next day cognitive function a clear relationship between SSRI induced sleep disruption and cognitive impairment has not always been established (Schmitt et al., 2002). Furthermore, it should be noted that effects in patient populations are likely to differ, especially those with impaired sleep and cognition, which is symptomatic of disorders such as depression, and for which SSRIs are a key treatment. Reviews of SSRI tolerability in affective disorders have indicated sleep disruption is associated with fluoxetine, paroxetine and sertraline, whilst also suggesting a relatively better profile for sertraline (e.g. Goldstein et al., 1998). However, escitalopram itself may be effective in reducing sleep disturbance in major depression (Lader et al., 2004). Anecdotal evidence indicates that users of SSRIs may switch from AM dosing to PM dosing to avoid extra-pyramidal effects such as day time subjective sedation, but that this may then lead to increased sleep disruption.

In conclusion, the study did not support a clear vigilance impairing effect of s-citalopram in comparison to sertraline, but may indicate a time of dosing/assessment dependent effect of s-citalopram, which is worthy of further investigation. Also, support was seen for a possible effect on focussed attention,

which was consistent with the hypothesised pattern. Whilst these speculative conclusions are of interest, it should be noted that the study was not designed to assess possible differential effects of AM Vs PM dosing and assessment, with this design employed solely as an economic factor, to allow assessment of a greater number of subjects per day. Therefore, it is not clear whether there was an influence of time of day of dosing, or of time of day of assessment, on cognition or related pharmacodynamic parameters, due to the confound between these potential effects. Furthermore, the lack of a placebo control means that, where a possible treatment effect occurred, it is not certain whether this resulted from effects of either or both treatments. Future studies should attempt to address whether any clear temporal relationship exists between SSRI treatment and effects on cognition, by repeated assessment over the study day. In addition, the potential for time of dosing to influence pharmacodynamic parameters, such as sleep and cognition, should also be investigated.

10 Dapoxetine has no Cognitive Interactions with Ethanol in Healthy Male Volunteers

10.1 Abstract

Dapoxetine is a novel selective serotonin reuptake inhibitor being investigated for the treatment of premature ejaculation. This study evaluated the potential pharmacokinetic and cognitive interactions of dapoxetine 60 mg with ethanol 0.5 g/kg in a single-center, double-blind, randomised, placebo-controlled crossover study in healthy adult male subjects (n=24). Dapoxetine was rapidly absorbed and eliminated; peak concentrations were noted 1.47 hours after administration and decreased with an alpha half-life of 1.33 hours and a terminal half-life of 15.6 hours. Pharmacokinetic parameters (Cmax, AUCinf, t1/2, and Tmax) of dapoxetine were not altered with concurrent ethanol consumption. Furthermore, coadministration of dapoxetine did not affect the pharmacokinetics of ethanol or significantly potentiate the cognitive and subjective effects of ethanol.

10.2 Introduction

While premature ejaculation is a common form of male sexual dysfunction (Laumann et al., 1999), no approved pharmacologic agent is currently indicated for its treatment. Selective serotonin reuptake inhibitor (SSRI) antidepressants are sometimes prescribed off-label for the treatment of premature ejaculation (Montague et al., 2004).

Dapoxetine is a short-acting SSRI developed as an on-demand treatment for premature ejaculation that is rapidly absorbed following oral administration, with peak plasma concentrations approximately 1 hour after administration. Dapoxetine elimination is rapid and biphasic; its alpha (distribution) half-life is approximately 1.4 hours, and its terminal half-life is approximately 20 hours. Dapoxetine-N-oxide, the primary circulating phase I metabolite, has weak serotonin receptor binding and transport inhibition in vitro (>250-fold less than dapoxetine), and does not contribute to clinical efficacy. Desmethyldapoxetine has a similar pharmacologic potency to dapoxetine in vitro, but accounts for <3% of circulating dapoxetine species.

Ethanol has known pharmacodynamic effects on cognitive function, such as impaired reaction time and recall (e.g. van Harten et al., 1992; Wesnes et al., 2000). Peak plasma ethanol concentrations are observed approximately 2 hours after oral administration, and a dose of 0.7 g/kg is associated with a maximum serum ethanol concentration of 502 mg/mL.

Given the potential for SSRIs to impair cognition and the possibility of additive or synergistic cognitive interactions with ethanol, and the likelihood of co-dosing in normal use, this study was designed to examine the potential for interactions between dapoxetine and ethanol in healthy volunteers.

10.3 Methods and Materials

10.3.1 Subjects

Healthy males (ages 18 to 45 years) within 20% of normal weight for height and body build, with a supine blood pressure (BP) of 90–140 mmHg systolic and 50–90 mmHg diastolic, with moderate ethanol intake (5-20 drinks/week) were eligible to participate. Subjects were excluded if they had clinically relevant abnormalities as determined by medical history, physical examination, blood chemistry, complete blood count, urinalysis, and electrocardiogram (ECG), or if they had a positive urine drug screen or alcohol breath test. All were required to use a medically accepted method of contraception throughout the study period and for 3 months after study completion. Consumption of alcohol, caffeine, or products containing grapefruit was not allowed within 48 hours before administration of study medication, and men who regularly consumed >450 mg of caffeine per day were excluded. Subjects with a history of smoking or tobacco use within the past 3 months were also excluded. Subjects were excluded if they had used any prescription or non-prescription

medications (excluding acetaminophen and multivitamins) within 7 days before study start and throughout the study period. Twenty-four subjects (mean age 25.5 ± 6.4 years) were enrolled. Four subjects discontinued early; one withdrew consent, 2 left for personal reasons, and 1 was discontinued for noncompliance.

10.3.2 Ethics

The study was approved by the Ethics Committee of Charterhouse Clinical Research Unit, Ravenscourt Park Hospital, Ravenscourt Park, London, UK, and conducted in accordance with Good Clinical Practice and the International Conference on Harmonization (ICH) Guidelines and Ethics Committee policies, including the ethical principles that have their origin in the Declaration of Helsinki on biomedical research involving human participants. Before participation, each subject was required to read, sign, and date an Ethics Committee approved consent form explaining the nature, purpose, and possible risks and benefits of the study, and the duration of participation.

10.3.3 Design

This was a single-center, double-blind, randomised, 4-treatment, 4-period, crossover study in healthy adult males.

10.3.4 Treatments

Subjects were assigned randomly to 1 of 4 treatment sequences and received each of the following 4 treatments:

1) a placebo tablet followed by ethanol 0.5 g/kg in ginger ale ("ethanol");
2) dapoxetine 60 mg followed by ethanol 0.5 g/kg in ginger ale ("dapoxetine + ethanol");
3) dapoxetine 60 mg followed by ginger ale as placebo for ethanol ("dapoxetine"); and
4) a placebo tablet followed by ginger ale ("placebo")

Ethanol or ginger ale was administered 30 minutes after dapoxetine or placebo, to provide peak concentrations of each at approximately the same time. For the placebo drinks, ethanol-soaked gauze was placed in a double-walled container used to carry the drinks, which were covered in plastic wrap; doses of ethanol in ginger ale were prepared in similar containers covered in plastic wrap. Participants drank doses of either ethanol or placebo through a straw that protruded through the plastic wrap. A washout period of 5 to 21 days was required between treatments.

10.3.5 Assessments

At the initial screening visit, a medical history was obtained, and a physical examination was performed, including vital signs (heart rate [HR], BP, and respiratory rate), 12-lead ECG, blood chemistry, complete blood count, and urinalysis. At each visit, participants were required to pass a urine drug screen and an alcohol breath test. Vital signs were measured at 0 (predose), 1, 2, 4, 6, 8, and 24 hours after administration of dapoxetine or placebo dapoxetine. At study termination, the physical exam, laboratory assessments, vital signs, and an ECG were repeated. All adverse events (AEs) were recorded and assessed in terms of severity and relationship to study drug, and followed until resolution or until the end of the study.

PLASMA ANALYSIS FOR DAPOXETINE AND ITS METABOLITES

Blood samples (5 mL) were collected at 0, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, and 72 hours after administration of dapoxetine for measurement of dapoxetine and its metabolites, desmethyldapoxetine and dapoxetine-N-oxide, using a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method, with a minimum quantifiable dapoxetine concentration of 1.00 ng/mL and minimum quantifiable desmethyldapoxetine and dapoxetine N-oxide concentrations of 0.200 ng/mL, using dapoxetine-d7, desmethyldapoxetine-d7, and dapoxetine-N-oxide-d7 as internal standards. Positive ions were monitored by MS/MS in the multiple reaction monitoring (MRM) mode for dapoxetine (m/z transition from 306.5 to 157.2), dapoxetine-d7 (m/z transition from 313.5 to 164.2), desmethyldapoxetine (m/z transition from 292.3 to 157.2), desmethyldapoxetine-d7 (m/z transition from 299.3 to 164.2), dapoxetine-n-oxide (m/z transition from 322.3 to 157.2), and dapoxetine-N-oxide-d7 (m/z transition from 329.3 to 164.2). The calibration curve was linear from 1.00-1000 ng/mL for dapoxetine and from 0.200-200 ng/mL for the metabolites.

Precision and accuracy were determined by replicate analyses of human plasma quality-control samples spiked with dapoxetine and the metabolites. Precision was measured as the percent coefficient of variation (% CV) of the quality control samples. The ranges of interassay precision were as follows: for dapoxetine, 6.01%-16.1%; for desmethyldapoxetine, 4.61%-16.0%; and for dapoxetine-N-oxide, 3.93%-18.2%. Accuracy was expressed as the percent difference between the mean value for each pool and the theoretical concentration (% bias). The ranges of interassay accuracy were as follows: for dapoxetine, -2.49% to 3.78%; for desmethyldapoxetine, -8.86% to 9.85%; and for dapoxetine-N-oxide, -1.25% to 3.47%.

PLASMA ANALYSIS FOR ETHANOL

Additional blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after administration of dapoxetine for ethanol analysis using a validated high-resolution gas chromatography with mass spectrometry (GC/MS) method, with a minimum quantifiable ethanol concentration of 2.74 µg/mL, and a calibration curve that was linear from 2.74-992.0 µg/mL. Precision and accuracy were calculated as described above; the range of interassay precision for ethanol was 2.4%-5.1%, and the range of interassay accuracy was -4.0% to 0.9%.

COGNITIVE AND SUBJECTIVE ASSESSMENTS

Cognitive function was measured using a battery of tasks from the Cognitive Drug Research (CDR) computerised cognitive assessment system. Prior to the first study day, cognitive function was measured 4 times, twice on each of 2 days, with 1 hour between tests, to familiarise participants with the tests and to minimize any learning effects during the study. The CDR tests were conducted before dosing and 0.5, 1.5, 2.5, 4, 6, 8, 12, and 24 hours after administration of study drug. The following tests were performed: Immediate Word Recall, Simple Reaction Time, Digit Vigilance, Choice Reaction Time, Visual Tracking, Spatial Working Memory, Numeric Working Memory, Delayed Word Recall, Word Recognition, Digit Symbol Substitution Test; and the Bond and Lader Visual-Analog Scale of Mood and Alertness, a questionnaire of 16 analogue scales that derives 3 factors that assess change in Self-rated Alertness, Self-rated Calmness, and Self-rated Contentment. Two composite scores from the CDR tests were also derived: 1) Power of Attention, which combines the reaction time measures from the 3 attention tests (i.e., Simple Reaction Time, Choice Reaction Time, and Digit Vigilance), and 2) Continuity of Attention, which combines the accuracy measures from the 3 attention tests (i.e., Simple Reaction Time, Choice Reaction Time, and Digit Vigilance). The primary consideration here will be the effects on the measures of attention.

10.3.6 Statistical Analysis

Maximum plasma concentration, Cmax; time to Cmax, Tmax; apparent half-life, t1/2; alpha and terminal t1/2; and area under the plasma concentration-versus-time curve, AUC; were estimated for ethanol, dapoxetine, desmethyldapoxetine, and dapoxetine-N-oxide. For dapoxetine and its metabolites, a 2-compartment model with first-order absorption and elimination was used (WinNonMix software, Version 2.0.1, Pharsight Corporation, Mountain View, CA).

Statistical analyses were conducted using SAS. Pharmacokinetic parameters for dapoxetine and ethanol were compared between the dapoxetine alone and dapoxetine + ethanol treatments using a mixed-effect ANOVA that included treatment, period, and sequence as fixed effects and subject-within-sequence as a random effect. Log-transformed dapoxetine AUCinf and Cmax values for

dapoxetine and dapoxetine + ethanol were compared using the least-square estimate of the mean parameters for the ratio of dapoxetine + ethanol to dapoxetine alone; ethanol alone was compared to dapoxetine + ethanol in the same manner.

For cognitive and subjective assessments, a repeated-measures ANOVA was used that included fixed effects of sequence, treatment, period, time, and treatment-by-time interaction, and random effect of subject-within-sequence. For each CDR measure, predose (baseline) data for each period were subtracted from those at each postdosing timepoint to derive difference from baseline scores, on which the analyses were performed. If the treatment-by-time interaction was significant ($P<0.01$), a standard ANOVA model for crossover designs, which included fixed effects of sequence, treatment, and period, and the random effect of subject-within-sequence, was used to compare the pharmacodynamic measure at each time point.

Statistical analyses were conducted on all available pharmacokinetic and pharmacodynamic data for the 20 participants who completed all 4 treatments.

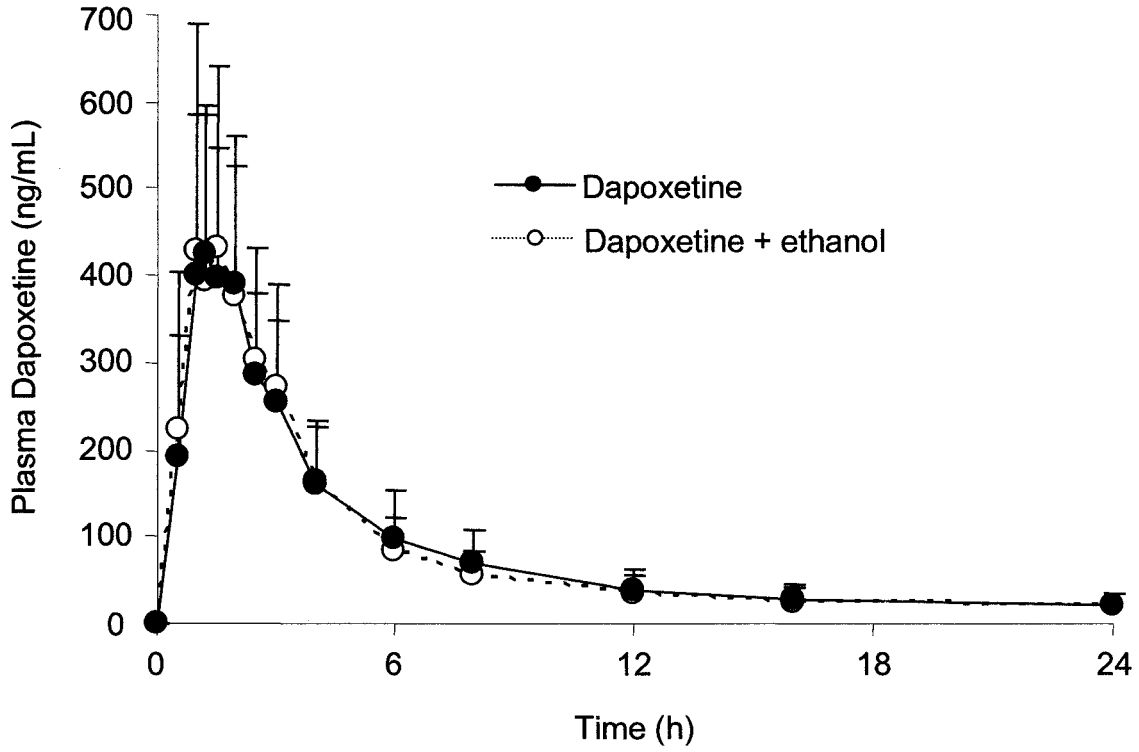
10.4 Results

10.4.1 Pharmacokinetic Analysis

DAPOXETINE

The pharmacokinetics of dapoxetine were not affected by coadministration of ethanol 0.5 mg/kg (Figure 29). Dapoxetine was rapidly absorbed, with maximal plasma concentrations 1.47 ± 0.51 hours after administration. Elimination was rapid and biphasic; the alpha and terminal half-life was 1.33 ± 0.14 and 15.6 ± 0.81 hours, respectively. By 24 hours after administration, plasma dapoxetine concentrations had decreased to 4.5% of Cmax. The pharmacokinetic parameter values for dapoxetine were not affected by ethanol coadministration, as noted by the similar peak concentrations and AUC values. The 90% CIs for the ratio of (dapoxetine + ethanol):(dapoxetine) for lnCmax and for lnAUCinf were within 80%–125%, indicating that ethanol did not affect the pharmacokinetics of dapoxetine.

Figure 29: Plasma concentration profile of dapoxetine administered alone and with ethanol



DESMETHYLDAPOXETINE AND DAPOXETINE-N-OXIDE

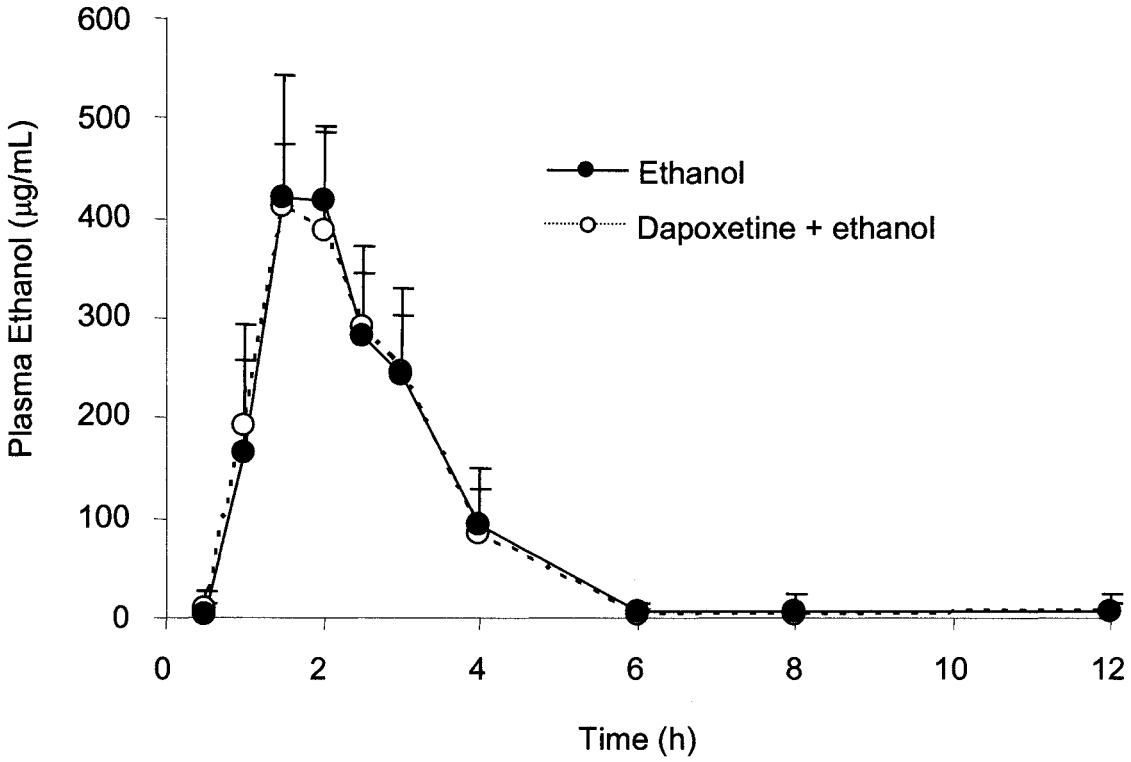
Ethanol did not affect the pharmacokinetics of desmethyldapoxetine or dapoxetine-N-oxide . For desmethyldapoxetine, the 90% CIs of the (dapoxetine + ethanol):(dapoxetine) ratio for lnCmax were within 80%–125%, while the 90% CI for lnAUCinf ratio was slightly outside the 80%–125% range, but the two treatments were not significantly different by ANOVA (P=0.258, dapoxetine vs dapoxetine + ethanol). For dapoxetine-N-oxide, the 90% CIs of the (dapoxetine + ethanol):(dapoxetine) ratios for lnCmax and for lnAUCinf were also within the 80%–125% no-effect boundary.

Coadministration of ethanol did not affect the metabolism of dapoxetine to desmethyldapoxetine or dapoxetine-N-oxide; the AUCinf ratio for (dapoxetine):(desmethyldapoxetine) was similar for dapoxetine and dapoxetine + ethanol (5.91 ± 1.4 and 6.57 ± 2.2, respectively), as was the AUCinf ratio for (dapoxetine):(dapoxetine-N-oxide) (1.23 ± 0.24 and 1.27 ± 0.24, respectively).

ETHANOL

Ethanol pharmacokinetics were not affected by coadministration of dapoxetine (Table II); plasma ethanol concentrations over time are shown in Figure 30. The 90% CIs of the (dapoxetine + ethanol):(ethanol) ratio for lnCmax and for lnAUCinf were within 80%–125%.

Figure 30: Plasma concentration profile of ethanol when coadministered with dapoxetine



10.4.2 Cognitive and Subjective Assessments

The analysis focused on the approximate time of peak plasma concentrations of both ethanol and dapoxetine (1.5 hours). Area under the effect curve analyses were conducted and no significant differences between treatments were observed; however, there was a high degree of variability across timepoints. Peak concentrations of dapoxetine occurred approximately 1.5 hours after administration, while peak concentrations of ethanol occurred approximately 1 hour after administration (ethanol was administered 30 minutes after dapoxetine, to match the time to peak concentration for both agents). Ethanol 0.5 g/kg impaired measures of attention, peaking at 1.5 hours and resolving by 4 hours, consistent with the plasma profile of ethanol.

Table 12: Effects of Ethanol and Dapoxetine at Tmax (1.5 hours)

Attentional Measures	Ethanol to placebo	Dapoxet-ine to placebo	Ethanol+ Dapoxet-ine to placebo	Dapoxet-ine to Ethanol	Ethanol+ Dapoxet-ine to Ethanol	Ethanol+ Dapoxet-ine to Dapoxet-ine
Simple Reaction Time	**0.0467**	0.8149	**0.0108**	**0.0277**	0.5411	**0.0056**
Digit Vigilance - Speed	*0.0706*	0.9333	**0.0161**	*0.0852*	0.5191	**0.0194**
Power of Attention	**0.0110**	0.5456	**0.0023**	**0.0485**	0.5639	**0.0118**
Continuity of Attention	**0.0092**	0.3671	**0.0180**	*0.0801*	0.8027	0.1295
Self-rated Alertness	0.1737	**0.0024**	**0.0002**	*0.0785*	**0.0099**	0.3862
Self-rated Contentment	**0.0392**	**0.0271**	0.4050	**0.0001**	**0.0046**	0.1588

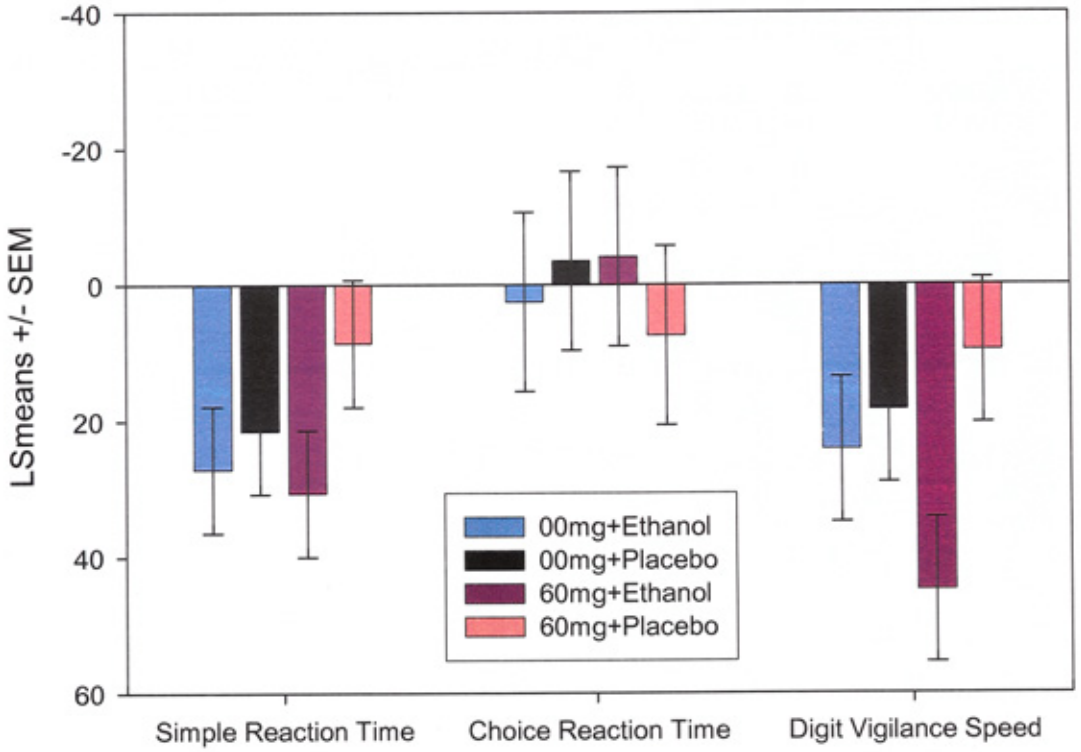
p-values for the comparisons at 1.5 hours comparing the study doses. Signals are in italics, significant effects are in **bold**.

Of the four measures from the attentional tasks, only Simple Reaction Time and Digit Vigilance Speed had significant main effects of treatment ($p<0.05$), the others being $p>0.1$.

The analysis at T_{max} indicated a significant main effect of treatment for the Simple Reaction Time task ($p=0.0102$). Both the ethanol alone (22.2 msec) and co-dosing (26.6 msec) showed highly significant declines compared to pre-dose (both $p=0.0001$), whereas there were no significant declines in the placebo (7.7 msec, $p=0.149$) or dapoxetine alone (6 msec, $p=0.2565$) conditions. The comparisons supported clear effects of ethanol, with decrements to performance indicated with ethanol alone, and co-dosing in comparison to placebo. In addition, performance with co-dosing was poorer when compared with dapoxetine alone, and poorer performance was seen for ethanol alone when compared to dapoxetine alone.

Within the Digit Vigilance task the speed measure showed significant comparisons for two conditions, indicating a decrement in performance for co-dosing in comparison with placebo, and in comparison with dapoxetine alone. Both ethanol alone (29.5 msec) and co-dosing (35.5 msec) showed highly significant declines compared to pre-dose (both $p=0.0001$), whereas the declines in the placebo (12.6 msec, $p=0.068$) or dapoxetine alone (13.4 msec, $p=0.053$) conditions missed significance. For the accuracy score on this task, a significant decline was seen for ethanol alone compared to pre-dose (−5.06%, $p=0.0063$), with a signal for co-dosing (-3 %, $p=0.0956$), but no significant changes for placebo (-1.73 msec, $p=0.3336$) or dapoxetine alone (−0.64 msec, $p=0.7176$).

Figure 31: Effects of Ethanol and Dapoxetine (60 mg) at Tmax (1.5 hours) on attentional task reaction times



The composite scores showed significant main effects for both attentional measures.

Power of Attention showed significant declines compared to pre-dose with ethanol (64 msec, p=0.0001), dapoxetine (28.3 msec, p=0.0336) and co-dosing (74.2 msec, p=0.0001), but not placebo (17.6 msec, p=0.1812). Importantly, the combined dose was not significantly different from ethanol alone.

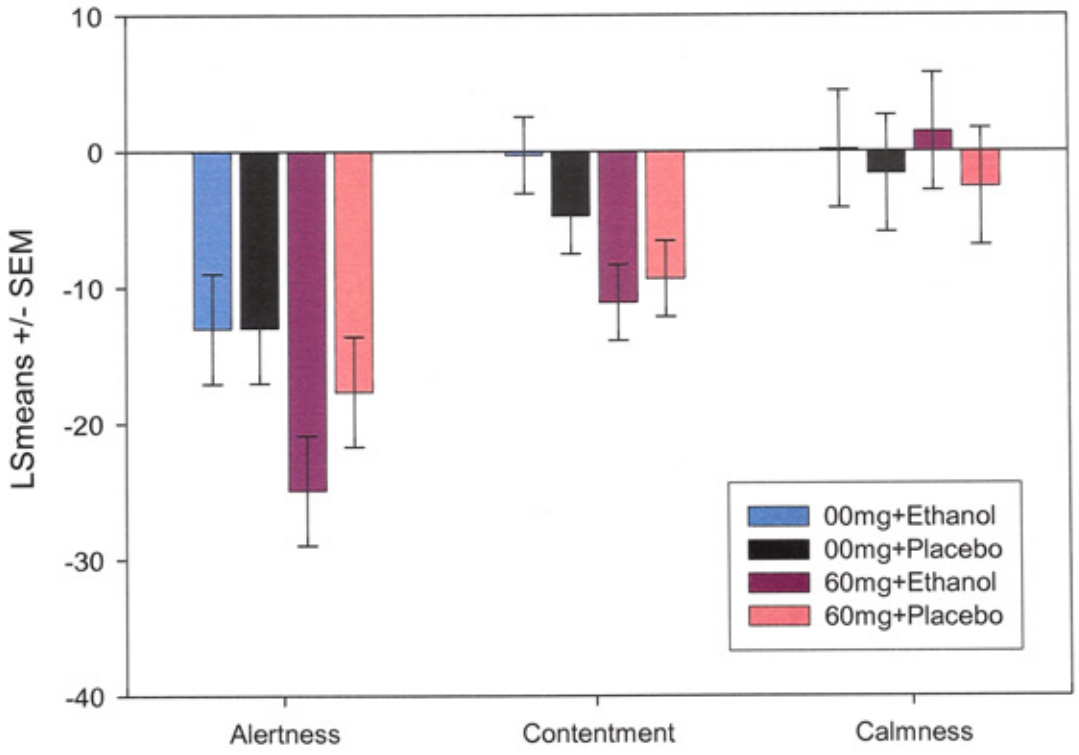
Continuity of Attention also showed declines compared to pre-dose with ethanol (-4.21 %, p=0.0001), dapoxetine (-2.11 %, p=0.0368) and co-dosing (-4 %, p=0.0001), but not placebo (-1 %, p=0.3165). Importantly, the combined dose was not significantly different from ethanol alone.

The analysis at T_{max} of performance within the VAS task indicated highly significant main effects for alertness and contentment.

Self-rated Alertness showed clear effects of dapoxetine, with decrements indicated with dapoxetine alone, and co-dosing. Furthermore, a decrement for co-dosing was seen when compared with ethanol alone. The declines from pre-dose were significant for all four conditions: (placebo -6.9 mm, p=0.0097; ethanol -11.7 mm, p=0.0001; dapoxetine -17.9 mm, p=0.0001; co-dosing −20.9 mm, p=0.0001). The effect in the combined condition can thus be seen not to be due an interaction, but to reflect the impairment seen with dapoxetine.

Self-rated Contentment showed clear effects of dapoxetine, with decreased (poorer) ratings seen for dapoxetine alone when compared to placebo, dapoxetine alone when compared to ethanol alone, and for co-dosing when compared to ethanol alone. In addition, self-rated contentment was seen to increase (improve) with ethanol alone when compared to placebo. In relation to pre-dose, contentment improved slightly with ethanol (3.5 mm, p=0.0802), while declining with placebo (-1.9 mm, p=0.3309), dapoxetine (-7.8 mm, p=0.0002), and co-dosing (-4.1 mm, p=0.042).

Figure 32: Effects of Ethanol and Dapoxetine (60 mg) at Tmax (1.5 hours) on self-ratings



Previous studies using the CDR system have found that doses of ethanol produce impairment on measures of attention and word recall/recognition. Van Harten et al. (1992), identified decrements with ethanol (40 g) to 3 task measures employed in the present study, Digit Vigilance Speed, Numeric Working Memory Sensitivity Index, and Immediate Word Recall Accuracy. Wesnes et al. (2000), identified decrements with ethanol (0.5 g/kg) to 3 task measures employed in the present study, Digit Vigilance Speed, Word Recognition Speed, and Self-rated Alertness (Table 13).

Table 13: Effects of ethanol in two comparison trials, and peak effects of ethanol versus placebo from the present study

Variable	Ethanol 40 g (van Harten et al., 1992)	Ethanol 0.5 g/kg (Wesnes et al., 2000)	Ethanol 0.5 g/kg (current study parametric analysis)	Ethanol 0.5 g/kg at 1.5 hours (current study non-parametric analysis)
Simple Reaction Time	X	X	X	▼
Choice Reaction Time	X	X	X	X
Digit Vigilance Targets Detected	X	X	▼	X
Digit Vigilance Speed	▼	▼	X	▼
Numeric Working Memory – Sensitivity Index	▼	X	X	X
Numeric Working Memory – Speed	X	X	X	X
Spatial Working Memory – Sensitivity Index	N/A	X	X	X
Spatial Working Memory – Speed	N/A	X	X	X
Immediate Word Recall – Accuracy	▼	X	▼	X
Delayed Word Recall – Accuracy	N/A	X	X	X
Word Recognition – Sensitivity Index	X	X	X	X
Word Recognition – Speed	X	▼	X	X
Self-rated Alertness	N/A	▼	X	▼

▲ Indicates benefit for treatment ▼ Indicates decrement for treatment

X Indicates no effect N/A Task/measure not included in study

10.5 Discussion

Results from this study demonstrated that consumption of ethanol had no effect on the pharmacokinetics of dapoxetine or its metabolites, desmethyldapoxetine and dapoxetine-N-oxide. Similarly, dapoxetine did not alter the pharmacokinetics of ethanol. Furthermore, measures of cognitive function did not support a consistent pattern of synergistic or additive interactions between ethanol and dapoxetine. However, there was a high degree of variability in the placebo data, making it difficult to draw clear conclusions. This was addressed to some extent by focussing on those data gathered at 1.5 hours post-dose. The closest cognition assessment timepoint to Tmax for both dapoxetine and ethanol. Here cognitive effects were more consistent and ethanol alone acted in the expected manner as a positive internal control.

Several cognitive measures were impaired following ethanol 0.5 g/kg, consistent with effects seen previously on the CDR measures (van Harten et al., 1992; Wesnes et al., 2000). The peak plasma ethanol concentrations (428–440 mg/mL) were comparable to those previously reported of 502 mg/mL and 640 mg/mL following doses of 0.7 g/kg10 and 40 mg, respectively. Dapoxetine administered alone was associated with declines in self-rated alertness, and self-rated contentment and whilst ethanol+dapoxetine resulted in statistically significant impairment on several cognitive measures compared with placebo, none were statistically significantly different to ethanol alone. These results suggested that the addition of dapoxetine did not result in impairment beyond that associated with ethanol.

SSRI antidepressants have not generally been shown to potentiate the cognitive effects of alcohol. Alcohol impaired measures of cognitive function, whereas 50 mg fluvoxamine did not potentiate the effects of alcohol, although 2 measures of cognitive function (speed of responding in the vigilance task and word recognition sensitivity) were possibly affected by fluvoxamine (van Harten et al., 1992). In another study, fluoxetine had no effect on auditory reaction time, DSST, or body sway with eyes open or closed, but did result in a decrease from baseline in immediate and delayed word recall. Coadministration of fluoxetine with alcohol significantly slowed auditory reaction time, reduced DSST, and increased body sway in both the eyes open and closed conditions, and produced further decreases in immediate and delayed word recall (Allen et al., 1988).

Conclusions

In healthy male subjects, coadministration of dapoxetine 60 mg with ethanol did not alter the pharmacokinetics of dapoxetine or ethanol. Whilst consistent, statistically significant effects of coadministration were not seen on cognitive or subjective measures there were some patterns for additional impairment. Dapoxetine alone

reduced self-ratings of alertness and contentment, neither of which were affected by coadministration of ethanol. These effects require further investigation.

11 Effectiveness of a 5-HT_6 Receptor Antagonist in Reversing Scopolamine-Induced Cognitive Impairment

11.1 Abstract

The aims of the present study were to establish the pharmacodynamic effects of a 5-HT_6 receptor antagonist on scopolamine induced deficits in cognitive function. This was a randomised, double-blind, placebo-controlled, two-way crossover, single oral dose study at two dose levels (Part 2), preceded by a Physostigmine Model Validation Phase (Part 1). Forty-eight healthy male subjects aged 18-40 years were studied. Twelve subjects in Part 1 received a single dose of scopolamine hydrobromide (0.5 mg) followed by either physostigmine (1.5 mg) or placebo in a two-way crossover design. Part 2 consisted of two groups of eighteen subjects. Group 2A received a single low dose (75 mg) or placebo in a two-way crossover design. Group 2B received a single high dose (300 mg) or placebo in a two-way crossover design. Cognitive function was assessed using a task battery from the Cognitive Drug Research computerised assessment system, including assessments of attention, working memory, secondary memory, and self-rated mood and alertness. Assessments were completed at one hour pre-scopolamine, and at 2, 3 and 4.5 hours post scopolamine in Part 1. In Part 2 assessments were completed at 1 hour prior to co-dosing of scopolamine and the 5-HT_6 antagonist, and at 1.5, 2.5, 4 and 6 hours post-dose. In line with previous work, physostigmine produced marked but temporary reversals of all the impairments to performance and self-rated alertness produced by scopolamine. For the 5-HT_6 antagonist, the 75 mg dose showed no effects. The 300 mg dose selectively reversed the effects of scopolamine on secondary memory. This effect was seen on each of the measures of accuracy from the four memory tasks. The potential basis for this selective effect and consequences for the treatment of Alzheimer's disease, particularly the symptomatic treatment of memory and attentional impairments respectively are discussed.

11.2 Introduction

It is now known that glutamatergic and cholinergic function are compromised in Alzheimer's disease (AD), and both are thought to contribute to the debilitating cognitive effects of the disease. Facilitation of glutamate has been shown to enhance memory (Staubli et al., 1994) and cholinergic function is important in both memory and attention (Perry et al, 1999).

The Cognitive Drug Research (CDR) computerised assessment system has been extensively validated in patients with AD (Simpson et al., 1991; Nicholl et al., 1995; Holland et al., 1997; Templeton et al., 1999; Wesnes, 2000) and other dementia's including Vascular Dementia (VaD) (Walker et al., 2000), Dementia with Lewy Bodies (DLB) (McKeith and Ayre, 1997; Walker et al., 1999; Walker et al., 2000), and Huntington's Disease (Mohr et al., 1996). It has shown high sensitivity to the cognitive deficits associated with Alzheimer's disease and has been able to differentiate AD from other forms of dementia.

Cholinesterase inhibitors increase the amount of ACh at the neuronal synaptic cleft by inhibiting acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of ACh and consequently improve neuronal transmission. The CDR system has shown sensitivity to identifying cognitive improvements with the cholinesterase inhibitors velnacrine (Goa and Fitton, 1994), and galantamine (Wesnes et al., 1994a; Wesnes et al., 1998) in AD and rivastigmine in DLB (McKeith et al., 2000; Wesnes et al., 2002).

Scopolamine is a muscarinic cholinergic receptor antagonist, which produces impairment to cognition in both young (Wesnes and Simpson 1988; Wesnes et al., 1988; Ebert et al., 1998) and elderly (Barker et al., 1995; Jones et al., 1991) subjects, as measured by the CDR system. These deficits match the cognitive profile of deficits seen in AD compared to controls, as predicted from the cholinergic dysfunction apparent in AD (Simpson et al., 1991; Walker et al., 2000; Ballard et al., 2002). Some of these deficits are also seen in other forms of dementia involving cholinergic dysfunction, such as deficits to attention in DLB.

The ability of 5-HT receptors to modulate glutamatergic and cholinergic function has highlighted the potential of particular 5-HT receptor subtypes as therapeutic targets in the treatment of the cognitive deficits associated with AD. For example Wyeth research is currently developing a 5-HT_{1A} antagonist (SRA-333), which increases glutamatergic and cholinergic neurotransmission (Patat at al., 2004).

Animal research demonstrates that 5-HT_6 receptors are predominantly expressed in the central nervous system (Roberts et al., 2002) and their function appears to involve the regulation or suppression of several neurotransmitter systems important to cognitive function (King et al., 2003). Several studies indicate 5-HT_6 receptors exert a tonic inhibitory effect on central cholinergic (ACh) neurotransmission, and 5-HT_6 antagonists may enhance ACh efflux (e.g. Shirazi-

Southall et al., 2002). A similar increase in glutamate release, independent of effects on ACh, has also been associated with administration of 5-HT_6 antagonists (Dawson et al., 2001).

The study compound has shown efficacy in various animal models of Alzheimer's disease and was well tolerated in animals. Moreover, the animal data has indicated a large margin between the doses of a 5-HT_6 receptor antagonist required to produce therapeutic effects and those inducing side effects (unpublished). This profile differs from the cholinesterase inhibitors for which efficacy is limited by dose dependent cholinergic side effects and possibly also disease progression (Giacobini, 2003).

Therefore, a 5-HT_6 receptor antagonist may be better tolerated than an acetylcholinesterase inhibitor and in addition, may be more efficacious in treating cognitive impairment associated with AD, due to its ability to elevate extracellular levels of glutamate as well as ACh.

11.3 Hypotheses

- It was hypothesised that scopolamine would produce a widespread impairment of cognition, which would be attenuated or reversed by physostigmine, validating the study paradigm in Part 1.
- It was hypothesised that the 5-HT_6 receptor antagonist would also show an ability to attenuate or prevent scopolamine induced impairment of cognition in Part 2.

11.4 Methods and Materials

11.4.1 Subjects

Forty-eight healthy male subjects (mean age 27.1, standard deviation 5.7 years) were studied in total, in two parts. In Part 1, twelve subjects were studied. In Part 2, two groups (2A and 2B) of eighteen subjects were studied.

11.4.2 Ethics

The study was conducted at a commercial phase I unit and supported by a sponsor pharmaceutical company. Ethical approval was obtained from the appropriate regulatory bodies.

11.4.3 Design and Treatments

This was a phase I, randomised, double-blind, placebo controlled, two-way crossover study of forty eight subjects in two parts.

In Part 1 twelve subjects received a single dose of 0.5 mg scopolamine hydrobromide, administered subcutaneously. Ninety minutes later a single dose of either physostigmine (1.5 mg) or placebo was administered. Subjects were randomly assigned to either sequence AB: physostigmine followed by placebo or BA: placebo followed by physostigmine. After a seven to fourteen day washout period the second part of the treatment was conducted.

In Part 2, two groups (2A and 2B) of eighteen subjects received a single dose of 0.5 mg scopolamine hydrobromide, administered subcutaneously (s.c.). Within each group subjects were randomly assigned to one of two sequences. In group 2A subjects were assigned to either sequence CD: a low dose (75 mg) followed by placebo or DC: Placebo followed by a low dose (75 mg). In group 2B subjects were randomly assigned to either sequence EF: a high dose (300 mg) followed by placebo or FE: placebo followed by a high dose (300 mg). The study compound was administered at the same time as scopolamine, with groups 2A and 2B being run sequentially.

11.4.4 Assessments

Schedule

Four training sessions were completed by each volunteer prior to the first day of dosing of the trial in order to ensure an optimal level of performance for the baseline assessment on the first study day (Wesnes and Pincock, 2002). For Part 1 the CDR assessments were completed immediately prior to the scopolamine injection and at 2, 3 and 4.5 hours post-dose. For Part 2 assessments were completed immediately prior to co-dosing of scopolamine and the study compound, and at 1.5, 2.5, 4 and 6 hours post-dose.

Cognitive Assessments

Parallel forms of the tasks were presented on each testing session to allow for equivalent repeated assessment. Tasks were computer-controlled, the information being presented on high resolution monitors, and the responses recorded via a response module containing two buttons, one marked 'NO' and the other 'YES'. In the word recall tasks the volunteer wrote down the words on a sheet of paper. The tasks were administered in the following order:

- Immediate Word Recall
- Simple Reaction Time
- Digit Vigilance
- Choice Reaction Time
- Numeric Working Memory
- Delayed Word Recall

- Word Recognition
- Picture Recognition

Task parameters and assessment procedures matched those for previous studies (e.g. Ebert et al., 1998).

Bond-Lader VAS of Mood and Alertness (Bond and Lader, 1974): This questionnaire of 16 analogue scales derives three scores that assess change in Self-rated Alertness, Self-rated Calmness and Self-rated Contentment. It has proven sensitivity to a wide range of compounds. A computerised version was employed, with the volunteer using a joystick.

11.5 Statistical Analysis

Formal statistical comparisons were performed on the data unadjusted for baseline, by study assessment time point, separately by dosing group (1, 2A, 2B). The model contained terms for sequence, period, dosing condition, and subject. All testing was two-tailed and a significance level of $p \leq 0.05$ adopted, with a level of $p \leq 0.1$ adopted for the purpose of assessing signals for significance.

11.6 Results

11.6.1 Part 1

Table 14: Physostigmine reversal of scopolamine induced impairment (mean ± SD)

		Study Assessment Time Point (Hours)			
	Dose	0	2	3	4.5
Simple Reaction Time (msec)	Placebo		295 (73)		
	Physostigmine		229 (22)**		
Choice Reaction Time (msec)	Placebo		459 (70)	456 (67)	461 (67)
	Physostigmine		417 (45)**	435 (52)**	441 (54)**
Digit Vigilance – Targets Detected (%)	Placebo		93.3 (8.1)	93.7 (5.0)	
	Physostigmine		98.3 (2.5)**	96.9 (4.7)**	
Digit Vigilance – Speed (msec)	Placebo		439 (66)	434 (42)	
	Physostigmine		400 (37)**	411 (45)**	
Numeric Working Memory – Sensitivity Index (SI)	Placebo		0.82 (0.10)		
	Physostigmine		0.89 (0.07)**		
Numeric Working Memory – Speed (msec)	Placebo		689 (111)	710 (159)	
	Physostigmine		588 (93)**	651 (120)**	
Immediate Word Recall – Accuracy (%)	Placebo		29.2 (10.3)	28.9 (8.4)	
	Physostigmine		40.0 (11.1)**	39.4 (12.5)**	
Delayed Word Recall – Accuracy (%)	Placebo		10.6 (11.5)		15.3 (12.0)*
	Physostigmine		17.8 (11.5)**		9.2 (8.3)
Word Recognition – Sensitivity Index (SI)	Placebo		0.44 (0.18)		
	Physostigmine		0.56 (0.21)*		
Word Recognition – Speed (msec)	Placebo		774 (124)		708 (65)*
	Physostigmine		676 (100)**		778 (154)
Picture Recognition – Speed (msec)	Placebo	725 (77)*	851 (157)	828 (168)	
	Physostigmine	756 (107)	712 (76)**	785 (119)*	
Self-rated Alertness (mm)	Placebo		39.2 (14.1)	42.5 (14.4)	
	Physostigmine		52.0 (15.9)**	48.8 (12.1)**	

Only values for which a significant difference **($p \leq 0.05$) or a signal for significance *($p \leq 0.1$) were identified are shown

Post scopolamine benefits were seen to measures of attention, working memory, secondary memory and self-rated alertness, for physostigmine Vs placebo. Benefits were evident on measures of both reaction time and accuracy in each cognitive domain. The data clearly validated the study paradigm, both in terms of the ability of scopolamine (0.5 mg s.c.) to produce widespread cognitive impairments, and the ability of physostigmine to temporarily reverse these impairments.

11.6.2 Part 2

Table 15: 5-HT_6 antagonist reversal of scopolamine induced impairment (mean ± SD)

Only values for which a significant difference **(p≤0.05) or a signal for significance *(p≤0.1) were identified are shown

		Study Assessment Time Point (Hours)				
	Dose	0	1.5	2.5	4	6
Digit Vigilance – Targets Detected (%)	Placebo					
	75 mg					
	Placebo			94.2 (5.3)**		94.2 (4.9)
	300 mg			90.3 (9.5)		95.6 (5.5)**
Numeric Working Memory - Sensitivity Index (SI)	Placebo					
	75 mg					
	Placebo			0.89 (0.10)**		
	300 mg			0.80 (0.19)		
Immediate Word Recall – Accuracy (%)	Placebo		27.8 (9.4)			37.4 (7.9)**
	75 mg		32.4 (8.5)**			30.0 (9.7)
	Placebo			28.5 (8.6)		32.2 (8.6)
	300 mg			34.1 (10.0)*		38.2 (6.8)**
Delayed Word Recall – Accuracy (%)	Placebo	29.6 (14.3)		7.0 (7.7)		
	75 mg	34.3 (13.0)*		12.9 (9.4)**		
	Placebo					10.9 (10.2)
	300 mg					17.6 (10.6)**
Word Recognition - Sensitivity Index (SI)	Placebo					
	75 mg					
	Placebo		0.45 (0.23)	0.48 (0.18)		
	300 mg		0.59 (0.19)**	0.56 (0.20)**		

Continued on following page

Picture Recognition – Sensitivity Index (SI)	Placebo					
	75 mg					
	Placebo			0.66 (0.18)		
	300 mg			0.74 (0.14)**		
Picture Recognition – Speed (msec)	Placebo				776 (191)	
	75 mg				723 (102)**	
	Placebo					
	300 mg					
Self-rated Contentment (mm)	Placebo				68.3 (14.5)**	69.0 (14.4)**
	75 mg				64.7 (16.0)	66.8 (17.0)
	Placebo					
	300 mg					

For the measures of attention, Digit Vigilance showed a significant impairment with 300 mg at 2.5 hours, and a significant improvement at 6 hours, in percentage of targets detected (accuracy). There was one significant effect on measures of working memory, which showed an impairment with 300 mg to Numeric Working Memory Sensitivity Index (accuracy) at 2.5 hours. For the measures of secondary memory there were a number of significant effects. There was an improvement in Immediate Word Recall Accuracy for 75 mg at 1.5 hours and an impairment at 6 hours, whilst for 300 mg there were benefits at 2.5 hours (signal) and 6 hours (significant). For Delayed Word Recall Accuracy there were improvements at 2.5 hours for 75 mg, and at 6 hours for 300 mg. For Word Recognition Sensitivity Index (accuracy), there were improvements for the 300 mg dose at 1.5 and 2.5 hours (Figure 36). For Picture Recognition Sensitivity Index (accuracy), there was a significant improvement at 2.5 hours for 300 mg. For the reaction time (speed) measure in this task, there was an improvement for 75 mg at 4 hours. For the self-ratings of mood and alertness, there was a signal for decreased Self-rated Calmness for 75 mg at 6 hours, and significant decreases in Self-rated Contentment for 75 mg at 4 and 6 hours.

Figure 33: Effects of Scopolamine + Physostigmine on Digit Vigilance Speed (mean +/-sem)

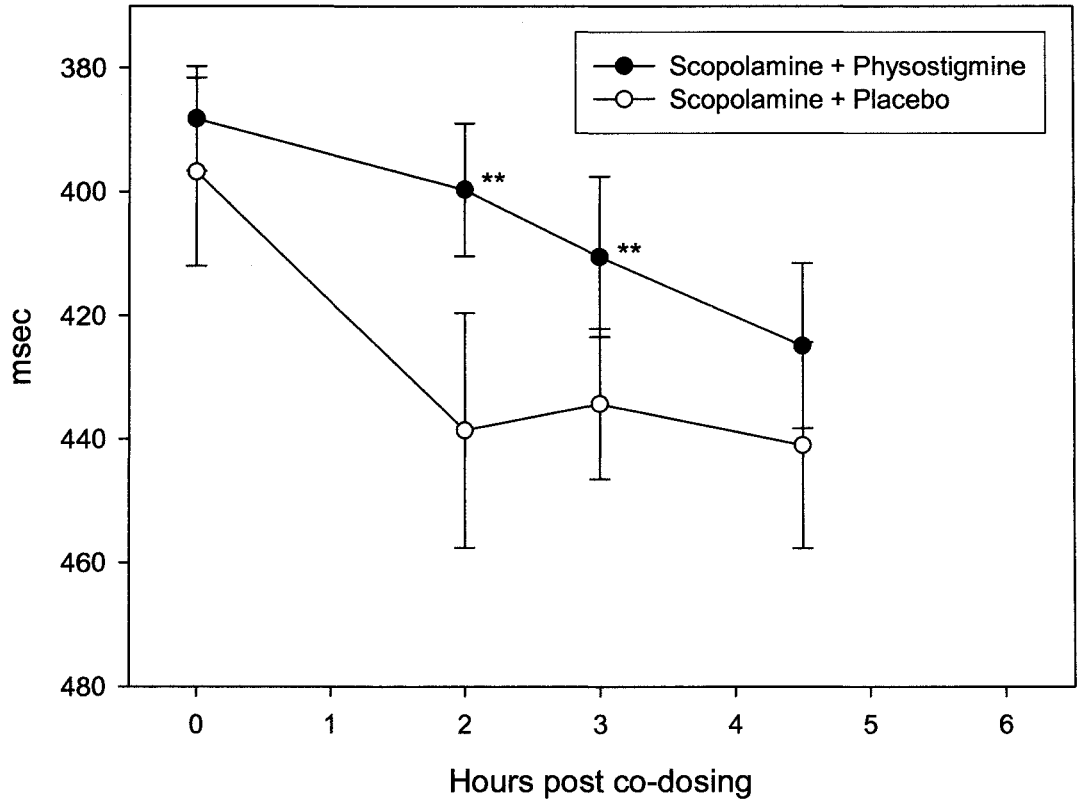


Figure 34: Effects of Scopolamine + 5-HT_6 antagonist on Digit Vigilance Speed (mean +/- sem)

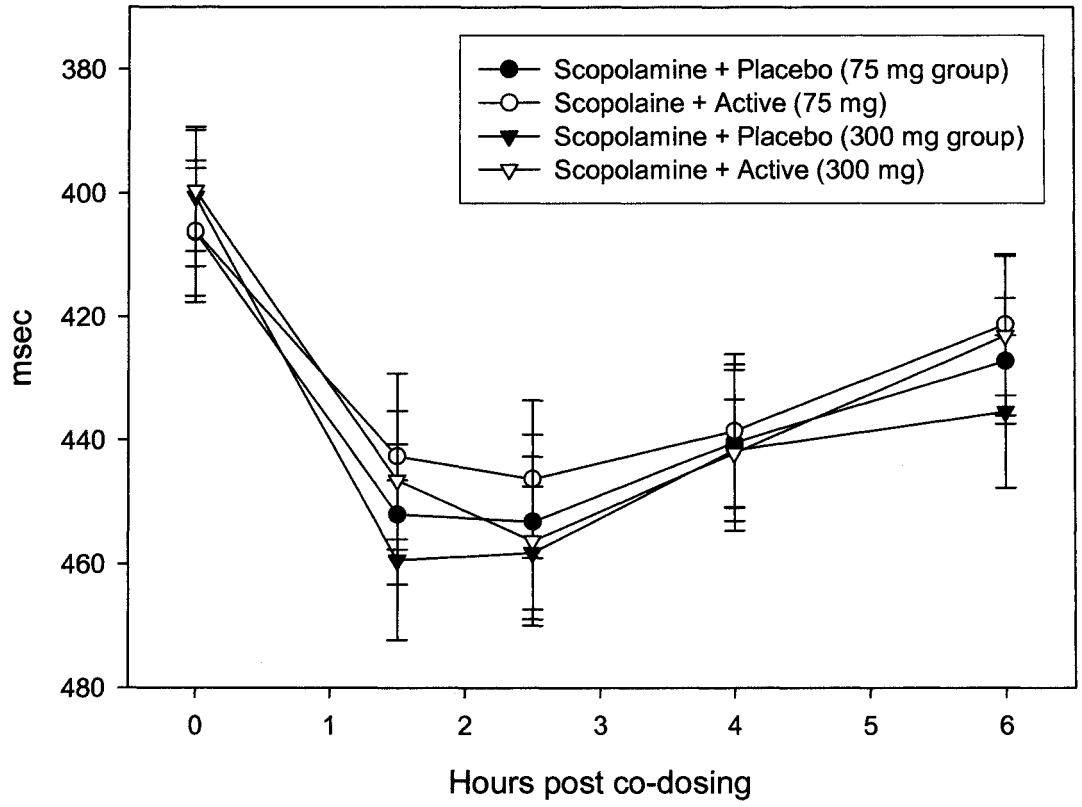


Figure 35: Effects of Scopolamine + Physostigmine on Word Recognition Sensitivity Index (mean +/- sem)

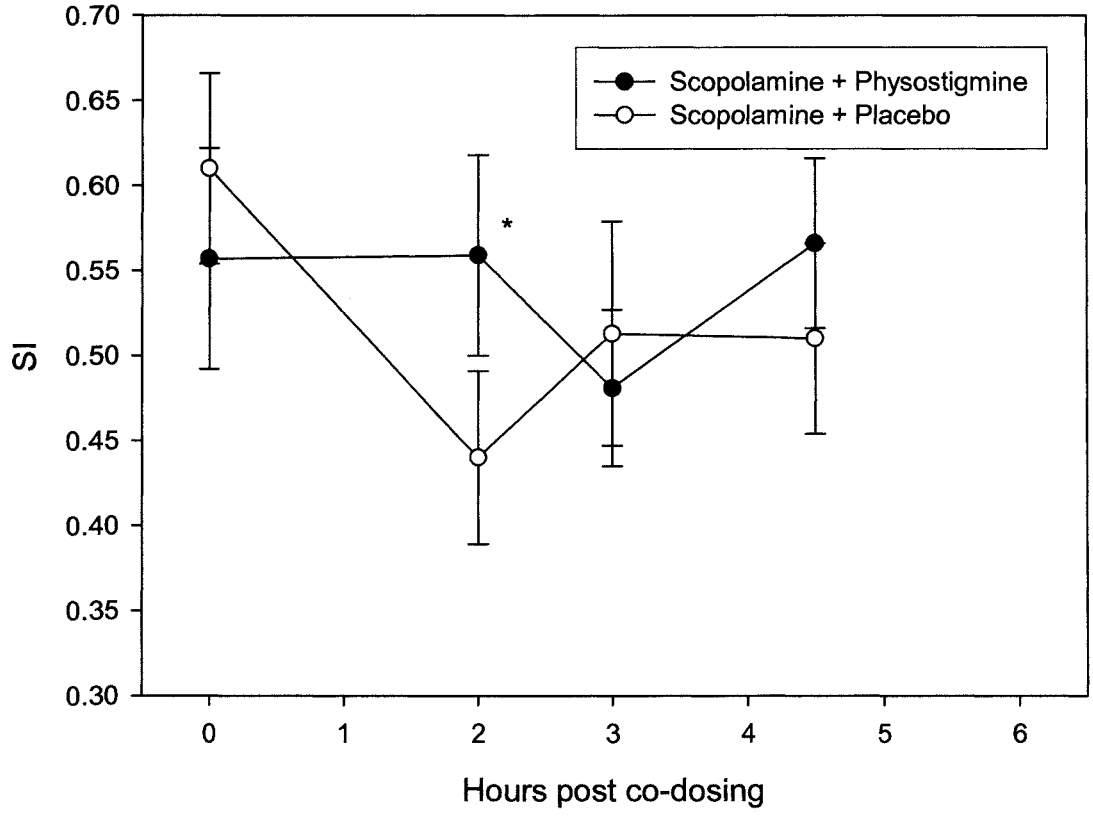
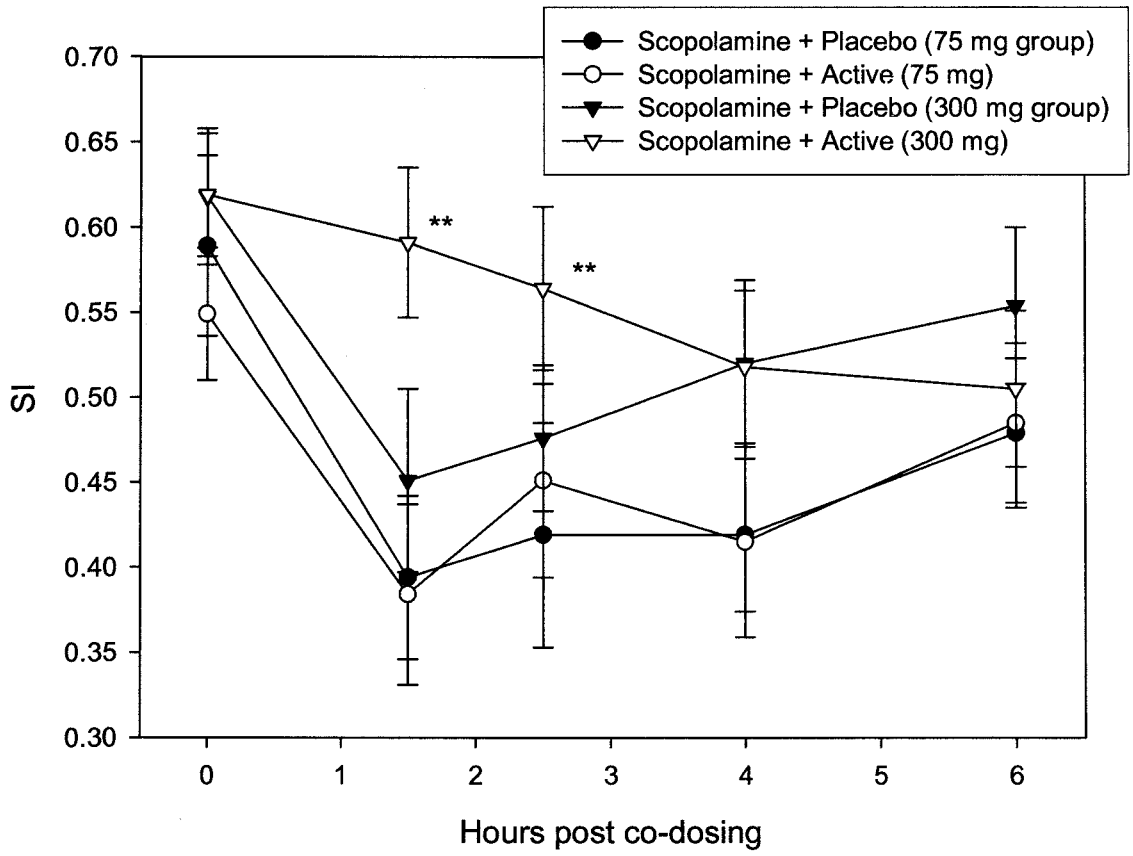


Figure 36: Effects of Scopolamine + 5-HT_6 antagonist on Word Recognition Sensitivity Index (mean +/- sem)



11.7 Discussion

Scopolamine (0.5 mg s.c.) in this study produced widespread cognitive impairments and showed a similar profile to that seen previously with 0.6 mg of the compound (e.g. Wesnes et al., 1988; 1991; Ebert et al., 1998). As in previous work, physostigmine 1.5 mg was able to counteract these effects (Wesnes et al., 1991; Ebert et al., 1998). The reversals were statistically reliable and for many variables represented a complete reversal of the scopolamine impairment (Figure 33). The reversals were also temporary, many of them having passed after one hour. It has been observed previously that reversal with physostigmine is temporary and that secondary memory performance (e.g. Word Recognition, Word Recall) does not completely reverse 30 minutes following physostigmine (Wesnes et al., 1991; Ebert et al., 1998), and the same was seen in the present study. Therefore, Part 1 of the study provided a clear validation of the scopolamine challenge paradigm.

In Part 2, the 75 mg dose was ineffective in reversing the effects of scopolamine. However, the 300 mg dose showed a beneficial profile, primarily on scores reflecting the accuracy of secondary memory. A large reduction in the scopolamine impairment was seen on the accuracy of Word Recognition at 1.5 hours and 2.5 hours. Verbal recall was also improved, Immediate Word Recall being beneficially affected at 2.5 hours and both Immediate and Delayed Word Recall at 6 hours. Picture Recognition sensitivity was enhanced with the 300 mg dose at 2.5 hours, though there was not a large decrement produced by scopolamine on this measure. The absence of effects on attention (Figure 34), working memory, the speed (reaction time) of recognition memory, and self-ratings of alertness, taken together with the clear effect on secondary memory, suggest a selective effect of the study compound.

Possible explanations for this selective effect of a 5-HT_6 antagonist on memory, whilst not influencing attention or working memory, are:

1. the dual effect on glutamatergic and cholinergic systems providing a 'dual' benefit to memory, but a 'single' benefit to attention/working memory
2. a relatively greater effect on glutamatergic as opposed to cholinergic systems
3. a combination of these factors
4. localisation of receptors in brain regions predominantly associated with memory

Facilitation of glutamatergic neurotransmission appears to be involved primarily in aspects of learning and memory (Staubli et al., 1994) as opposed to attention, while cholinergic function is critical to attention, whilst also being implicated in memory (Perry et al, 1999). Therefore, it might be supposed that memory would be preferentially affected by the 'dual action' of 5-HT_6 antagonism. The finding further supports this idea that D-Cycloserine, which modulates NMDA receptor activity specifically improved memory in the scopolamine challenge model, whilst attention

was unaffected (Jones et al., 1991; Wesnes et al., 1991). A further factor may be the importance of 5-HT_6 receptors in influencing ACh outflow, with evidence indicating that 5-HT_6 antagonists may not significantly increase ACh efflux (Shirazi-Southall et al., 2002). Related to this is the high level of expression in the hippocampus, which also suggests a role in memory processes (Wooley et al., 2001).

This selective profile of effects, whilst positive in terms of memory, does not support efficacy in terms of treating the attentional deficits in AD. However, this apparent gap in efficacy may be addressed by the use of this compound and other 5-HT antagonists as combination therapies in AD, alongside the established cholinesterase inhibitors. This may allow treatment to have both a greater benefit, by allowing for greater enhancement of cholinergic function before dose limiting side effects emerge, and a broader benefit, by influencing a range of neurotransmitter systems important to cognitive function. This approach is already being investigated (e.g. Patat et al., 2004) and may prove to be an important development in the treatment of cognitive symptoms in AD.

12 Cognitive performance in humans during a smoked cocaine binge-abstinence cycle

12.1 Abstract

Seventeen non-treatment seeking cocaine-dependent individuals participated in one of two different 3-week in-patient studies of sleep and cognition. During a 3-day period, they were allowed to self-administer smoked (5 subjects) or intravenous (12 subjects) cocaine. All smoked-cocaine participants completed three days of drug-free baseline followed by three days of cocaine self-administration and two weeks complete abstinence. Half of the intravenous cocaine participants followed this same schedule while the other half completed two weeks abstinence prior to receiving cocaine and all self-administered placebo on days the other half received cocaine. Cognition assessments were completed daily, using the CDR computerised cognitive assessment system. During the second week of abstinence cocaine users performed more poorly on attention tests. Similarly, attentional performance differentiated cocaine users' performance from controls. On two attention tasks combining cognitive effort with vigilance, cocaine users' performance did not differ from normative values on 'Binge' drug days but became markedly poorer by the second week of abstinence. It is hypothesized that chronic cocaine users' attention, although degraded by chronic cocaine use, may be maintained at normal levels by continued drug use with deficits becoming unmasked during abstinence. Cognitive deficits may be related to sleep quality deficits that also emerge with abstinence and both may contribute to relapse.

12.2 Introduction

Much concern has been raised recently regarding possible adverse effects of cocaine on cognitive function (e.g. Bolla et al., 1998, 1999, 2000). In general, these studies have compared abstinent cocaine abusers with matched controls with or without a history of other substance use disorders (Horner, 1999). Several of these studies have documented poorer performance in abstinent cocaine users on measures of cognitive function including attention, vigilance and reaction time, psychomotor speed, and spatial and verbal memory (e.g. Bauer, 1994).

Although there is much agreement about the presence of heterogeneous neurocognitive impairments in many chronic cocaine users compared to controls, impairment to particular cognitive domains or on specific neuropsychological tests is not a consistent finding (for a review of attentional findings see Horner, 1999). Inconsistencies occur even in studies which are well designed and that monitor abstinence closely (Horner, 1999). Possible sources both of impairments and inconsistencies have been investigated by examining cognitive function in conjunction with cocaine use and abstinence.

One source of heterogeneity is variability in time elapsed from cessation of use to the point of neurocognitive assessment (Horner, 1999). While repeated measures can address this variability, previous test-retest intervals have been chosen primarily to detect signs of long-term recovery from putative neurotoxicity associated with cocaine abuse, rather than the naturalistic variability in cognition seen in cycles of drug use and abstinence. For example, Bauer (1994) re-tested after 1 week, 2 weeks, and 2 months of abstinence and DiSclafani et al. (2002) re-tested after 6 weeks and 6 months. In contrast, in the present study a laboratory model of cocaine binge use and abstinence has been used (Foltin and Fischman, 1997) together with multiply repeated, computerised cognitive assessment (CDR system).

This is the first longitudinal, twice-daily assessment of cognitive functioning in chronic cocaine users under controlled, in-patient conditions that include laboratory administration of cocaine followed by two weeks of confirmed abstinence. Also, unlike any prior study, sleep has been measured in parallel with cognitive assessments.

12.3 Hypotheses

The study was planned to characterise the profile of impairments associated with chronic cocaine use including the variability in cognition over time, associated with a cycle of binge use and abstinence. In addition, possible relationships with sleep were investigated using parallel assessment.

12.4 Methods and Materials

12.4.1 Subjects

All subjects were non-treatment seeking cocaine-dependent individuals whose major route of administration was smoking crack cocaine. In total 17 subjects were studied. New York City subjects were 4 males and one female, 4 African American and one Caucasian, mean age 35.4 (SD 4.4, range 30-41) with a mean 12 years of education (SD 3, range 9-16). New Haven subjects were 10 male and 2 female, 9 African American and 3 Caucasian, mean age 39 years (SD 7, range 24-49) with a mean 15 years of education (SD 2.3, range 9-18). Average self-reported cocaine use was 9 g/wk (SD 3.0, range 5-12) for New York subjects and 8.8 g/wk (SD 5.5, range 1.2-20) for New Haven subjects (computed from self-reported weekly spending and estimated per-gram street price at time of study). All subjects were free of major medical illness, other DSM IV Axis I disorders, psychiatric medication, diagnosed sleep disorders and neurological conditions. All subjects were current cocaine users and none expressed a desire for treatment of cocaine abuse or dependence at the time of study participation. All reported using cocaine in a "binge" pattern. All reported concurrent alcohol use and smoked tobacco cigarettes. Other reported drug use was infrequent. All subjects passed medical and psychological evaluation prior to the study, and none were receiving psychiatric treatment.

Those passing an initial structured telephone interview received a physical exam, ECG, medical history evaluation and psychiatric interview. Only those judged psychiatrically and physically healthy were accepted.

12.4.2 Ethics

Each subject signed a consent form approved by the Institutional Review Boards of the College of Physicians and Surgeons of Columbia University and The New York State Psychiatric Institute (New York participants) or Yale University School of Medicine Human Investigations Committee (New Haven participants). of age, were solicited through word-of-mouth referral and newspaper advertisements.

12.4.3 Design

The 17 Cocaine users in the current study participated in 22-23 day protocols as in-patients in hospital clinical research units. Five New York participants were studied at Columbia University College of Physicians and Surgeons and 12 New Haven participants at Yale University School of Medicine (Table 16).

Table 16: Study Days

Study Phase	Washout Phase (WA)		Binge Phase (BI)		Early Abstinence Phase (ABE)			
Study Day	1	2	3	4	5	6	7	8
Study Phase	Late Abstinence Phase (ABI)							
Study Day	9	10	11	12	13	14	15	16
Study Phase	Late Abstinence Phase (ABI)							
Study Day	17	18	19	20	21	22	23	24

Subjects were admitted on a Friday (Day 1) and were screened for sleep disorders the following night (Day 2) using polysomnography (PSG). Subjects went to bed at the same time each night and were awakened at the same time each morning. Sleep was recorded using the Nightcap ambulatory sleep monitoring system (Ajilore et al., 1995) on all nights and PSG on 11 of the 21 nights. On awakening, subjects recorded dream content, rated the subjective quality of their sleep, filled out the Beck Depression Inventory-II (Beck, Steer, and Brown, 1996), and indicated the presence or absence of cocaine withdrawal symptoms. Shortly thereafter they completed their morning (AM) battery of cognitive tasks (see below).

Cocaine Sessions

During experimental sessions, each participant was seated in a reclining lounge chair in front of a computer monitor. The electrocardiogram was continuously monitored via chest electrodes (Tektronix 413 Monitor®, Beaverton, OR; MAC PC®, Marquette Electronics, Milwaukee, WI), while heart rate (HR) and blood pressure (systolic, SP; diastolic, DP) were recorded every two minutes (Sentry II - Model 6100 automated vital signs monitor, NBS Medical, Costa Mesa, CA) beginning 20 min prior to drug administration. An Apple Macintosh IIci computer located in an adjacent room was used for automated data collection.

Experimental sessions were conducted at approximately 1100 and 1400, and began with 30 minutes of baseline measurement of cardiovascular activity and mood. Cocaine (smoked or intravenous) was administered up to six times in each of the daily sessions. Cocaine was not given on any trial in which cardiovascular activity was above our criteria for safe drug administration (HR < 130, DP < 100, SP < 165).

12.4.4 Treatment

Cocaine was provided by The National Institute on Drug Abuse (Foltin et al., 1990). Subjects generally opted to consume the full amount of cocaine made available.

12.4.5 Assessments

A repeatable battery of computerised tasks was used to measure subjects' cognitive functioning on the morning and evening of every protocol day except abstinence days 9-10 and 16-17. For 5 subjects (New York), morning (AM) tasks began from 0540 to 1119 (mean = 0836, median = 0904) and afternoon (PM) tasks began from 1204 to 1816 and all but two sessions began between 1425 and 1729. The overall PM mean start time was 1539 (median = 1533). On binge days (Days 4-6), the test batteries were given prior to their first (AM) cocaine administration session (see above) and following their PM session. For 12 subjects (New Haven) only PM cognitive assessments were conducted.

CDR battery tests:

- Immediate Word Recognition
- Simple Reaction Time
- Choice Reaction Time
- Digit Vigilance
- Spatial Working Memory
- Numeric Working Memory
- Delayed Word Recognition

Only the results of the CDR battery attentional tasks (Simple and Choice Reaction Time, and Digit Vigilance) are reported here.

12.4.6 Statistical Analysis

Performance in each of the cocaine users in the total sample (N=17) pooled from New York and New Haven studies was compared to group means for healthy controls of the same age and sex. These normative data were from the CDR database and gathered from clinical trials in Europe and North America. Among the different test variables, age- and sex-matched normative sample sizes ranged from 7 to 169 individuals (mean=58, SD=42, median=38).

Each New York participant's mean binge performance was the average of afternoon tests on Days 4-6 while their mean abstinence performance was the average for afternoon tests during the second week of abstinence (mean of the five experimental days tested: 15 and 18-21; equivalent to abstinence days 9 and 12-15). New Haven early-binge participants' binge and abstinence means were calculated for these same periods, however, tests results were available and

averaged in all seven days of the second week of abstinence (experimental days 15-21, equivalent to abstinence days 9-15). New Haven late-binge participants' average abstinence scores were the mean for experimental days 9-15 (abstinence days 9-15), while their average binge score was the mean of experimental days 18-20.

Mean binge and abstinence scores for the 17 cocaine users were compared to those of 17 age- and sex-matched normative samples using Bonferroni corrected unpaired t-tests (alpha = 0.05), and confirmed using Mann-Whitney U (MWU) tests corrected for ties. Mean binge and abstinence scores for cocaine users were compared using repeated measures ANOVA (equivalent to paired t-tests).

12.5 Results

Comparison of cocaine users at binge and abstinence to norms

Cocaine users showed significantly longer reaction time than controls for Simple Reaction Time during both binge (t-test p=0.0004; MWU p=0.0009) and abstinence (t-test p=0.003; MWU p<0.0001). For Digit Vigilance, neither reaction time (speed) nor accuracy (targets detected) differed between cocaine users and controls at binge, however, there was a trend for cocaine users to perform more poorly on both at abstinence (t-test p=0.02 and 0.01 respectively; MWU p=0.04 and 0.05 respectively). Similarly, for Choice Reaction Time no difference was seen between cocaine users and controls at binge, but cocaine users showed longer reaction time than norms during abstinence (t-test p=0.004; MWU p=0.004).

Comparison of performance at binge and abstinence in cocaine users

Reaction times were shorter (better task performance) during binge compared to abstinence for Digit Vigilance [$F(1,15)=24.4$, $p=0.0002$] and Choice Reaction Time [$F(1,15)=11.2$, $p=0.005$] as well as a trend for Simple Reaction Time [$F(1,15)=3.8$, $p=0.07$]. In addition, Digit Vigilance accuracy (targets detected) was greater during binge [$F(1,16)=10.5$, $p=0.005$].

Figure 37: Cocaine Users Versus Controls on Simple Reaction Time

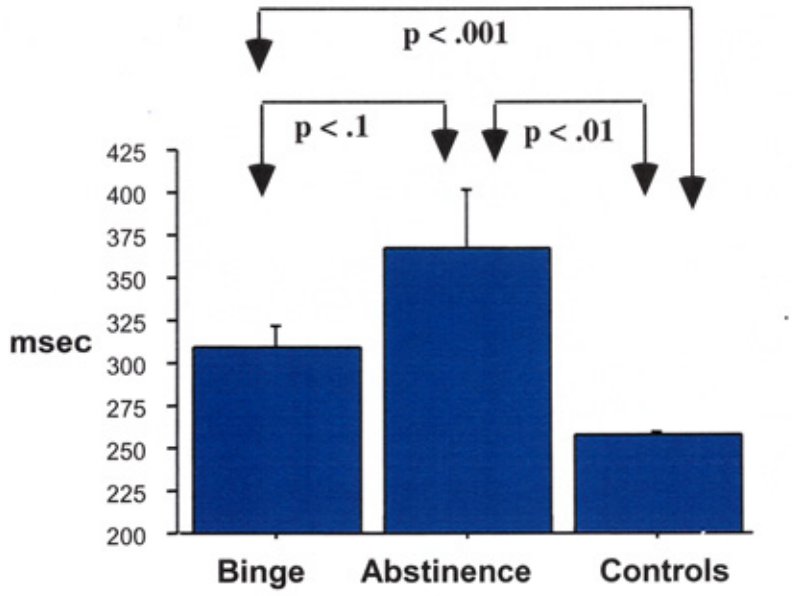


Figure 38: Cocaine Users Versus Controls on Choice Reaction Time

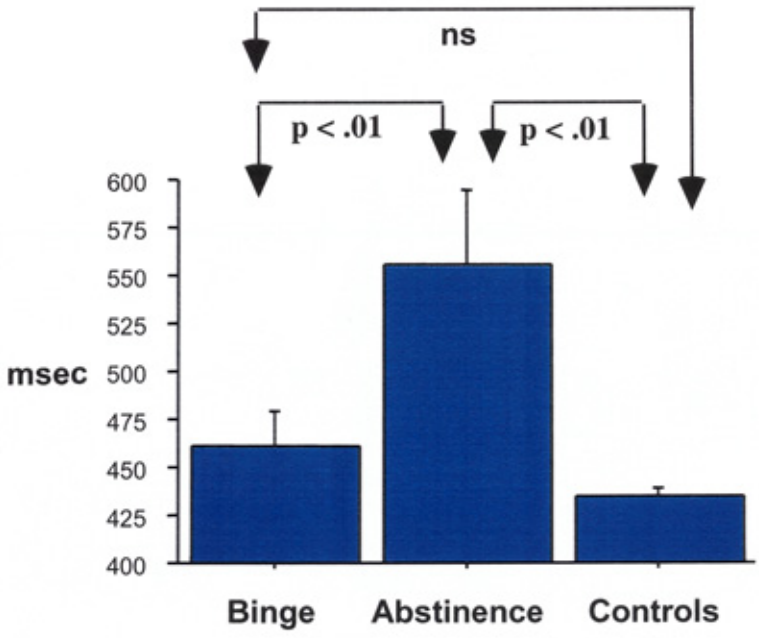


Figure 39: Cocaine Users Versus Controls on Digit Vigilance Targets Detected

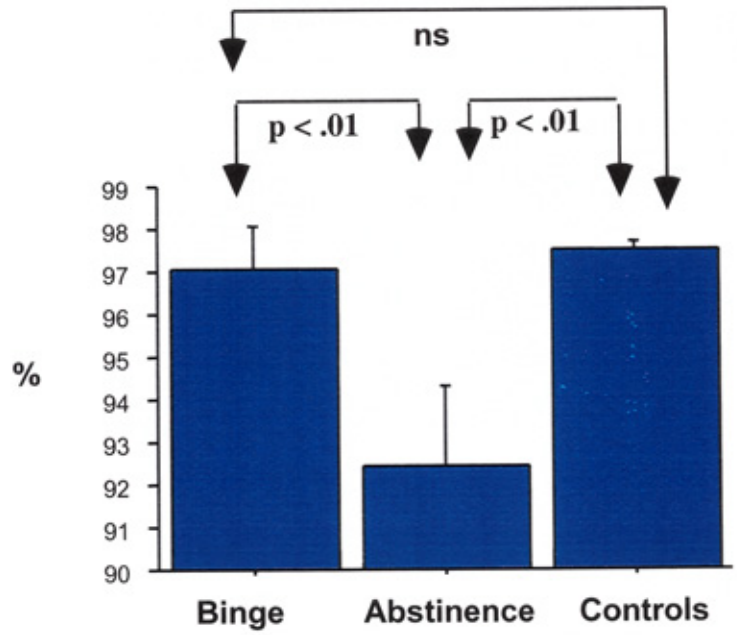
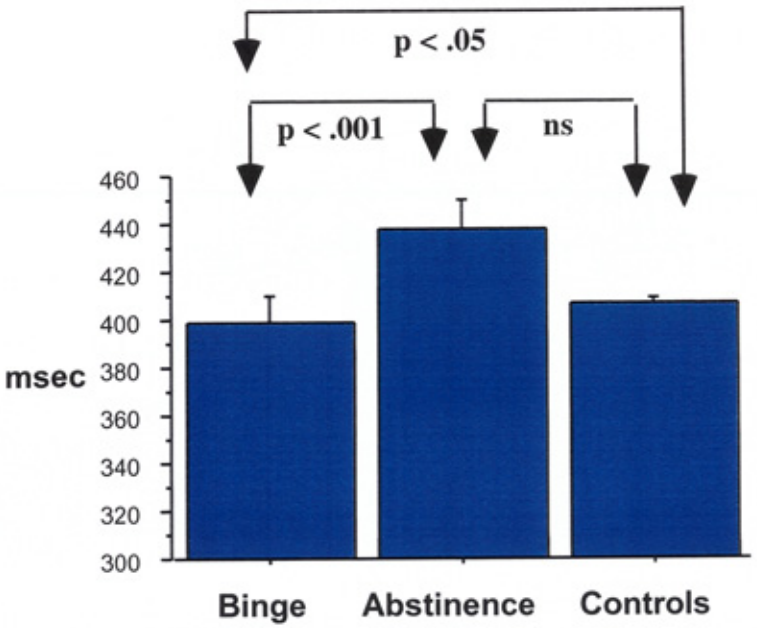


Figure 40: Cocaine Users Versus Controls on Digit Vigilance Speed



12.6 Discussion

Summary of Results

During binge days, cocaine users performed more poorly than age and sex matched population norms on the Simple Reaction Time measure only. However, by the second week of abstinence, cocaine users performed more poorly Simple, Choice and Digit Vigilance reaction times as well as Digit Vigilance accuracy. Samples from two cocaine-using populations suggest that, in chronic cocaine users, reaction time (and in some cases accuracy) on attentional tasks has declined by the second week of abstinence compared to periods when drug is being used. Notably, those tests that differentiated cocaine users from norms (Simple, Choice and Digit Vigilance) were identical to those that declined from binge days to the last week of abstinence in the pooled sample.

Greater impairment relative to norms during the second week of abstinence compared to binge days suggests that, to some extent, a decline occurs in some cognitive domains when cocaine-dependent individuals discontinue use of drug. Specifically, Choice and Digit Vigilance reaction time and accuracy measures that reflect sustained attention, psychomotor speed and information processing were not distinguishable from norms on days during which cocaine was used (binge) but reflected poorer-than-normal performance during the second week of abstinence.

Observed cognitive declines were previously reported to be temporally associated with sleep quality declines in these same subjects (Pace-Schott et al., 2003). Across binge-abstinence, mean core sleep quality variables (sleep duration, efficiency and latency) changed significantly in the direction of poorer sleep when analysed in the same manner as the cognitive changes described above. These findings replicate previous in-patient studies (Johanson et al., 1999; Kowatch et al., 1992). Over this same period, REM latency significantly decreased. Moreover, when averaged over the last week of abstinence, sleep quality and REM latency (which were low but marginally normal during binge) declined to levels suggesting clinically significant sleep disturbance (Pace-Schott, et al. 2003).

Sleep effects on cognition are increasingly documented in the domains of vigilance, attention and psychomotor performance (e.g. Van Dongen et al., 2003; Jewett et al., 1999). If some of the developing cognitive impairment observed here is indeed a consequence of deteriorating sleep quality, it is not surprising that these effects are exaggerated in the afternoon (see above), and less obvious in the morning.

Although no direct measures of neuronal changes were made in this study, the findings are consistent with the hypothesis that abstinent chronic cocaine users experience an elevation of CNS "allostatic load" (McEwen and Wingfield, 2003) due to chronic extrinsic perturbation of brain aminergic systems. Such long-term

perturbations may ultimately come to degrade the brain substrate of neurobehavioral and cognitive integrity in a manner analogous to an increasing "disease load" in aging (Karlamangla et al., 2002). Allostatic load may be further exacerbated by sleep loss (Van Cauter and Spiegel, 1999) that, itself, may result from chronic aminergic perturbation and neural compensatory reactions.

As has been hypothesized for dysregulated reward circuitry in addiction (Ahmed et al., 2002), adequate sleep regulation as well as cognitive capacity in the chronic cocaine user may come to require the periodic synaptic elevation of aminergic neuromodulators associated with cocaine use. During abstinence, the absence of periodic extrinsic aminergic elevations may cause both sleep quality and cognitive performance to decline.

Both the theory of allostatic load and the theory of dysregulated reward circuitry rest on disruptions of aminergic neurotransmitter systems through chronic cocaine use. That cocaine use impacts on cognition is clearly predicted by the wide evidence for a role for aminergic systems as mediators of cognition, and more specifically DA as a key modulator of attentional function.

Damage to serotonergic systems, which also occurs in chronic cocaine abuse (Battaglia and Napier, 1998; Fleckenstein et al., 2000), may play an important role in abstinence-related sleep quality deterioration since serotonin is believed to be more intimately involved in sleep regulation than dopamine (Pace-Schott and Hobson, 2002).

The finding that cognition and sleep dysregulation may be temporally associated has important implications for research into the effects of recreational drug use on cognition. It is not sufficient to investigate cognition alone in drug users. Given that drug use may influence both sleep and cognition, and that sleep may influence cognition, sleep assessment may be critical to the understanding of effects on cognition. Furthermore, the temporal and circadian nature of these effects may be important as differential influences may be seen over the day or across cycles of drug use and abstinence.

13 Sleep and cognition during recreational 'ecstasy' use: An acute use pilot study of cognitive function assessment, and actigraphy, temperature and saliva analyses

13.1 Abstract

Cognitive deficits in drug users need to be quantified, and the involvement of sleep quality determined. A previous study has identified poorer attentional task performance in chronic cocaine users during periods of drug abstinence, which were also temporally related to declines in sleep quality. Here, a similar paradigm was employed in 'ecstasy' users in a pilot study to assess the feasibility of cognition and sleep assessment in this drug using group in a naturalistic setting.

Chronic cocaine users (data reported in preceding chapter) were studied in clinical research units in the US. Subjects participated in a 3-week protocol with cocaine "Binge" followed by drug "Abstinence". During Binge, subjects consumed cocaine, twice daily in smoked form or once daily intravenously. Subjects repeatedly completed cognitive tests of attention and memory. Four self-reported 'ecstasy' users were studied at home (UK, 1 male and 3 female, mean age 34, range 27-44). Users completed the cognitive battery, and wore actigraphy devices pre and post recreational 'ecstasy' use. 'Ecstasy' use was confirmed using saliva samples. Test scores for both groups were compared to age and sex matched normative data. Mean Binge and Late Abstinence test scores for the cocaine users were compared to those of the matched norms using Bonferroni corrected unpaired t-tests. Data for the MDMA users was unsuitable for formal statistical analyses.

Cocaine users showed significantly longer reaction times than normals on tasks assessing attention, and significantly poorer word recognition. No differences between normals and cocaine users were seen for accuracy measures on tasks assessing working memory. 'Ecstasy' users showed poorer performance post use on all tasks, and were >1 standard deviations poorer than normals both pre and post use on most reaction time measures.

During cocaine use, attention and secondary memory significantly declined from Binge to Abstinence, and across the Abstinence phase. This was not consistently seen for working memory. This suggests that attention and recognition memory were selectively impaired in cocaine users, especially during Abstinence. Reported sleep quality declines seen in cocaine users during Abstinence may contribute to cognitive deficits. Recreational drug use is likely to involve poly-drug use rather than single substance misuse. Advances in assessment technology provide opportunities for studying sleep and cognition in 'real-world' recreational settings.

13.2 Introduction

There is extensive evidence to suggest that (±)3,4-methylenedioxymethamphetamine (MDMA; 'Ecstasy') is a potent and selective brain serotonin (5-HT) neurotoxin in both animals and humans.

Animal Studies

A number of studies in animals demonstrate that MDMA produces toxic effects on 5-HT neurons (e.g. Stone et al., 1986 and 1987), which may be directly relevant to long-term behavioural effects in human recreational users.

Supporting evidence is found using a number of different experimental techniques and demonstrates; lasting decreases in axon markers unique to 5-HT neurons, lasting decreases in vesicular monoamine transporters, reduced anterograde transport and evidence of 'pruning' (a reorganisation of ascending 5-HT projections similar to that observed in lesioning techniques). Taken together, this evidence provides very strong support for specific and long lasting 5-HT injury in animals resulting from MDMA administration.

The relevance of this evidence to humans is addressed by covering three areas of research findings. First, a range of species is known to be sensitive to MDMA-induced 5-HT injury, and there is no evidence to suggest humans are insensitive to it. Second, the route and schedule of drug administration does not seem likely to offer any protection to human users of MDMA. There appears to be no difference between the level of neurotoxicity produced by oral and parenteral (other route) drug administration. Also, doses as low as 5 mg have been found to produce 5-HT neurotoxicity in non-human primates. Third, the typical human single dosages of 75-125 mg fall squarely in the neurotoxic range predicted by the interspecies scaling method. Therefore, not only is there clear evidence of MDMA-induced 5-HT injury in animals, there is also the possibility that humans are similarly sensitive to the neurotoxic effects of the drug.

There is some evidence for factors, which may mediate the level of neurotoxicity. For example, some research indicates neurotoxicity may be related to hyperthermic response. Colado et al. (1999) showed pentobarbitone to have a neuroprotective effect against MDMA-induced 5-HT injury in rats. It is proposed that this neuroprotective effect resulted from counteracting MDMA-induced hyperthermia. In addition Malberg et al. (1998) demonstrated that small changes in ambient temperature can produce large changes in MDMA-induced 5-HT injury in rats. Body temperature, when taking MDMA, may then be one factor influencing the level of 5-HT injury experienced.

Human Physiological Studies

Studies of human physiology focus on evidence for MDMA-induced neurotoxicity in humans, and functional abnormalities that may be related to 5-HT injury such as

cognitive deficits, altered sleep, altered neuroendocrine function, altered behavioural response to 5-HT selective drugs and increased impulsivity.

A review of clinical studies by McCann et al. (2000) report on a number of areas of research including; cerebrospinal fluid (CSF) studies, positron emission tomography (PET) studies, pharmacological challenge studies, and sleep studies. In addition, evidence of altered EEG response in MDMA users will be outlined. Evidence for cognitive deficits, psychiatric disturbances, and increased impulsivity will be dealt with separately in later sections.

In reviewing cerebro spinal fluid studies it was concluded that nonhuman primates with MDMA–induced 5-HT lesions display selective deficits in CSF 5-HIAA with no alterations in CSF homovanillic acid (HVA) or 3-methoxy 4-hydroxyphenylglycol (MHPG), the major metabolites of dopamine and norepinephrine, respectively. These deficits in CSF 5-HIAA can be seen as an index of MDMA-induced 5-HT injury . Early studies in humans find both evidence of CSF 5-HIAA deficit in MDMA users and also no deficit. However, these early studies failed to control for factors influencing CSF 5-HIAA such as diet, activity, age, gender, affective disorder, drug use, season, and sample volume. Once these factors were controlled for, further studies found results, which reflected those identified in monkeys with MDMA-induced 5-HT injury. Specifically a reduction in CSF 5-HIAA in MDMA users, without reductions in CSF HVA or MHPG (McCann et al., 1994).

McCann et al. (1998) conclude that PET studies, similarly to CSF studies, are also capable of detecting MDMA induced neurotoxicity. Studies with [11C]McN-5652, a 5-HT transporter ligand, indicate humans with a history of MDMA use show decreased [11C]McN-5652-labelled 5-HT transporter sites. This decrease is correlated with level of MDMA exposure.

Dafters et al. (1999) report that MDMA use was positively correlated with absolute power in the alpha band, which has been shown to be inversely related to mental function. Also MDMA use was negatively correlated with EEG coherence, which, has been associated with disorders such as dementia, white-matter disease and normal aging.

McCann et al. (1999) report data employing the 5-HT precursor L-tryptophan as a pharmacological challenge. This L-tryptophan challenge paradigm showed some evidence of altered neuroendocrine function in MDMA users. This lack of consistency may be explained by a lack of sensitivity of the technique, or possible reinnervation of hypothalamic 5-HT neurones as observed over time in MDMA-induced 5-HT injury in nonhuman primates.

A further study by Gerra et al. (1998) showed blunted prolactin response in MDMA users following administration of fenfluramine, which is thought to act by releasing 5-HT from presynaptic neurons. This is suggestive of MDMA-induced 5-HT neurotoxicity in humans altering hypothalamic 5-HT function. McCann et al. (2000) also cite work by their group showing blunted plasma prolactin and cortisol

responses, greater levels of 'positive' and lesser levels of 'negative' symptoms following intravenous infusion of meta-chlorophenylpiperazine (m-CPP). Pre-treatment with 5-HT neurotoxins alters neuroendocrine and behavioural response to m-CPP in animals. Neuroendocrine and behavioural responses are also found in human populations who are administered m-CPP, which is thought to act at postsynaptic 5-HT2C receptors and in addition release 5-HT.

Schifano (2000) reported on a large-scale study of polydrug (including MDMA) users attending a Public Health Addiction Treatment Unit. Longer-term, larger-dose MDMA users were found to be at high risk of developing a number of psychopathological disturbances. These disturbances included depression, psychotic disorders, cognitive disturbances, bulimic episodes, impulse control disorders, panic disorders, and social phobia.

Sleep studies

There is a clear relationship between 5-HT and sleep. In animals, lesions of 5-HT cell bodies have been shown to induce insomnia whilst pharmacologic brain 5-HT depletion results in decreased non-rapid eye movement (NREM) sleep, and to a lesser extent rapid eye movement (NREM) sleep. Evidence from human studies has produced inconsistent data. L-tryptophan treatment has shown to decrease, increase and produce no change in REM sleep time, in different studies. In a single study of sleep in MDMA users, a decrease in an early stage of NREM sleep was identified. In attempting to confirm and extend these findings McCann et al. (2000) cite work by their group, which found altered sleep in MDMA users. However, the MDMA users were found to spend more time asleep (attributable to later stages than those in the previous study) and to have greater sleep efficiency. In rats, repeated exposure to MDMA has been shown to interfere with the ability of serotonin to phase shift the circadian clock, which in humans may also cause disordered sleep, cognition and mood (Biello and Dafters, 2001). Furthermore, it has been suggested that the damage to serotonergic systems, which also occurs in chronic cocaine abuse (Battaglia and Napier, 1998; Fleckenstein et al., 2000), may play a role sleep quality deterioration since serotonin is believed to be more intimately involved in sleep regulation than dopamine (Pace-Schott and Hobson, 2002). Whilst it has not been demonstrated that abstinence related declines in attention in cocaine users were related to sleep measures (Pace-Schott et al, in press), given the relationships between sleep parameters and attention, this is an area worthy of further investigation. Furthermore, Allen et al. (1993) identified persistent differences versus controls, which may have been due to serotonergic neurotoxicity. Whilst Huxster et al., (2006) have identified subjective cognitive impairment in the sub-acute (post ecstasy use) phase, which was no longer significant after controlling for ecstasy related sleep disruption, suggesting this sleep disruption is capable of causing cognition impairment.

Human Cognitive Function Studies

Parrott (2000) provides a summary of cognitive deficits, identified in drug free MDMA users, divided into three categories: memory, higher executive ability, impulsiveness and other cognitive functions.

Memory has been found to be impaired on a range of tasks, with significant decrements identified by several different research groups (e.g. Verkes, 2001). These decrements have been found in comparison to non-drug using controls and polydrug (non-MDMA using controls).

Tasks assessing executive function again identify decrements in MDMA users performance, in comparison to controls (e.g. Wareing et al., 2000, 2004).

Studies of impulsiveness show that MDMA users are more impulsive in comparison to both non-drug using controls and polydrug (non-MDMA using) controls.

Parrott (2000) concludes that there is little evidence for impairments on more basic information processing tasks such as Stroop, Vigilance, Simple Reaction Time, Choice Reaction Time, and other simple cognitive tasks, of an attentional nature.

Therefore, clear evidence is found for functional abnormalities in MDMA users in comparison to control populations. This evidence comes from altered physiology (CSF, PET, pharmacological challenge, EEG) and altered behaviour (sleep and cognition studies). This evidence is suggestive of lasting MDMA-induced 5-HT injury in human recreational users.

Given the inter-relationships identified between crack cocaine use and sleep parameters in the study outlined previously, and the putative cognitive and sleep effects of recreational MDMA use, a clear question arises as to the potential for sleep/cognition interactions in this second drug using population. However, this topic has not received detailed attention. Furthermore, there are a number of criticisms of the research regarding the self-report nature of much of the data on drug use and other parameters, and lack of control for potential confounds (e.g. Cole et al., 2002). Researchers in the area argue that studies of recreational users are vital and that some methodological difficulties are inevitable outside the laboratory, requiring empirical data, rather than criticism of methods employed (e.g. Parrott and Fox, 2003). Therefore, a pilot study was conducted to look at the possibility of collecting objective drug use and sleep data in a recreational ecstasy using population, in a non-laboratory setting.

13.3 Hypotheses

The pilot study was planned to investigate the viability of conducting cognitive and sleep assessments in recreational 'ecstasy' users, associated with a cycle of binge use and abstinence, in a naturalistic setting.

13.4 Methods and Materials (Pilot Study Outline)

13.4.1 Subjects

Recreational ecstasy users were recruited via word of mouth. Four subjects (3 female and 1 male) were recruited into the pilot study. Age ranged from 27 to 42.

13.4.2 Ethics

Ethical approval for the study was granted by the Joint Ethics Committee (Newcastle and North Tyneside Health Authority, University of Newcastle upon Tyne, Northumbria University). Subjects provided verbal consent to the study procedures, but were not required to provide signed consent due to concerns over legal issues surrounding the possession of controlled substances. The study was supported by University of Northumbria at Newcastle and Cognitive Drug Research Ltd.

13.4.3 Design

Subjects were visited in their own homes on an afternoon or evening prior to using ecstasy. Training and baseline assessments were performed and the actigraph fitted, and the subjects then continued in their normal behaviour. On the day following ecstasy use subjects were revisited, repeated the assessments and also supplied details of the previous nights drug use, and gave a saliva sample.

13.4.4 Treatments

Subjects used recreational drugs in their own possession and were instructed that participation in the study should not influence their patterns recreational drug use.

13.4.5 Assessments

Training and baseline assessments were performed on a short computerised battery of cognitive function tasks assessing attention, working memory and secondary memory (CDR Ltd.). Subjects then completed a drug use questionnaire and were fitted with an Actiwatch-TS, actigraphy and skin temperature recording device (Cambridge Neurotechnology Ltd.).

On the day following ecstasy use subjects were revisited, repeated the cognitive test battery, supplied details of the previous nights drug use, and gave a saliva sample (Oral fluid test kits and laboratory analyses were provided by Cozart Bioscience Ltd.).

CDR battery tests:

- Immediate Word Recall
- Simple Reaction Time
- Choice Reaction Time
- Digit Vigilance

- Spatial Working Memory
- Numeric Working Memory
- Delayed Word Recall
- Delayed Word Recognition
- Delayed Picture Recognition

13.4.6 Statistical Analysis

Data were summarised for each subject and qualitative assessment made. In addition, data were presented and standardised z-scores and compared to reliable change indices with correction for practice effects.
Z=(score – reference population score)/reference population SD

(Reaction times were multiplied by –1 to standardise to positive values)

A reliable change index was then calculated to derive an index of meaningful change and allow for comparative review of performance over the assessment period. Reliable change indices (RCIs) incorporate measures of test-retest reliability and practice effects into formulae for calculating the range in which an individual score is likely to fluctuate as a result of measurement error. A simple RCI with the addition of correction for practice effects has previously been shown to perform as well as a number of different regression models and outperform RCI alone when assessing neuropsychological test performance (Temkin et al., 1999; Hinton-Bayre, 2000). In correcting for practice effects the RCI with correction may also allow for meaningful assessment of performance without prior training on assessments.

RCI 90% confidence interval = (SE_{diff})*(+/-1.64)+practice effect, where:

$SE_{diff} = \sqrt{[2(SE_m)^2]}$

$SE_m = SD_1 \sqrt{[1-r_{xx}]}$

SD_1 = standard deviation of reference population at baseline

r_{xx} = test-retest reliability coefficient between reference population follow-up and baseline scores

Practice effect = mean difference between reference population follow-up and baseline scores

It has previously been established that performance on CDR assessments plateaus following four training assessments (e.g. Wesnes and Pincock, 2002). Therefore, the scores at the first assessment were compared to those at the fifth (following complete training), for all data as performance was not expected to alter through practice effects with further repeated assessment.

13.5 Results

Initial data were obtained from four subjects:

Chronic and Acute Ecstasy Use

Saliva samples confirmed the presence of MDMA in saliva on the day following ecstasy use for all subjects. Two subjects gave samples containing MDMA at 125 and 149 ng/ml respectively (the first volunteer had ingested 2 white pills with a bird logo and the second one white pill). Two subjects gave samples containing 180 ng/ml MDMA and 51 and 85 ng/ml MDA (methylenedioxyamphetamine) respectively (both subjects had ingested 1.5 white pills with an 'E' logo). These results were consistent with the use of ecstasy tablets containing ring-substituted amphetamines.

Figure 41: Ecstasy tablet images (Ecstasy testing project EcstasyData.org)

Internet source

All four subjects reported having used 'ecstasy' previously on between 50 and 400 occasions, with an average intake of 2 to 3 tablets per occasion, and a maximum use of between 3 and 12 tablets.

Chronic and Acute Drug Use – Other

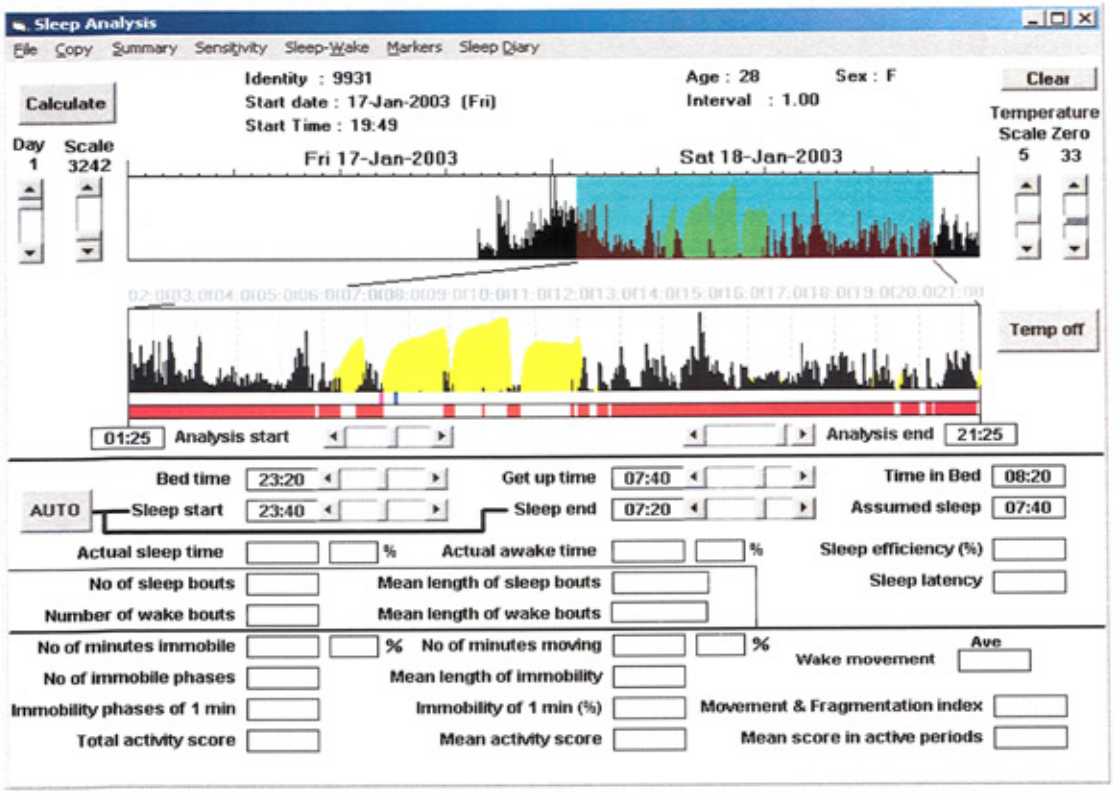
All subjects used other recreational drugs (including cocaine, heroin and cannabis) in addition to alcohol and tobacco, during the acute period of 'ecstasy' use.

Drug use questionnaire data showed that all subjects were habitual 'poly-drug users' using a variety of scheduled and legal recreational drugs for a number of years.

Sleep

The sleep analyses showed that the subjects stayed awake (active) throughout the night during the period of acute 'ecstasy' use. Sleep occurred during the mid to late-morning of the following day (approx. 0900 to 1200) for 2 to 4 hours.

Figure 42: Actigraphy data for single subject



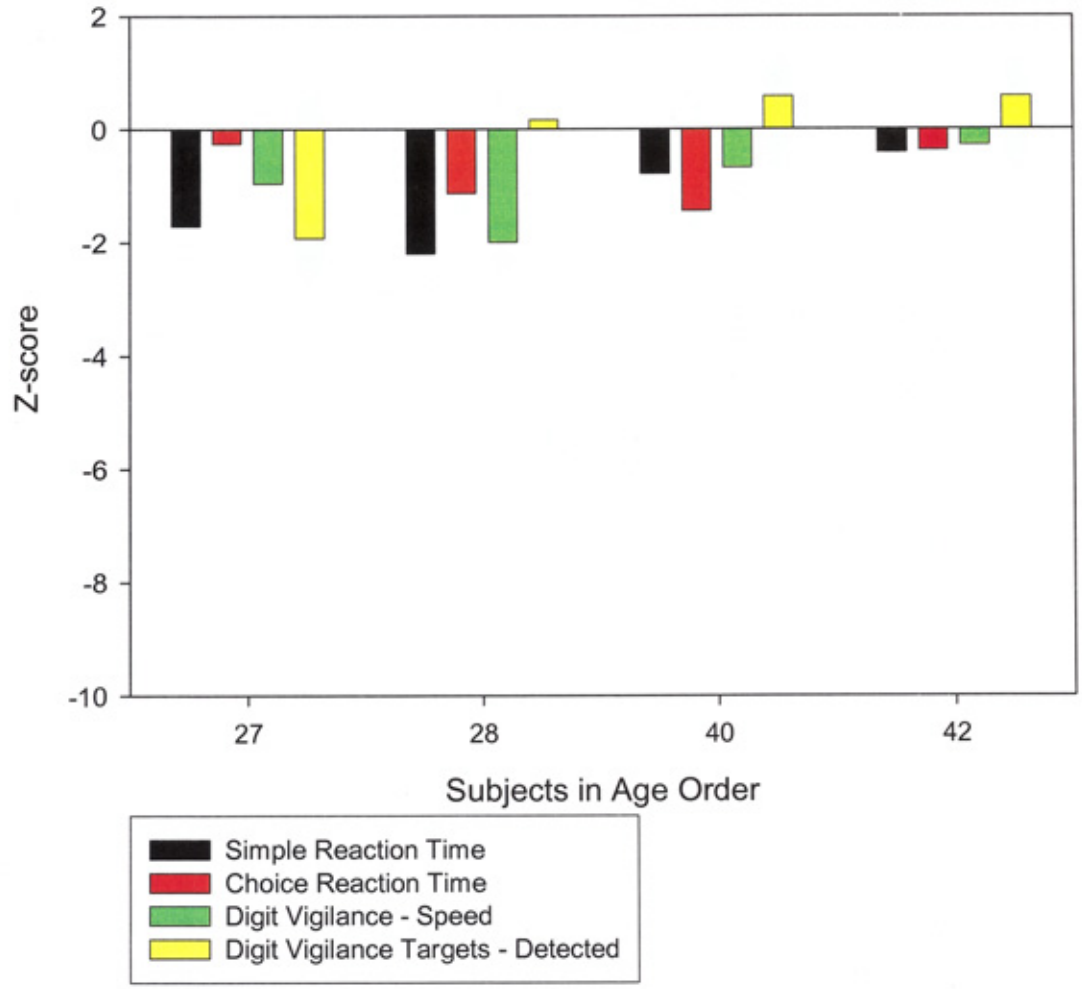
Black histograms indicate actigraphy recordings with sleep indicated by reduced activity.

Yellow shading indicates skin temperature.

Cognitive Function

Standardised z-scores using normative data from a reference population of healthy volunteers aged 21 to 50 years of age (mean = 30.4, N=3299) showed that reaction time performance at the first assessment was generally poorer in the MDMA users. However, comparative deficits were not as great as those seen in smoked cocaine users in comparison to the same normative reference population. As with the smoked cocaine users, there was no indication that the reference population was inappropriate due to the range of ages, with general decrements seen for all subjects, and the least decrement with the eldest.

Figure 43: Z-scored Attentional Measures for all MDMA Users



Each of the subjects generally showed poorer scores on all measures on the day following recreational drug use. The use of RCIs showed that reliable decline was not evident for any of the attentional measures. Though a decline approaching the RCI was seen for one subject on the Simple Reaction Time task, this was not evident for other measures and subjects, and one subject showed a reliable improvement in Digit Vigilance task performance on the accuracy measure (targets detected).

Figure 44: Simple Reaction Time with RCI as Reference Line (MDMA)

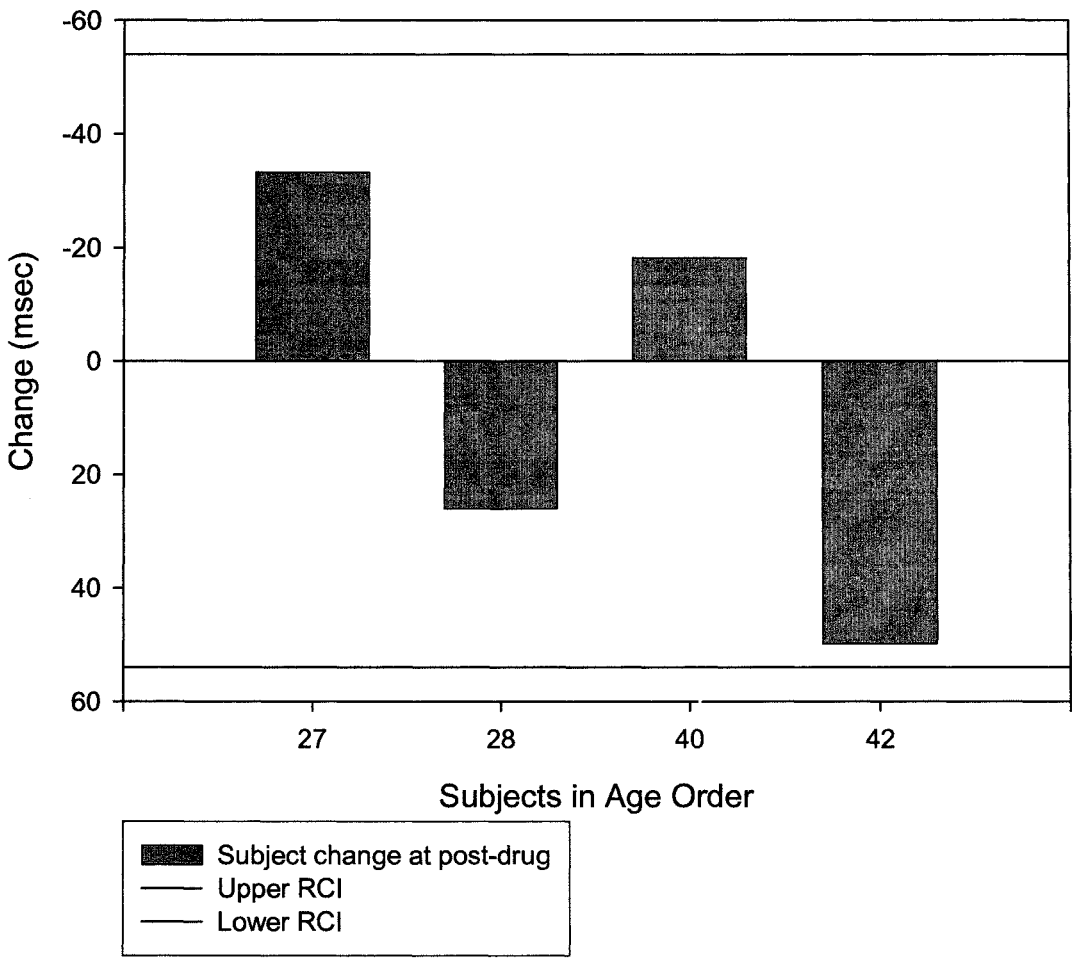


Figure 45: Choice Reaction Time with RCI as Reference Line (MDMA)

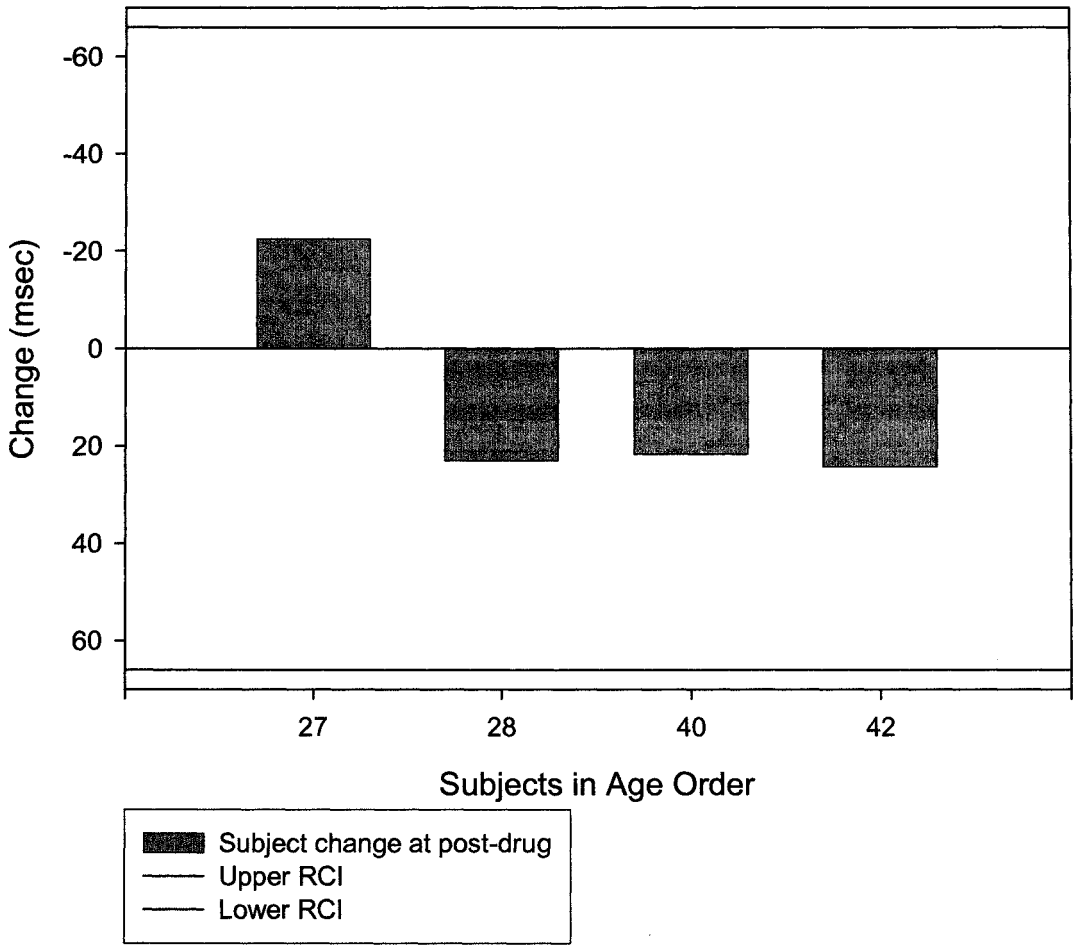


Figure 46: Digit Vigilance Targets Detected with RCI as Reference Line (MDMA)

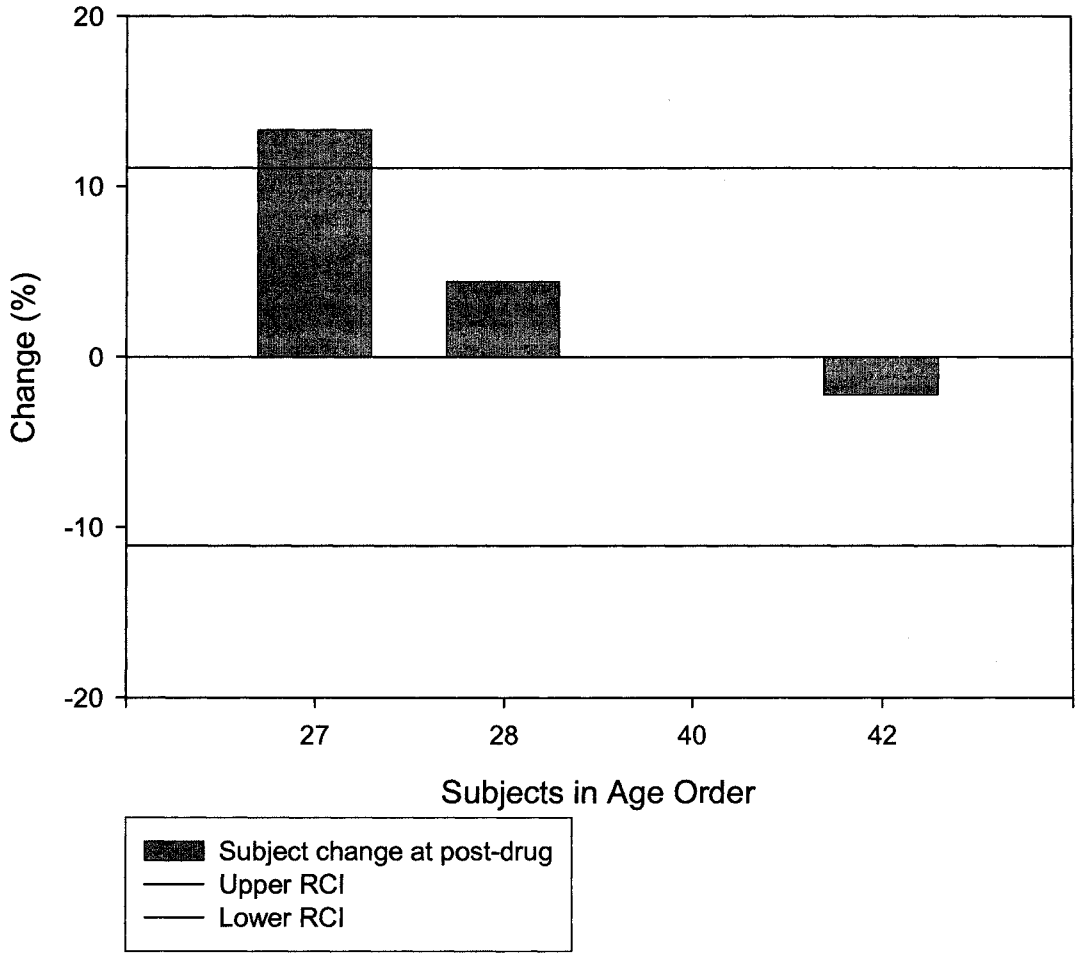
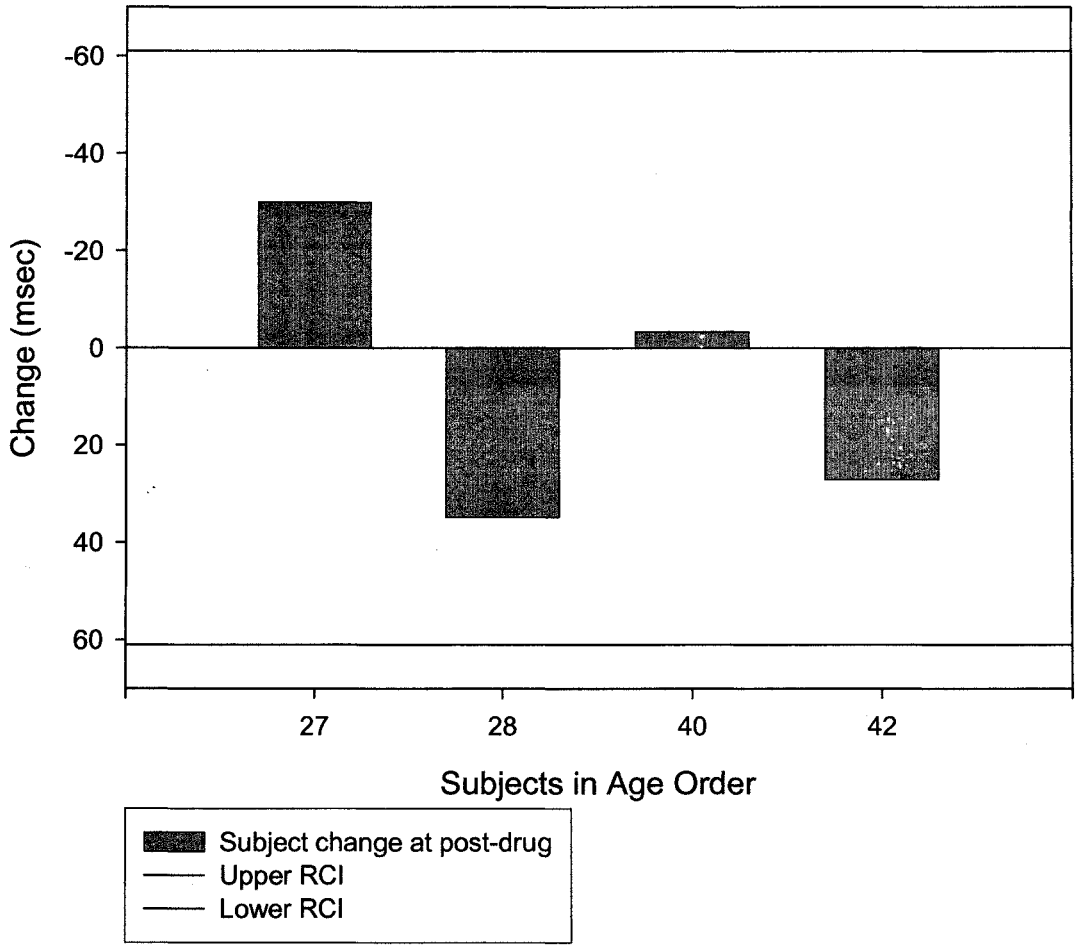


Figure 47: Digit Vigilance Speed with RCI as Reference Line (MDMA)



13.6 Discussion

The data provided broad support for the utility of the methodology employed in the study.

Saliva sampling allowed for confirmation of 'ecstasy' use. This was an important addition to this type of recreational study in a 'real-world' setting, as a common criticism of this type of study is a failure to gather data other than self-report, on drug use. However, it must be recognised that the saliva sampling technique only offers limited confirmatory evidence. The metabolic pathways of the ring-substituted amphetamines have been shown to be complex (Table 1 in Parrott, 2004) with both MDMA and MDA (the ring-substituted amphetamines identified) candidates as potential parent drugs and metabolites. Therefore, whilst confirming use of amphetamines consistent with 'ecstasy'/MDMA, there is no firm data

available regarding plasma levels or pill constituents. Also, it should be noted that the technique itself is expensive, and may be prohibitively so for many studies.

General patterns of drug use in the four subjects indicated repeated 'poly-drug' use. This is a large methodological problem for research into the potential effects of MDMA. However, 'poly-drug' use is the reality of recreational drug use for the majority of 'ecstasy' users (Rodgers et al., 2003). Whilst the issue of 'poly-drug' use and multiple unknown pill constituents cannot be ignored, research into recreational use should focus on investigation of drug use as it occurs. Unfortunately, instead the research has tended to focus on attempts to abstract unrepresentative drug using populations, or rigidly apply knowledge of individual compounds gained in the laboratory, to more complex 'real-world' situations.

The sleep analyses indicated a disrupted sleep pattern against that of what may be considered normal (i.e. night-time sleep, of a duration of around 8 hours). Again, the implications of the data were primarily methodological, as there was no comparative data to indicate what was normal sleep in the subjects studied. However, the data demonstrated the utility of sleep assessment. It is well known that stimulant drugs diminish and extinguish, for periods of time, the need for sleep or even the ability to sleep. Anecdotally, 'ecstasy' is a drug associated with extended (night-time) periods of activity and wakefulness. Whilst it is acknowledged that sleep disruption has a number of adverse effects on cognition and that it is associated with affective disorders such as depression, there is a paucity of research into the potential interaction of 'ecstasy'/MDMA effects on sleep and cognition, acutely, sub-acutely and chronically. The present paradigm has provided a potential method for addressing this.

The cognitive function assessment did not support next-day declines in attentional performance using the RCI methodology. Whilst in smoked cocaine users a number of declines in reaction time performance were seen during abstinence, which exceed the RCI limits, this was not evident here in the change from baseline to post-drug. However, the RCI technique, whilst identifying reliable statistical change does not allow for any interpretation of what may be a clinically significant effect, nor does it provide an insight into what might be statistically significant in conventional analyses of group change with larger sample sizes. Indeed, the RCI limits of approximately 50 to 60 msec for the reaction times from the attentional tasks are in excess of those produced acutely by relevant doses of sedating compounds on these same measures (e.g. alcohol 0.5g/kg, Wesnes et al., 2000c). Therefore, in a study on this scale firm conclusions cannot be drawn.

The assessment methodology itself was sound, but a particular difficulty was identified in scheduling next-day assessments, with subjects less willing to be tested next-day, than during the initial baseline assessments. It is likely that in a full-scale study, considerable attrition would result from change in compliance from pre to post acute drug use assessment. An alternative to direct participant visits for next day assessment of cognition, such as telephone or internet testing may result

in greater compliance and/or allow a greater volume of data to be captured more economically to account for attrition rates.

In conclusion, this pilot study has demonstrated that saliva sampling and actigraphy are important and utilisable techniques in recreational studies of acute 'ecstasy' use. Lengthy assessment of subjects outside of an institutional setting was problematic, particularly in the period immediately following drug use. However, given this caveat, the pilot study has outlined a paradigm in which cognition and sleep could be assessed in recreational 'ecstasy' users in a non-laboratory environment, and obtain detailed and well controlled data. As highlighted in recent debate on research in this area (Parrott and Fox, 2003), methodological and theoretical concerns regarding research in this area need to be addressed via empirical studies, and the methods outlined here provide techniques to gather that data.

In continuing this work it would be of interest to combine study of both cocaine and MDMA users. A study assessing baseline cognitive performance and sleep in these two groups could help to explore dissociable effects on sleep and cognition between what is primarily chronic dopaminergic neurotoxicity and chronic serotonergic neurotoxicity, though with possible secondary involvement of the other neurotransmitter system. Whilst it is clear from the literature that poly-drug use is the norm, groups mainly using cocaine or ecstasy could be identified and could help answer some of the current issues regarding the relative contribution of each drug to the neuropsychological problems associated with recreational drug use, as well as the theoretical questions regarding the mechanisms involved. For example, dissociable neuropsychological effects could support primary serotonergic neurotoxicity related to ecstasy versus dopaminergic neurotoxicity related to cocaine, whilst lack of dissociation could support more general neurotoxicity, or mechanisms such as allostatic load and bioenergetic stress (Parrott 2006). Further data on the relationship between sleep and cognition may be gathered by exploration of the acute and sub-acute phases of both drugs of abuse, by building up a profile of usage patterns in which use is followed by a period of abstinence, which may also have a unique neuropsychological profile. This information may also help investigate further the mechanisms involved with the possibility that unique neurotransmitter effects are more apparent in the sub-acute phase e.g. during the period of 5-HT depletion from the vescicle, whilst chronic perturbation effects (allostatic load / bioenergetic stress) become more important not just after longer periods of drug abuse, but also only emerge clearly during longer periods of abstinence when acute and sub-acute effects do not mask them. This data could also allow a clearer picture of the social and economic consequences of these patterns of drug use to emerge, with differential effects in each of these phases of drug use.

14 General Discussion

14.1 Outcome of studies

It is clear that the attentional task battery of the CDR system is a sensitive and valid measure of attention. Dopamine (DA) is widely considered to play a key role in the regulation of cognition and attention (e.g. Nieoullon, 2002) and evidence from studies of amphetamines suggests that the cognitive effects of these drugs depend on activation of the DA system, resulting in enhanced locomotor stimulation and behavioural activation (Willner and Scheel-Kruger eds, 1991). The reaction times from the CDR attentional battery were found to be sensitive to the effect of altered DA transmission produced by an amphetamine, whilst the sensitivity of the CDR attentional battery to pharmacological disruption of DA has also been established (e.g. Beuzen et al., 1999; Legangneux et al., 2000). Sensitivity of the CDR attentional tasks has also been established in Parkinson's Disease (PD) Parkinson's Disease Dementia (PDD) and Dementia with Lewy Bodies DLB (e.g. Walker et al., 1999, 2000a, 2000b, 2000c; Ballard et al., 2002), which have underlying dopaminergic pathology. Furthermore, the version of the Mackworth Clock task used in this series of studies was demonstrated to closely match versions with established sensitivity to d-amphetamine (Mackworth, 1965) and more recently, differential SSRI effects (Schmitt et al., 2002) and effects of acute tryptophan depletion (Schmitt et al., 2000). Therefore, it is clear that the tasks and measures employed are sensitive to the cognitive effects of changes in DA function in man. This sensitivity to changes in DA function is important because one possible mechanism via which serotonergic function may influence attention, is through inhibition of the DA system. However, despite this sensitivity to DA manipulation and pathology, no effects were identified on attentional measures either from the CDR system or the Mackworth Clock task, following acute tryptophan depletion (ATD) or acute tyrosine/phenylalanine depletion (ATyrD); manipulations with indirect and direct influences on DA. This was in contrast to the expected improvements to selective and sustained attention, from a reduction of 5-HT neurotransmission through acute tryptophan depletion, and impairments from a reduction of DA neurotransmission through acute tyrosine / phenylalanine depletion.

The lack of a clear differentiation between the SSRIs s-citalopram and sertraline also failed to support the predicted hypotheses. It was predicted that s-citalopram would impair attention through increased 5-HT neurotransmission inhibiting DA neurotransmission, whilst for sertraline this effect would be attenuated by the additional action of DA reuptake inhibition. The minimal differentiation may suggest that SSRIs have relatively slight or unpredictable effects on attention with sub-chronic dosing. However, it is also apparent that there may have been confounding factors, with the possibility of an interaction between sleep, circadian

rhythm, and attention. Research supports effects of sleep effects on vigilance, attention and psychomotor performance (e.g. Van Dongen et al., 2003; Jewett et al., 1999), whilst serotonin is believed to be intimately involved in sleep regulation (Pace-Schott and Hobson, 2002). SSRIs are known to influence REM sleep, probably through an increase in serotonin in the brain stem (Ridout et al., 2003; Feige et al., 2002; Schlosser et al., 1997). Sleep disruption has been associated with fluoxetine, paroxetine and sertraline, whilst a relatively better profile has been seen for sertraline (e.g. Goldstein et al., 1998), and evidence has suggested escitalopram itself may be effective in reducing sleep disturbance in major depression (Lader et al., 2004). Anecdotal evidence indicates that users of SSRIs may switch from AM dosing to PM dosing to avoid extra-pyramidal effects such as day time subjective sedation, but that this may then lead to increased sleep disruption. A recent study showed that a 5-HT_{1A} agonist suppressed REM sleep but that these effects were AM dosing dependent, and this same mechanism is thought to underlie the effects of SSRIs on sleep (Wilson et al., 2005). In the current SSRI study an interaction was seen between treatment and time of dosing / assessment, such that vigilance (Mackworth Clock task) appeared to be impaired with AM dosing and assessment, with s-citalopram, but improved with PM dosing and assessment. This may then suggest a differential effect of SSRI treatment dependent on time of dosing/assessment. However, given that the study was not designed to investigate these effects, the fact that they were restricted to the Mackworth Clock task and the potentially complex interactions between these factors, must temper the strength of any conclusion, and further research is needed. Despite this, the issues raised are relevant to studies sub-chronic dosing in healthy subjects and patient studies, and may inform patient reports of impaired alertness. Future studies should attempt to address whether any clear temporal relationship exists between SSRI treatment and effects on cognition.

Acute effects of dapoxetine 60 mg alone were evident for self-ratings of alertness and contentment, but not for objective attentional measures, whilst ethanol alone produced a pattern of cognition impairment consistent with that seen previously. Whilst there were some indications of additional cognitive effects during coadminstration, the general variability in the data (particularly that under placebo) and the lack of statistical support for interactions and the absence of any pharmacokinetic interactions suggest that no cognitive interactions were present. Though cognitive interactions with alcohol have not generally been seen for the SSRIs, they may be associated with attentional impairment (e.g. van Harten et al., 1992) and there is some limited evidence for interactions associated with cognitive impairment (e.g. Allen et al., 1988). Therefore, alcohol interaction studies remain an important consideration in the safety assessment of novel and existing SSRIs where there is potential for coadministration.

Findings from the cocaine and 'ecstasy' studies supported the possibility of sleep disruption in both these populations, which may be related to theories of CNS

"allostatic load" either in terms of resultant sleep disruption, or exacerbation by sleep loss (Van Cauter and Spiegel, 1999). This may be related to damage to serotonergic systems, which occurs in chronic cocaine abuse (Battaglia and Napier, 1998; Fleckenstein et al., 2000), and potentially 'ecstasy' use. These potential effects are clearly relevant to studies of recreational 'ecstasy' users. Given the evidence for 5-HT neurotoxicity in animals and the evidence for disruptions to 5-HT function in human recreational users, there is a clear need to investigate sleep parameters in addition to cognition in this population. These considerations should be used to inform research into recreational drug use, and the need to identify both the scope and aetiology of cognitive impairment may be critical in treatment strategies for drug users. For example, if cognitive impairment results from, or is exacerbated by disrupted sleep, then assessment of sleep will be critical in understanding cognition in these drug users.

The cognitive effects of a 5-HT_6 antagonist in the scopolamine challenge model of cognitive dysfunction were focussed on memory rather than attention. This may have been due to the specific actions of 5-HT_6 antagonism on glutamatergic and cholinergic systems providing a preferential benefit to memory over attention, with these neurotransmitter systems, both having strong involvement in memory functions. In addition, the localisation of the 5-HT_6 receptors in brain areas associated with learning and memory over those associated with other aspects of cognition including attention is an important factor. The key issue raised is the potential cognitive and other specificity of receptor sub-type specific compounds. This provides both potential treatment benefits in improving the 'focus' of a treatment, and reducing extra pyramidal effects. Yet it is also important to recognise that cognitive dysfunction often occurs in more than one domain, such as the marked memory and attentional deficits in AD, both of which require treatment.

14.2 Methodological Issues

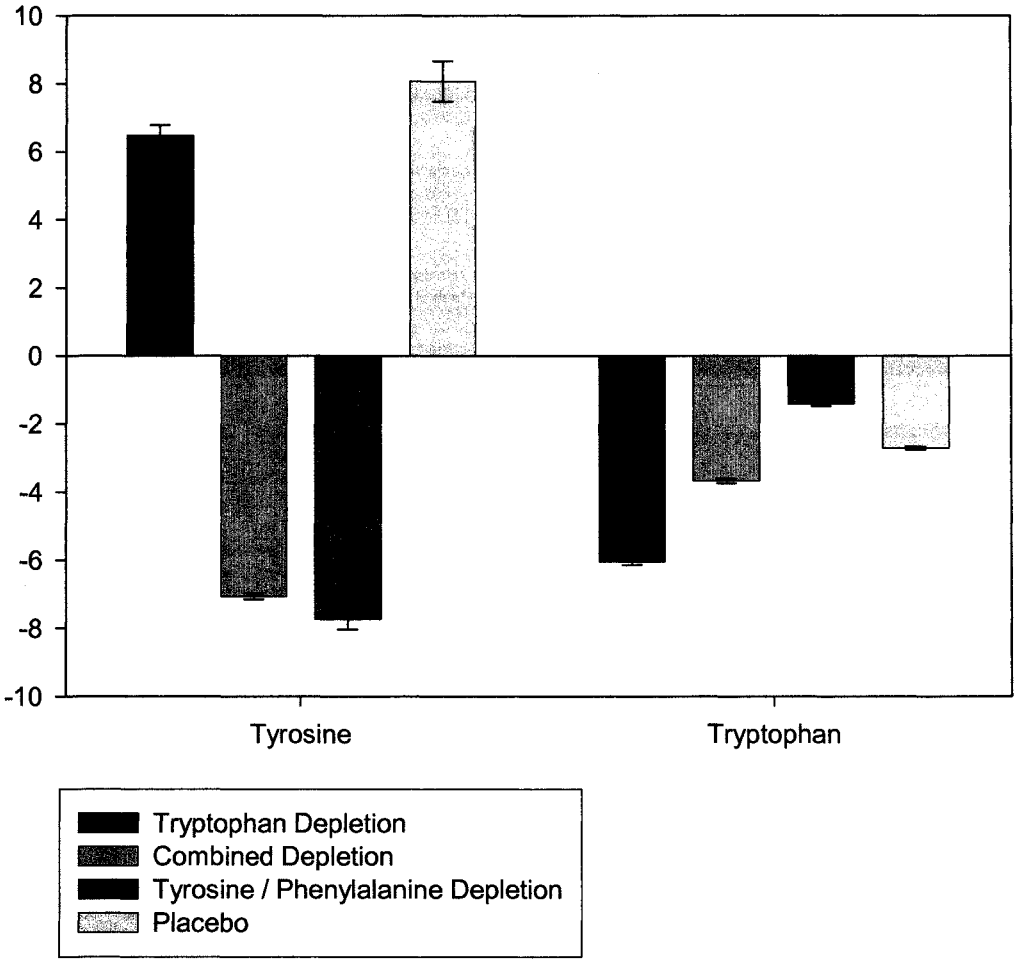
The study of the amphetamine analogue provided data supporting the sensitivity of the CDR attentional measures (reaction times) to enhanced dopaminergic neurotransmission, consistent with previously reported effects. Possible criticisms of this study were the small sample size (N=8) and the transferability of these data to other populations given the small sample and the population characteristics (middle aged subjects mean age 51.8). In respect of the sample size, the main consideration was one of risk exposure and selection of a small sample size was intended to reduce the level of risk by exposing fewer subjects to the treatment, with the study primarily powered on pharmacokinetic and safety measures. However, in order to retain power a cross-over design was used, such that all 8 subjects completed each treatment allowing for a more powerful within subjects analysis, whilst two placebo periods were completed doubling the sample size for this treatment. The success of this was demonstrated by the statistically significant

effects observed and whilst these were restricted to reaction time measures, there was not a consistent pattern of effects in other measures, which it could be argued may have failed to reach significance due to the small sample size. With regard to the population itself, a mixed sex sample was used, but these were older adults (40 to 80 years of age). Therefore, whilst we would expect results to generalise to males and females there is a question over specific sex differences and whether young subjects (<40) or elderly subjects (>80) would show similar effects. One possible argument may be that these effects may only be evident in a 'sub-optimal' system e.g. when a neurotransmitter system has degenerated with ageing. In respect to the specific methodology of this study, this is a non-issue, as it serves to illustrate the sensitivity of these assessments to this type of pharmacological manipulation (a validation study) and was not attempting to investigate the underlying mechanisms involved. However, this is a potentially important theoretical issue and the applicability of findings in particular populations to other populations is important when considering both the mechanisms by which serotonergic function may influence attention and in the clinical evaluation of serotonergic compounds.

The monoamine depletion study failed to support the proposed hypotheses with respect to cognition. This lack of support for the hypotheses does not necessarily contradict theories implicating serotonergic inhibition in modulating attention and potential explanations involve the reliability of the experimental manipulation itself, in terms of producing effects on neurotransmission, the manipulations having relatively slight or unpredictable effects on attention, the sensitivity of the measures employed and the nature of the population under study. It is unlikely that the lack of effects resulted from insensitivity of the task measures, given the clearly established sensitivity to DA manipulation and the sensitivity of these measures in previous studies (e.g. Schmitt et al., 2000; Harrison et al., 2004; Matrenza et al., 2004). Furthermore, whilst particular populations may be more sensitive to these effects (e.g. females or populations with a family history of affective disorder / serotonergic dysfunction), studies of both male (Gallagher et al., 2003), female (e.g. Harrison et al., 2004), mixed populations (e.g. Schmitt et al. 2000) and healthy subjects with a family history of depression have reported effects (e.g. Riedel et al., 2003). Though in the latter case there may be dissociation in the nature of the effects on certain measures. Therefore, the remaining explanations centre on the reliability of the experimental method. For both types of manipulation (acute tryptophan and acute tyrosine / phenylalanine depletion), results have failed to be consistent with both supportive and contradictory data for an influence on attention reported (e.g. Harrison et al., 2004; Lythe et al., 2005). It is possible that the effects seen in previous studies of acute tryptophan depletion resulted from influencing neurotransmitters other than serotonin. The data gathered from this and other studies have shown that in depleting tryptophan and hence reducing competition for the transporter, levels of tyrosine are increased. In fact, Figure 48

below shows that in the present study ratio of tyrosine was increased in both the tryptophan depletion and placebo conditions, whilst ratio of tryptophan was reduced to differing extents in all conditions. Any increase in tyrosine ratio could in turn increase DA synthesis and result in the attentional benefits identified in previous work. In addition, it is clear that the placebo / control arms of these studies do not have a neutral effect in that large ratio changes may be evident for these treatments also. Therefore, the effects seen previously might depend as much on increasing tyrosine as they do on decreasing tryptophan. Given the potential importance of ratios as opposed to absolute levels of each individual large neutral amino acid (LNAA), further study of the source and impact of these changes is required in order to make this technique a reliable probe of cognitive function, which produces consistent research findings. However, what is clear is that the experimental paradigm does not provide a method for investigating the effects of serotonergic function on attention, as tryptophan depletion can result in tyrosine loading, therefore any effects seen might be directly dopaminergic, and furthermore there is an even greater confound if trying to investigate if serotonergic effects on attention operate via the dopaminergic system.

Figure 48: Change in the Ratio of Tyrosine (TYR) and Tryptophan (TRP) to other Large Neutral Amino Acids for each Drink Condition (mean +/- sem)



In respect to the two studies of SSRI effects the major methodological concerns arise from the same issue, but in two separate forms. For the sub-chronic dosing study with escitalopram and sertraline, the data showed a lack of differentiation, which was supportive of a safe profile in terms of no cognition impairment being associated with the compounds. However, the lack of a placebo arm makes it difficult to draw firm conclusions as it is only possible to speculate on the likely position of a placebo arm relative to both active treatments. The placebo arm was not included due to the cost involved in adding a complete additional arm to the study. Clear effects in the predicted direction on the attentional measures would have provided support to the theoretical hypotheses concerning serotonergic and dopaminergic influences on attention, but in the absence of clear effects interpretation is difficult and conclusions about the mechanisms of effect are not possible, as we cannot clearly state that either treatment has had any effects. In contrast, for the acute SSRI study, the major problem was the marked variability

under placebo treatment, which called into question the validity of the conclusions regarding effects of the active treatments. This problem was addressed by focussing on the Tmax for the treatments and thus reducing the variability, which was seen at later timepoints in the study. The source of this variability is difficult to identify, but may stem from the conditions in which the data were gathered. Noise and distraction within a busy clinical research unit may have a marked effect on performance and the importance of tightly controlled experimental conditions should not be underestimated. Given the widespread use of cognition assessment in clinical research a thorough programme of investigation to define the optimal conditions of clinical cognition assessment could lead to better research procedures improving the quality of data collection. However, at this time the source of the placebo variability seen in this study is unknown.

In respect of the study of 5-HT_6 antagonism the results were clear in identifying a modulatory effect on memory, but not on attention during scopolamine challenge. This raises two important questions; the first of which is the importance of the scopolamine challenge paradigm, which may elucidate the mechanisms important to this effect. It is probable that the cholinergic pathway, which mediates both the cognition impairment with scopolamine and is thought to be positively modulated by antagonism at the 5-HT_6 receptor, is an important one in terms of the cognitive effects seen. This then raises issues with regard to other circumstances in which a positive modulation of cognition would be evident. For example this might be more useful to a disease such as Alzheimer's disease with an underlying cholinergic pathology, than to cognitive dysfunction where a different aetiology is suspected. Furthermore, it may require that a neurotransmitter system is operating sub-optimally, before a benefit can emerge. However, both of these issues it is possible to address with clinical studies in the target indication, with the data here serving as proof of concept. One possible flaw in the methodology used, is the more recent finding of positive modulation of attentional task performance in the rat. Whilst it is difficult to map animal models of cognition directly on to human models, in a test of attentional performance Hatcher at al. (2005) used an analogue of the Wisconsin Card Sorting task in rodents, which uses food reward to train digging behaviour with discrimination based on odour and media (material in which the food was buried). Intra and extra-dimensional shifts were achieved with changes to odour and material. Two 5-HT_6 antagonists were found to reduce errors and the number of trials required to reach a criterion level of performance, supporting a benefit to attentional set-shifting with the compounds. This aspect of attention may have clinical relevance to the treatment of some cognitive disorders (e.g. schizophrenia). However, it was not an aspect of cognition assessed in the current studies, which have concentrated on measures of focused and sustained attention, rather than other more executive aspects such as the allocation of attentional resource, shift in attentional resource and inhibition, which may influence attentional function, but are not measured well using the measures

employed. Therefore, it is possible that some relevant aspects of attentional function pertinent to manipulations of the serotonergic system may be missed unless other types of task measure are employed.

The studies of cocaine and MDMA use must be acknowledged as pilot studies, which cannot provide significant contributions to theories of mechanisms underlying cognitive dysfunction in these drug using groups. However, they do provide support for methodologies, which could be used to study these areas in more detail. A study assessing baseline cognitive performance and sleep in these two groups could help to explore dissociable effects on sleep and cognition between what is primarily chronic dopaminergic neurotoxicity and chronic serotonergic neurotoxicity; though possible secondary involvement of the other neurotransmitter system could also be considered. Whilst it is clear from the literature that poly-drug use is the norm, groups mainly using cocaine or ecstasy could be identified and could help answer some of the current issues regarding the relative contribution of each drug to the neuropsychological problems associated with recreational drug use, as well as the theoretical questions regarding the mechanisms involved. For example, dissociable neuropsychological effects could support primary serotonergic neurotoxicity related to ecstasy versus dopaminergic neurotoxicity related to cocaine, whilst lack of dissociation could support more general neurotoxicity, or mechanisms such as allostatic load and bioenergetic stress (Parrott 2006). Consideration of the literature has shown a trend for a shift away from serotonergic neurotoxicity as the key theory explaining differences between 'ecstasy' users and the various control groups, to recognition of a more complex pattern of poly-drug use and understanding of the difficulty in confirming 'ecstasy' tablet content. In the late 1990's studies of 'ecstasy' users tended to speculate that serotonergic neurotoxicity was the likely cause of the effects seen (e.g. Parrott, 1998; Morgan, 1999; McCann, 1999; Dafters, 1999). Over time debate has shifted opinion, such that the evidence for neurotoxicity in humans is considered to be less strong, as opposed to that for changes in serotonergic function. In addition, concomitant use of other drugs is seen as an important mediator of effects (for review see Morton, 2005) and concerns over tablet content have been raised (Cole et al., 2002). Studies have begun to converge more on consideration of 'ecstasy' users or 'poly-drug users' as a recreational drug-using group with potential neurological and psychobiological problems, as opposed to a group in which a distinct and specific neurotoxicity may be present. This has lead to a wider consideration of the effects of dysregulated neurotransmitter function, as opposed to a focus on a theory of serotonergic neurotoxicity and its predicted effects. Further data on the relationship between sleep and cognition may be gathered by exploration of the acute and sub-acute phases of both drugs of abuse, by building up a profile of usage patterns in which use is followed by a period of abstinence, which may also have a unique neuropsychological profile. This information may also help investigate further the mechanisms involved with the

possibility that unique neurotransmitter effects are more apparent in the sub-acute phase e.g. during the period of 5-HT depletion from the vescicle, whilst chronic perturbation effects (allostatic load / bioenergetic stress) become more important not just after longer periods of drug abuse, but also only emerge clearly during longer periods of abstinence when acute and sub-acute effects do not mask them. This data could also allow a clearer picture of the social and economic consequences of these patterns of drug use to emerge, with differential effects in each of these phases of drug use.

14.3 Sleep and circadian interactions with cognition

The potential interactions between serotonergic influences on circadian rhythm / sleep and cognition may be critical to future research into cognitive effects of SSRIs. This is true both for patient studies and chronic and sub-chronic (multiple dose) studies in healthy subjects, both of which may show influences of circadian effects and altered sleep due to the time course of the studies. The limited detailed research in this area of circadian/sleep/cognition interaction with SSRIs may explain the prior identification of effects on vigilance performance, which have been attributed to direct serotonergic influence on attention (via dopaminergic modulation), but which have not been replicated here. Similarly, research into the chronic effects of MDMA on cognition and attention may benefit from a more structured approach to sleep and circadian parameters, given the mechanism of action of the compound and the potential for serotonergic neurotoxicity in recreational users. Whilst, these effects cannot be considered relevant to studies of acute monoamine depletion and acute effects of serotonergic compounds, they are worthy of consideration in the development of serotonergic compounds as therapeutic treatments, where there is reason to believe a compound may influence sleep or circadian rhythm. Both sleep parameters and cognition (in particular focussed and vigilant attention) are worthy of investigation either during early repeated dosing studies and/or later patient trials of sub-chronic or chronic dosing. The CDR system has been demonstrated to be sensitive to attentional impairment in populations with disordered sleep including primary insomnia (Ashurst et al., 2004; Wesnes et al., 2005), and shift-work sleep disorder. In addition, improvements to attention have been seen in these populations with armodafinil (Wesnes et al., 2005). Furthermore, the attentional tasks have shown sensitivity to sleep deprivation and reversal of these deficits by modafinil (Wesnes and Macher 2004). Therefore, the assessments employed here may be good candidates for further research where there may be an interaction between treatment effects on sleep parameters and attention. An additional and important consideration is the co-morbidity of sleep disorders in many disease states, in which the serotonergic system may be a therapeutic target. Disordered sleep is common in both depression and AD and the potential for treatments to normalise sleep and aid cognition, perhaps in addition of other efficacious actions is an exciting one.

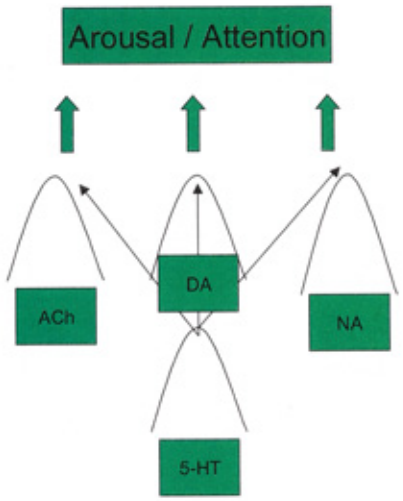
14.4 Research Implications

14.4.1 Theoretical Implications

The nature of the structural and functional interactions between the serotonergic system and other neurotransmitter systems should be the primary consideration when looking at potential serotonergic influences on cognition. The majority of research into serotonin and cognition has been focussed on the areas of learning and memory (see Meneses, 1999 for review), and research into serotonergic compounds intended to treat cognitive dysfunction has also focussed primarily on these areas of cognition, as opposed to attentional function.

It has been demonstrated that attention is mediated by a number of neurotransmitter systems primarily, dopamine, noradrenaline and acetylcholine, with 5-HT systems inhibiting each of these. Robbins (1997) in opening the abstract to the review states "Unitary concepts of arousal have outlived their usefulness and their psychological fractionation corresponds to a similar chemical differentiation of the reticular formation of the brain." This is supported in the review by a well developed series of studies demonstrating that different aspects of attentional task performance may be influenced by manipulations of cholinergic, noradrenergic and dopaminergic systems respectively. In this respect this empirical evidence and the conclusions are consistent with Allport's earlier (1992) theory that there are many individual sub-processes, which contribute to overall attentional function. However, in respect of the serotonergic system specifically, Robbin's concludes that "the 5-HT systems may serve to dampen the actions of each of the others [ACh, NA, DA], for example by promoting behavioural inhibition and cortical dearousal." This latter conclusion is consistent with theories put forward by several reviewers in various forms such as Jacobs and Fornal (1999) who propose that the primary function of serotonin is to facilitate motor output, whilst simultaneously suppressing sensory information processing to inhibit input which might disrupt motor behaviour. Yet it is notable that a unitary concept of arousal has been invoked for the serotonergic system, though it has fallen out of favour in general terms and is not thought by Robbins to explain the data seen for other neurotransmitter systems. Therefore, it is interesting to apply the data gathered from the current studies to unitary arousal theories for the role of serotonin.

Figure 49: Theoretical inhibition of neurotransmitter systems important in arousal/attention by serotonin



After Robbins, 1997

These theories suggest that in particular SSRIs might have generally deleterious effects on attention through inhibition of ACh, NA, DA, whilst tryptophan depletion might be expected to have the opposite effect. As was seen, this was not supported by the data. The possible methodological issues have been discussed and lead to the conclusion that monoamine depletion may not provide a reliable paradigm for investigating these effects in humans. However, it was also apparent that the SSRI effects were not clearly apparent as 'generally deleterious'. Accepting the methodology of those studies, there are two possible implications for these theories of 'general arousal' with respect to serotonin. Either they are insufficient to explain the data and a more complex understanding of serotonergic influences on attention is required, or they do have sufficient complexity to explain the data.

Taking this second point, the data seen may be explained by considering each neurotransmitter system as an inverted U with respect to optimal function (Figure 49). These Us in turn feed into attentional/arousal processes via level of function of the underlying neuropsychological processes, which they control. Increased or decreased function of any one neurotransmitter system may serve either to optimise or de-optimise any given process and thus arousal/attention, dependent on where that system starts from on the curve, from sub-optimal through optimal to supra-optimal. In addition, serotonin then provides an additional

influence by modulation the function of the other systems. Thus, accepting that all systems are unlikely to be operating optimally at all times, it may be seen, particularly in healthy subjects, how experimental manipulations might result in limited, subtle or inconsistent effects by optimising and de-optimising different underlying systems, which feed into more gross measures of attentional function/arousal. This also predicts that in cases of underlying dysfunction (sub optimal function) more consistent effects might be expected. Therefore, the theories as they stand can be sufficient to explain the results of the present SSRI and monoamine depletion studies, and inconsistencies in the published data on these paradigms.

The first point, that a general theory of inhibition for serotonin is insufficient, requires either evidence that for a specific role for serotonin in governing underlying neuropsychological functions relevant to attention, consistent evidence indicating that increased serotonergic function may serve to increase arousal/attention or vice versa, or evidence for specific and differing roles of the different receptor subtypes. Whilst this thesis has not attempted to investigate specific neuropsychological processes with which serotonin may be directly involved, there is some evidence for this, some of which Robbins (1997) outlines himself with respect to response inhibition, suggesting that serotonin might have a role in task performance where inhibition of particular responses is required, which then could contribute to measures of overall task performance and hence attentional function/arousal. What is pertinent to the present study is possible evidence for consistent opposite effects to general inhibition, or differing receptor subtype specific effects on attention/arousal. This might have come either from effects contrary to the proposed hypotheses in the SSRI or monoamine depletion studies, or clear effects on attention in the study of the 5-HT_6 antagonist. However, this evidence was not seen. Therefore, from the present evidence the general theory is still the best supported. Yet it must be acknowledged that other data challenge this and that further studies may result in more complex theories. This may be seen from three strands of research. The first is evidence for serotonergic influences on specific neuropsychological processes. The second is a greater understanding of the nature and complexity of the functional interactions between serotonin and other neurotransmitter systems. It is clear that this interaction goes far beyond the dopamine system, but also the noradrenergic and cholinergic systems and includes e.g. glutamate and GABA, and that the relationship is not always inhibitory but may be stimulatory also (Table 1). Taking 5-HT_6 antagonism as the example, here antagonism was being used to enhance cholinergic and glutamatergic function, consistent with the inhibitory theory. No positive effect on attention was seen, which may either be due to factors to do with the paradigm, or may indicate that the general model is insufficient by demonstrating an example of a receptor subtype where antagonism does not result in consistent attentional enhancement / increased arousal, thus differing from the theory of general inhibition. However,

more convincing data would need to come from consistent findings in this respect and as noted evidence for improved attentional set-shifting has been seen in the rat with 5-HT_6 antagonism (Hatcher et al., 2005). Stronger evidence challenging the general theory might be examples of e.g. 5-HT_3 or $_4$ receptor agonists, which were able to positively influence attention/arousal on the basis of their functional interaction with the dopaminergic or cholinergic systems.

Future studies should then seek to investigate this by building up a greater understanding of the functional interactions between the serotonin receptor subtypes and both attentional/arousal as a gestalt and as specific to particular neuropsychological processes contributing to these overall measures. In addition, this must follow alongside a greater understanding of the underlying neurobiology of these interactions in man.

14.4.2 Clinical drug development

Over the last decade, as research has identified the effective mechanisms in each therapeutic area, there has been a general shift from non-specific treatments with several modes of action and a number of extra-pyramidal effects, to increasingly specific targets. The development of antidepressant treatments illustrates this trend (Burke and Preskorn, 1995). The earlier treatments, the TCAs and MAOIs, had wide pharmacological effects and a broad profile of undesirable extra-pyramidal effects. Later treatments e.g. the SSRIs focussed on more specific targets maintaining or improving efficacy, whilst attempting to minimise side effects.

Development of Antidepressant Treatments by Serendipity (after Burke and Preskorn, 1995):

Tricyclic Antidepressants (TCAs):

- Mechanisms believed not to mediate antidepressant response: Acetylcholine (ACh), Histamine (H_1, H_2), Alpha-1.
- Mechanisms believed to mediate antidepressant response: Norepinephrine (NE), Serotonin (5-HT).

Monoamine Oxidase Inhibitors (MAOIs):

- Mechanisms believed to mediate antidepressant response: Norepinephrine (NE), Serotonin (5-HT), Dopamine (DA).

Refinement and Testing

Bupropion:

- Mechanisms believed to mediate antidepressant response: Norepinephrine (NE), Dopamine (DA).

Venlafaxine:

- Mechanisms believed to mediate antidepressant response: Norepinephrine (NE), Serotonin (5-HT).

Selective Serotonin Reuptake Inhibitors (SSRIs):

- Mechanisms believed to mediate antidepressant response: Serotonin (5-HT).

Serotonin Receptor Subtype Specific (e.g. 5-HT_2 antagonists, 5-HT_{1A} agonists):

- Mechanisms believed to mediate antidepressant response: Serotonin (5-HT).

This same trend can be observed in clinical research with serotonergic compounds, with the emergence of several receptor subtype specific ligands under investigation for the treatment of various different disorders.

Table 17: 5-HT receptor sub-type specific compounds in current development for CNS indications

Company	Compound	Type	Indication	Phase
GSK	742457	$5HT_6$ antagonist	schizophrenia and dementia	I
Wyeth	Lecozotan (SRA-333)	$5\text{-}HT_{1A}$ antagonist	Alzheimer's	2
Vernalis/Roche	-	$5\text{-}HT_{2C}$ agonist	obesity	0
Arena	APD356	$5\text{-}HT_{2C}$ agonist	obesity	2
Arena	APD125	$5\text{-}HT_{2A}$ inverse agonist	insomnia	1
Organon	Org 50081	$5\text{-}HT_2$ 'blocker'	insomnia	2

Information obtained from public 'product pipeline' data.

Alzheimer's disease (AD), can be used as a specific example of this trend. Until recently in the dementias, the primary therapeutic target was been the cholinergic system. Post-mortem studies in the mid-1970s identified severely reduced cholinergic activity in the brains of AD patients, and these losses were linked to the cognitive deficits seen in the disease. The cholinergic hypothesis of memory dysfunction in dementia, reflected the view that dementia was predominantly a memory disorder (Bartus et al., 1982). Research efforts focussed primarily on the cholinergic system, and increasing levels of ACh at the receptor through the use of the anti-cholinesterase's to inhibit enzymatic metabolism. This has led to several treatments becoming available, which are effective in treating memory impairments associated with AD (e.g. galantamine, donepezil, rivastigmine). Later it became recognised that attentional impairment was an important part of the profile of cognitive impairment in the dementias, and that the cholinergic system was an important modulator of attentional function in addition to memory function. An important feature of this research has been computerised assessment using millisecond reaction time recording as critical in order to measure attentional function. The benefits of the anti-cholinesterases to attention have now been identified in several forms of dementia, including AD (Wesnes, 2001). However, the effectiveness of the anti-cholinesterases appears limited and research into different therapeutic targets has continued. The 5-HT receptor sub-types have emerged as one class of novel therapeutic targets, as understanding of their modulatory actions has grown and research has highlighted multiple neurotransmitters systems as involved in cognition and compromised in the dementias.

Wyeth's "Lecozotan" is being developed specifically with the purpose of treating cognitive dysfunction in AD (see table above). The purpose is to attempt to enhance the activity of neurotransmitter systems compromised in AD through progressive neurodegeneration. A learning impairment is seen following cholinergic blockade and lesions in rats and primates, which may be attenuated by 5-HT_{1A} antagonists, suggesting potential efficacy in treating AD (Carli at al., 1997). These effects may be mediated through 5-HT_{1A} inhibitory input influencing both glutamate and acetylcholine (Barnes and Sharp, 1999). Facilitation of glutamate has been shown to enhance memory (Staubli et al., 1994) and cholinergic function is important in both memory and attention (Perry et al, 1999). Both glutamatergic and cholinergic function are compromised in AD and are thought to contribute to the debilitating cognitive effects of the disease. However, as the studies in the present thesis have outlined, the general theory serotonergic of inhibition may be challenged and 5-HT antagonism may not always result in reduced inhibition and consequently enhanced cognition. Therefore, it will be important to consider several interacting factors such as the functional interactions with the receptor subtype, its location in brain tissue and the possibility of differential effects on attention versus learning and memory. It is known that both attentional and memory functions are compromised in AD (e.g. Ballard et al, 2002). Therefore, it is critical in the development of these compounds to determine whether attentional function is benefited, in addition to learning memory. Indeed, this is already a key benefit of several of the current treatments in AD e.g. galantamine (De Deyn et al., 2004). Were attentional function to be unaffected, the possibility of combination therapy (e.g. anticholinesterase + Lecozotan), could be considered to attempt to provide a broader cognitive benefit.

Therefore, future studies must be concerned with ensuring comprehensive assessment of cognition in the development of serotonergic compounds intended for the treatment of cognitive disorders.

14.4.3 Conclusions

General theories of inhibition have been demonstrated to be sufficient to account for the data from the studies presented in this thesis. However, the issues raised with regard to methodology question the evidence gathered from studies of SSRIs and chronic MDMA use in man, which have not adequately investigated possible interactions between the experimental manipulations and both sleep and cognition. Furthermore, studies of monoamine depletion may be more fundamentally flawed in being unable to influence any one amino acid in isolation, particularly in that changes are seen in both typtophan and tyrosine in all treatments in a four-way crossover investigating single, combined and placebo depletion treatments. Whilst only limited evidence is seen here for possible differential effects of receptor

subtypes, with a 5-HT_6 antagonist failing to improve attention, wider evidence does indicate a possible role for serotonin in specific neuropsychological processes underlying attention/arousal and for differential effects of the receptor subtypes. This indicates that further investigation of the relationships between serotonin and attention in man is warranted in order to develop more complex and accurate models useful in informing clinical research.

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16 Conference presentation/attendance during the course of this thesis

British Association for Psychopharmacology Summer Meeting, July 2001

Annual Scientific Meeting of the Psychobiology Section of the British Psychological Society, September 2001

British Association for Psychopharmacology Summer Meeting, July 2002

Annual Scientific Meeting of the Psychobiology Section of the British Psychological Society, September 2002

British Association for Psychopharmacology Summer Meeting, 2003

Third Dutch Endo-Neuro-Psychopharmacology Meeting, June 2004

CINP Meeting, June 2004

British Association for Psychopharmacology Summer Meeting, 2004

British Association for Psychopharmacology Summer Meeting, 2005

American Academy of Neurology Annual Meeting, San Diego April 2006

17 Training course/workshop attendance during the course of this thesis

CNPS/BAP Workshop: Psychopharmacology of Cannabis and Ecstasy, April 2002

Repeated Measures Analysis Course: Statistical Services Centre, Reading University, June 2002

Psychopharmacology from Laboratory to Clinic: University of Maastricht, November 2003

Fourth Graduate Course on Neuropsychopharmacology: Euron Graduate Schools, April 2004

Innovating Clinical Drug Development: A focus on biostatistics: SMi London, January 2006

18 Appendix 1: Summary Statistics for Chapter 7

The MEANS Procedure

Analysis Variable : Simple Reaction Time

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	267.4	16	30.39	7.597	232.2	332.8
	1.5 hour	16	279.7	16	38.91	9.727	226.0	357.7
	2.5 hour	16	289.8	16	46.81	11.70	234.2	388.0
05 mg	0.0 hour	8	271.9	8	35.78	12.65	220.4	321.1
	1.5 hour	8	273.4	8	28.78	10.18	229.1	312.6
	2.5 hour	8	295.4	8	39.19	13.86	240.1	356.1
15 mg	0.0 hour	8	269.8	8	32.77	11.59	231.9	329.9
	1.5 hour	8	270.6	8	32.70	11.56	227.6	331.8
	2.5 hour	8	279.1	8	33.33	11.79	242.5	337.3
30 mg	0.0 hour	8	264.7	8	41.61	14.71	226.7	355.2
	1.5 hour	8	271.3	8	45.39	16.05	219.7	370.5
	2.5 hour	8	274.8	8	34.82	12.31	236.5	352.5

The MEANS Procedure

Analysis Variable : Simple Reaction Time Standard Deviation

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	37.60	16	18.46	4.614	14.37	79.03
	1.5 hour	16	43.20	16	18.11	4.527	23.62	77.92
	2.5 hour	16	48.95	16	28.47	7.117	22.19	131.5
05 mg	0.0 hour	8	41.40	8	21.10	7.461	14.36	78.11
	1.5 hour	8	34.42	8	17.87	6.317	17.14	71.98
	2.5 hour	8	46.69	8	25.55	9.032	22.31	84.28
15 mg	0.0 hour	8	37.25	8	18.09	6.396	20.64	75.44
	1.5 hour	8	44.23	8	22.49	7.951	18.44	85.85
	2.5 hour	8	45.21	8	19.90	7.036	22.11	72.39
30 mg	0.0 hour	8	33.90	8	29.48	10.42	13.46	105.3
	1.5 hour	8	39.97	8	21.54	7.616	20.94	83.74
	2.5 hour	8	50.46	8	22.03	7.787	30.87	89.05

The MEANS Procedure

Analysis Variable : Choice Reaction Time

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	432.9	16	55.43	13.86	376.5	547.1
	1.5 hour	16	439.1	16	60.81	15.20	389.4	597.6
	2.5 hour	16	450.4	16	74.02	18.51	382.9	622.9
05 mg	0.0 hour	8	439.6	8	61.20	21.64	384.7	566.8
	1.5 hour	8	432.9	8	55.70	19.69	345.8	540.2
	2.5 hour	8	446.0	8	49.43	17.48	397.5	548.7
15 mg	0.0 hour	8	441.7	8	52.65	18.62	389.8	551.9
	1.5 hour	8	429.8	8	48.25	17.06	382.4	535.0
	2.5 hour	8	438.2	8	80.21	28.36	382.5	629.2
30 mg	0.0 hour	8	442.4	8	107.3	37.95	379.7	701.2
	1.5 hour	8	420.5	8	62.58	22.12	368.4	560.0
	2.5 hour	8	439.2	8	75.78	26.79	389.4	619.2

The MEANS Procedure

Analysis Variable : Choice Reaction Time Accuracy

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	97.63	16	1.962	0.491	94.00	100.0
	1.5 hour	16	95.75	16	2.517	0.629	90.00	100.0
	2.5 hour	16	96.13	16	2.680	0.670	90.00	100.0
05 mg	0.0 hour	8	95.25	8	2.121	0.750	92.00	98.00
	1.5 hour	8	97.75	8	0.707	0.250	96.00	98.00
	2.5 hour	8	96.50	8	2.070	0.732	94.00	100.0
15 mg	0.0 hour	8	96.00	8	2.138	0.756	94.00	100.0
	1.5 hour	8	95.25	8	1.035	0.366	94.00	96.00
	2.5 hour	8	96.00	8	3.024	1.069	92.00	100.0
30 mg	0.0 hour	8	97.25	8	2.121	0.750	94.00	100.0
	1.5 hour	8	96.00	8	2.619	0.926	92.00	98.00
	2.5 hour	8	97.00	8	2.390	0.845	92.00	100.0

The MEANS Procedure

Analysis Variable : Choice Reaction Time Standard Deviation

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	80.63	16	24.76	6.189	52.06	143.2
	1.5 hour	16	88.48	16	31.65	7.913	59.75	178.1
	2.5 hour	16	85.46	16	34.21	8.553	45.13	168.7
05 mg	0.0 hour	8	84.08	8	36.91	13.05	52.76	170.3
	1.5 hour	8	78.88	8	28.83	10.19	56.52	139.8
	2.5 hour	8	91.75	8	36.64	12.95	59.48	166.2
15 mg	0.0 hour	8	80.45	8	25.94	9.170	44.95	133.2
	1.5 hour	8	83.68	8	29.50	10.43	50.92	137.8
	2.5 hour	8	85.58	8	45.98	16.26	49.43	190.4
30 mg	0.0 hour	8	82.98	8	62.62	22.14	45.42	236.1
	1.5 hour	8	73.37	8	23.27	8.229	46.31	123.1
	2.5 hour	8	91.68	8	56.22	19.88	52.46	225.1

The MEANS Procedure

Analysis Variable : Digit Vigilance Targets Detected

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	97.78	16	4.445	1.111	82.22	100.0
	1.5 hour	16	98.06	16	3.129	0.782	88.89	100.0
	2.5 hour	16	98.47	16	2.778	0.695	91.11	100.0
05 mg	0.0 hour	8	99.72	8	0.785	0.277	97.78	100.0
	1.5 hour	8	98.61	8	1.149	0.406	97.78	100.0
	2.5 hour	8	97.78	8	3.143	1.111	91.11	100.0
15 mg	0.0 hour	8	98.61	8	2.358	0.834	93.33	100.0
	1.5 hour	8	97.50	8	4.666	1.650	86.67	100.0
	2.5 hour	8	98.61	8	2.358	0.834	93.33	100.0
30 mg	0.0 hour	8	96.95	8	5.305	1.876	84.44	100.0
	1.5 hour	8	99.17	8	1.149	0.406	97.78	100.0
	2.5 hour	8	97.78	8	2.058	0.728	93.33	100.0

The MEANS Procedure

Analysis Variable : Digit Vigilance Speed

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	420.3	16	45.05	11.26	367.4	512.6
	1.5 hour	16	432.5	16	54.28	13.57	369.8	553.9
	2.5 hour	16	451.1	16	65.17	16.29	380.3	566.3
05 mg	0.0 hour	8	432.0	8	43.31	15.31	396.5	523.6
	1.5 hour	8	439.3	8	46.77	16.54	397.3	528.1
	2.5 hour	8	455.7	8	57.71	20.40	388.4	567.6
15 mg	0.0 hour	8	422.9	8	41.17	14.56	376.4	500.2
	1.5 hour	8	421.1	8	45.13	15.96	378.1	490.6
	2.5 hour	8	429.7	8	53.91	19.06	371.3	512.7
30 mg	0.0 hour	8	433.6	8	38.91	13.76	380.2	500.4
	1.5 hour	8	421.1	8	58.82	20.80	354.7	521.8
	2.5 hour	8	424.4	8	38.50	13.61	387.1	501.4

The MEANS Procedure

Analysis Variable : Digit Vigilance False Alarms

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	1.125	16	1.204	0.301	0	4.000
	1.5 hour	16	1.438	16	1.861	0.465	0	5.000
	2.5 hour	16	0.625	16	0.885	0.221	0	3.000
05 mg	0.0 hour	8	0.625	8	1.061	0.375	0	3.000
	1.5 hour	8	2.000	8	1.414	0.500	0	4.000
	2.5 hour	8	1.125	8	1.246	0.441	0	3.000
15 mg	0.0 hour	8	0.875	8	1.356	0.479	0	3.000
	1.5 hour	8	0.875	8	0.991	0.350	0	3.000
	2.5 hour	8	1.250	8	0.707	0.250	0	2.000
30 mg	0.0 hour	8	1.375	8	1.061	0.375	0	3.000
	1.5 hour	8	0.625	8	0.744	0.263	0	2.000
	2.5 hour	8	1.000	8	0.926	0.327	0	2.000

The MEANS Procedure

Analysis Variable : Digit Vigilance Standard Deviation

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	64.69	16	17.98	4.494	42.19	98.96
	1.5 hour	16	60.48	16	19.59	4.897	36.14	102.2
	2.5 hour	16	65.50	16	25.39	6.346	34.89	115.8
05 mg	0.0 hour	8	53.29	8	11.76	4.156	38.79	71.81
	1.5 hour	8	58.34	8	26.75	9.459	31.96	111.3
	2.5 hour	8	76.91	8	23.46	8.294	39.70	119.5
15 mg	0.0 hour	8	60.12	8	23.21	8.207	36.81	111.0
	1.5 hour	8	54.70	8	14.33	5.066	33.84	77.52
	2.5 hour	8	66.82	8	20.28	7.170	44.96	93.52
30 mg	0.0 hour	8	60.36	8	22.17	7.840	32.26	96.91
	1.5 hour	8	70.95	8	29.58	10.46	30.09	125.5
	2.5 hour	8	62.28	8	19.22	6.796	32.46	92.26

The MEANS Procedure

Analysis Variable : Cognitive Reaction Time

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	165.5	16	39.75	9.938	105.9	239.7
	1.5 hour	16	159.3	16	35.81	8.952	123.6	239.9
	2.5 hour	16	160.6	16	46.56	11.64	92.09	248.7
05 mg	0.0 hour	8	167.7	8	43.90	15.52	102.5	245.7
	1.5 hour	8	159.5	8	55.51	19.62	58.20	229.7
	2.5 hour	8	150.7	8	36.68	12.97	100.4	192.6
15 mg	0.0 hour	8	171.9	8	45.75	16.17	105.9	226.4
	1.5 hour	8	159.2	8	26.85	9.492	127.5	203.2
	2.5 hour	8	159.1	8	62.00	21.92	103.0	291.9
30 mg	0.0 hour	8	177.7	8	74.20	26.23	121.0	346.0
	1.5 hour	8	149.2	8	32.66	11.55	95.92	189.4
	2.5 hour	8	164.4	8	47.81	16.90	118.6	266.6

The MEANS Procedure

Analysis Variable : Power of Attention

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	1121	16	119.2	29.81	1003	1391
	1.5 hour	16	1151	16	145.7	36.43	1023	1462
	2.5 hour	16	1191	16	173.6	43.39	1045	1559
05 mg	0.0 hour	8	1144	8	126.7	44.81	1049	1359
	1.5 hour	8	1146	8	109.9	38.87	1034	1343
	2.5 hour	8	1197	8	128.8	45.55	1078	1411
15 mg	0.0 hour	8	1134	8	102.9	36.37	1058	1341
	1.5 hour	8	1122	8	114.9	40.62	1031	1357
	2.5 hour	8	1147	8	150.1	53.06	1013	1475
30 mg	0.0 hour	8	1141	8	177.5	62.77	993.4	1557
	1.5 hour	8	1113	8	156.9	55.49	975.7	1452
	2.5 hour	8	1138	8	141.0	49.84	1040	1473

The MEANS Procedure

Analysis Variable : Continuity of Attention

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	91.69	16	2.549	0.637	85.00	95.00
	1.5 hour	16	90.56	16	2.827	0.707	84.00	95.00
	2.5 hour	16	91.75	16	2.696	0.674	86.00	95.00
05 mg	0.0 hour	8	91.88	8	1.642	0.581	90.00	94.00
	1.5 hour	8	91.25	8	1.669	0.590	88.00	93.00
	2.5 hour	8	91.13	8	3.044	1.076	87.00	95.00
15 mg	0.0 hour	8	91.50	8	2.391	0.845	86.00	94.00
	1.5 hour	8	90.63	8	2.065	0.730	86.00	93.00
	2.5 hour	8	91.13	8	2.850	1.008	87.00	94.00
30 mg	0.0 hour	8	90.88	8	3.227	1.141	84.00	94.00
	1.5 hour	8	92.00	8	1.690	0.598	89.00	94.00
	2.5 hour	8	91.50	8	1.927	0.681	88.00	94.00

The MEANS Procedure

Analysis Variable : Response Variability

COND	VISIT	N Obs	Mean	N	Std Dev	Std Error	Min	Max
00 mg	0.0 hour	16	584.4	16	110.3	27.56	411.8	745.8
	1.5 hour	16	544.4	16	90.82	22.70	363.7	672.0
	2.5 hour	16	589.2	16	131.0	32.76	394.2	904.0
05 mg	0.0 hour	8	587.0	8	129.5	45.79	362.2	742.5
	1.5 hour	8	600.6	8	120.7	42.68	417.0	735.2
	2.5 hour	8	554.5	8	143.6	50.76	360.1	710.8
15 mg	0.0 hour	8	601.6	8	135.6	47.95	445.1	884.4
	1.5 hour	8	576.9	8	153.5	54.27	396.3	853.0
	2.5 hour	8	597.1	8	158.9	56.18	357.9	871.4
30 mg	0.0 hour	8	648.5	8	169.3	59.87	331.7	897.9
	1.5 hour	8	622.1	8	126.8	44.84	485.5	870.7
	2.5 hour	8	573.5	8	143.4	50.69	306.5	758.0

19 Appendix 2: Summary Statistics for Chapter 8

Tyrosine (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	50.788	49.835	3.2563	13.0250	32.12	80.27
	Post-dose	16	124.160	133.835	7.2405	28.9618	65.50	164.83
ATDATyrD	Pre-dose	16	50.058	49.565	3.4810	13.9241	30.64	87.27
	Post-dose	16	18.554	15.350	2.5550	10.2201	7.39	40.02
ATyrD	Pre-dose	16	50.778	51.660	3.6384	14.5537	27.45	86.98
	Post-dose	16	16.016	16.575	1.3395	5.3580	6.45	27.50
placebo	Pre-dose	16	49.444	50.315	3.1694	12.6777	28.98	80.48
	Post-dose	16	130.009	134.305	7.6054	30.4216	50.52	164.26

Summary Statistics of CDR Data

Valine (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	208.648	206.040	9.2215	36.8861	154.54	282.01
	Post-dose	16	394.002	384.510	17.6280	70.5121	282.26	502.29
ATDATyrD	Pre-dose	16	213.464	215.170	8.1105	32.4422	160.06	273.87
	Post-dose	16	369.835	361.575	18.5418	74.1671	231.96	537.16
ATyrD	Pre-dose	16	219.966	215.525	9.6030	38.4119	167.12	296.04
	Post-dose	16	416.079	416.290	17.5736	70.2943	285.72	540.49
placebo	Pre-dose	16	214.101	220.785	8.6777	34.7108	158.60	291.32
	Post-dose	16	363.043	391.670	22.3292	89.3167	174.54	514.14

Summary Statistics of CDR Data

Isoleucine (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	71.803	71.890	3.3180	13.2721	43.86	96.75
	Post-dose	16	87.794	86.080	5.1092	20.4369	58.87	115.14
ATDATyrD	Pre-dose	16	72.846	71.085	3.4465	13.7862	48.01	97.83
	Post-dose	16	85.046	78.735	6.7689	27.0755	50.28	170.76
ATyrD	Pre-dose	16	73.324	73.100	3.8091	15.2363	50.55	107.44
	Post-dose	16	93.218	88.460	5.3309	21.3237	64.01	140.49
placebo	Pre-dose	16	69.851	70.525	3.2005	12.8021	41.06	86.62
	Post-dose	16	79.551	75.115	6.7477	26.9910	39.35	119.03

Summary Statistics of CDR Data

Phenylalanine (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	53.802	54.180	2.0213	8.0850	41.02	66.74
	Post-dose	16	65.179	63.500	2.0925	8.3699	56.54	85.10
ATDATyrD	Pre-dose	16	52.781	50.525	1.9711	7.8843	44.02	73.05
	Post-dose	16	29.638	28.590	2.3200	9.2799	12.26	48.80
ATyrD	Pre-dose	16	53.218	53.160	2.3464	9.3855	39.73	77.50
	Post-dose	16	27.383	29.775	1.9359	7.7434	8.81	35.56
placebo	Pre-dose	16	52.928	51.810	1.8242	7.2967	39.61	67.35
	Post-dose	16	59.636	60.685	3.4077	13.6308	43.33	83.48

Summary Statistics of CDR Data

Tryptophan (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	38.906	40.270	1.7404	6.9614	26.44	51.38
	Post-dose	16	13.328	14.515	1.4318	5.7271	4.23	25.62
ATDATyrD	Pre-dose	16	38.058	37.260	1.6596	6.6384	28.08	50.80
	Post-dose	16	24.714	23.440	1.6882	6.7530	13.91	39.08
ATyrD	Pre-dose	16	39.019	37.805	1.6136	6.4544	30.01	50.91
	Post-dose	16	45.738	45.685	1.5664	6.2656	36.57	56.31
placebo	Pre-dose	16	36.690	35.850	1.5550	6.2199	24.50	48.21
	Post-dose	16	36.016	35.115	1.5853	6.3411	25.83	50.16

Summary Statistics of CDR Data

Leucine (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	126.476	128.400	5.9062	23.6246	79.27	177.52
	Post-dose	16	187.866	181.455	10.0999	40.3997	122.57	249.17
ATDATyrD	Pre-dose	16	128.830	125.665	5.3303	21.3210	99.34	175.24
	Post-dose	16	193.419	189.760	12.8895	51.5579	125.22	349.59
ATyrD	Pre-dose	16	128.490	125.160	6.4463	25.7853	90.76	183.33
	Post-dose	16	217.745	230.610	11.9428	47.7710	99.08	276.57
placebo	Pre-dose	16	126.528	127.865	4.3785	17.5141	96.69	153.62
	Post-dose	16	206.789	193.090	17.5995	70.3979	88.67	350.44

Summary Statistics of CDR Data

Tyr+Phe (nm/ml)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	104.590	106.895	4.9862	19.9450	74.73	147.01
	Post-dose	16	189.339	197.565	8.5956	34.3822	122.38	235.99
ATDATyrD	Pre-dose	16	102.839	100.070	5.0497	20.1986	75.63	160.32
	Post-dose	16	48.192	44.510	4.7289	18.9156	22.64	86.07
ATyrD	Pre-dose	16	103.996	101.800	5.7838	23.1350	67.18	164.48
	Post-dose	16	43.398	44.270	2.9777	11.9109	15.26	60.08
placebo	Pre-dose	16	102.372	103.395	4.3588	17.4354	73.43	147.83
	Post-dose	16	189.646	197.300	9.2599	37.0397	97.18	245.49

Summary Statistics of CDR Data

Prolactine (U/I)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	15	0.211	0.198	0.0151	0.0584	0.12	0.35
	Post-dose	15	0.180	0.171	0.0122	0.0473	0.10	0.27
ATDATyrD	Pre-dose	15	0.223	0.211	0.0169	0.0656	0.15	0.40
	Post-dose	15	0.162	0.159	0.0117	0.0453	0.09	0.25
ATyrD	Pre-dose	15	0.233	0.219	0.0146	0.0566	0.14	0.37
	Post-dose	15	0.178	0.160	0.0190	0.0735	0.09	0.38
placebo	Pre-dose	15	0.207	0.202	0.0135	0.0522	0.12	0.28
	Post-dose	15	0.187	0.179	0.0168	0.0649	0.10	0.32

Summary Statistics of CDR Data

Tyr:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	10.105	10.147	0.4003	1.6012	6.94	13.81
	Post-dose	16	16.586	16.748	0.6995	2.7981	11.12	22.11
ATDATyrD	Pre-dose	16	9.875	9.829	0.5565	2.2259	6.70	15.42
	Post-dose	16	2.809	2.235	0.4703	1.8813	0.96	7.03
ATyrD	Pre-dose	16	9.807	9.742	0.4939	1.9756	6.84	13.59
	Post-dose	16	2.068	2.036	0.1983	0.7933	0.76	3.57
placebo	Pre-dose	16	9.915	9.557	0.5699	2.2796	6.16	15.53
	Post-dose	16	17.983	18.013	1.1602	4.6407	10.14	26.36

Summary Statistics of CDR Data

Val:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	61.442	60.951	1.8396	7.3585	48.23	75.77
	Post-dose	16	82.623	83.067	1.4873	5.9490	71.28	93.65
ATDATyrD	Pre-dose	16	62.576	62.956	1.3495	5.3982	52.51	74.44
	Post-dose	16	106.387	105.290	4.1562	16.6250	68.43	131.81
ATyrD	Pre-dose	16	64.197	65.048	1.4379	5.7516	55.78	72.73
	Post-dose	16	104.319	103.635	1.9259	7.7036	88.88	117.15
placebo	Pre-dose	16	63.814	64.184	1.3375	5.3502	49.79	73.01
	Post-dose	16	71.432	71.355	2.9928	11.9712	54.85	97.59

Summary Statistics of CDR Data

Ile:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	14.970	14.972	0.2950	1.1800	12.48	17.36
	Post-dose	16	11.138	11.253	0.3539	1.4158	9.22	13.92
ATDATyrD	Pre-dose	16	15.043	15.117	0.3497	1.3989	11.79	16.94
	Post-dose	16	13.197	13.293	0.5199	2.0796	9.98	18.49
ATyrD	Pre-dose	16	14.902	14.616	0.3742	1.4969	12.70	18.40
	Post-dose	16	12.841	12.579	0.3818	1.5274	11.09	16.45
placebo	Pre-dose	16	14.556	14.528	0.4652	1.8606	10.36	18.16
	Post-dose	16	9.838	10.325	0.4430	1.7719	6.89	12.48

Summary Statistics of CDR Data

Phe:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	10.921	10.756	0.2921	1.1683	9.33	13.09
	Post-dose	16	8.246	7.994	0.3284	1.3138	6.54	10.61
ATDATyrD	Pre-dose	16	10.535	10.514	0.2097	0.8386	8.65	11.82
	Post-dose	16	4.525	4.209	0.4722	1.8888	1.13	9.35
ATyrD	Pre-dose	16	10.472	10.389	0.2760	1.1040	8.70	12.22
	Post-dose	16	3.634	3.883	0.3378	1.3511	1.04	6.07
placebo	Pre-dose	16	10.719	10.537	0.2808	1.1233	9.54	13.41
	Post-dose	16	7.559	7.158	0.4675	1.8700	5.04	11.03

Summary Statistics of CDR Data

Trp:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	7.685	7.349	0.3216	1.2862	6.16	10.04
	Post-dose	16	1.642	1.482	0.2302	0.9209	0.46	4.17
ATDATyrD	Pre-dose	16	7.400	7.192	0.2801	1.1204	6.22	9.72
	Post-dose	16	3.723	3.585	0.3489	1.3954	1.29	7.35
ATyrD	Pre-dose	16	7.478	7.312	0.2007	0.8029	6.27	8.88
	Post-dose	16	6.059	5.819	0.2620	1.0478	4.16	7.86
placebo	Pre-dose	16	7.201	6.950	0.2702	1.0807	5.27	9.30
	Post-dose	16	4.484	4.405	0.3144	1.2575	3.26	8.46

Summary Statistics of CDR Data

Leu:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	29.803	30.468	0.6681	2.6724	24.60	33.79
	Post-dose	16	27.282	27.767	0.5520	2.2082	23.65	31.67
ATDATyrD	Pre-dose	16	30.136	30.117	0.3361	1.3444	28.19	33.43
	Post-dose	16	36.320	35.164	1.1899	4.7596	29.19	46.96
ATyrD	Pre-dose	16	29.367	29.245	0.4175	1.6699	26.98	33.45
	Post-dose	16	36.104	35.899	1.2598	5.0391	23.01	43.63
placebo	Pre-dose	16	29.998	30.046	0.5126	2.0504	26.95	33.58
	Post-dose	16	30.673	30.184	1.8096	7.2385	21.58	46.36

Summary Statistics of CDR Data

Tyr+Phe:LNAA

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	32.854	32.165	1.0281	4.1124	24.12	39.84
	Post-dose	16	38.499	38.027	1.3145	5.2581	30.86	48.24
ATDATyrD	Pre-dose	16	31.771	31.027	1.0803	4.3211	25.53	42.87
	Post-dose	16	10.694	9.069	1.4210	5.6839	3.14	24.92
ATyrD	Pre-dose	16	31.328	29.485	1.1529	4.6117	25.89	39.15
	Post-dose	16	8.115	8.577	0.6861	2.7443	2.59	12.82
placebo	Pre-dose	16	32.150	30.657	1.1795	4.7182	26.26	43.80
	Post-dose	16	40.805	38.032	2.2156	8.8625	28.53	60.14

Summary Statistics of CDR Data

Stroop Card 1 (sec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	38.533	38.321	1.5860	6.3438	29.58	50.58
	Post-dose	16	39.424	39.096	1.4396	5.7583	28.98	50.38
ATDATyrD	Pre-dose	16	37.277	36.738	1.1034	4.4135	28.89	45.00
	Post-dose	16	38.639	38.506	1.4293	5.7172	28.77	48.04
ATyrD	Pre-dose	16	37.724	37.935	0.8544	3.4177	31.09	43.10
	Post-dose	16	39.392	39.292	1.2400	4.9599	31.18	48.20
placebo	Pre-dose	16	38.460	38.672	0.8717	3.4868	33.66	46.32
	Post-dose	16	39.190	38.957	1.0327	4.1310	33.72	50.04

Summary Statistics of CDR Data

Stroop Card 2 (sec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	46.295	47.378	1.6273	6.5090	35.90	57.10
	Post-dose	16	44.680	43.894	1.7483	6.9930	33.57	60.29
ATDATyrD	Pre-dose	16	47.168	47.032	1.7393	6.9570	37.66	59.56
	Post-dose	16	45.914	44.009	2.1475	8.5902	32.86	66.99
ATyrD	Pre-dose	16	46.978	46.973	1.6577	6.6306	38.27	62.75
	Post-dose	16	47.217	46.312	1.6680	6.6721	36.55	61.98
placebo	Pre-dose	16	46.676	46.091	1.3430	5.3722	37.49	58.13
	Post-dose	16	47.518	47.282	1.5525	6.2101	37.28	59.02

Summary Statistics of CDR Data

Stroop Card 3 (sec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	67.017	65.274	3.6676	14.6705	44.65	99.82
	Post-dose	16	64.263	64.418	2.7796	11.1186	45.23	85.87
ATDATyrD	Pre-dose	16	66.199	64.182	3.4098	13.6391	48.63	95.35
	Post-dose	16	66.445	64.728	3.0588	12.2354	43.70	90.12
ATyrD	Pre-dose	16	66.832	63.837	3.0422	12.1687	49.51	92.29
	Post-dose	16	65.574	62.470	3.2614	13.0457	49.09	93.90
placebo	Pre-dose	16	68.472	66.511	3.5097	14.0390	45.99	98.58
	Post-dose	16	68.705	65.950	3.1109	12.4437	53.63	95.06

Summary Statistics of CDR Data

Simple Reaction Time (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	274.281	270.070	7.0572	28.2289	224.58	329.40
	Post-dose	16	281.739	273.870	12.1508	48.6031	212.92	419.85
ATDATyrD	Pre-dose	16	278.719	268.040	9.2658	37.0631	224.74	377.73
	Post-dose	16	278.281	277.815	7.5393	30.1571	232.63	343.49
ATyrD	Pre-dose	16	275.013	267.125	6.4782	25.9127	249.02	347.92
	Post-dose	16	285.403	273.295	13.7291	54.9162	223.69	467.76
placebo	Pre-dose	16	276.312	269.630	12.2906	49.1623	216.04	442.22
	Post-dose	16	282.466	281.240	9.6214	38.4855	221.35	394.98

Summary Statistics of CDR Data

Simple Reaction Time - Coefficient of Variance (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	15.946	15.194	1.4809	5.9235	8.30	27.41
	Post-dose	16	17.534	17.357	1.8332	7.3327	6.37	35.30
ATDATyrD	Pre-dose	16	16.301	16.503	0.9862	3.9448	9.82	23.32
	Post-dose	16	16.304	16.080	1.3252	5.3009	8.05	25.15
ATyrD	Pre-dose	16	14.966	14.335	1.2704	5.0815	7.87	25.52
	Post-dose	16	18.078	14.477	3.6452	14.5810	6.67	69.32
placebo	Pre-dose	16	16.464	14.639	2.3755	9.5022	8.95	48.31
	Post-dose	16	19.413	16.435	2.5933	10.3730	9.23	52.84

Summary Statistics of CDR Data

Digit Vigilance - Targets Detected (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	96.391	97.780	2.0271	8.1086	66.67	100.00
	Post-dose	16	94.863	97.780	2.3894	9.5576	62.22	100.00
ATDATyrD	Pre-dose	16	96.807	97.780	1.1471	4.5884	82.22	100.00
	Post-dose	16	95.696	96.670	1.4122	5.6489	77.78	100.00
ATyrD	Pre-dose	16	97.084	98.890	1.8999	7.5996	68.89	100.00
	Post-dose	16	95.973	97.780	1.5343	6.1374	75.56	100.00
placebo	Pre-dose	16	97.084	98.890	1.2945	5.1779	82.22	100.00
	Post-dose	16	94.862	95.560	1.5008	6.0030	77.78	100.00

Summary Statistics of CDR Data

Digit Vigilance - Speed (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	421.536	415.890	11.1009	44.4037	343.22	550.27
	Post-dose	16	430.847	426.875	14.6892	58.7566	361.82	602.36
ATDATyrD	Pre-dose	16	428.958	424.460	13.7955	55.1820	364.27	597.05
	Post-dose	16	444.846	451.145	12.7181	50.8722	378.53	563.51
ATyrD	Pre-dose	16	419.458	418.165	12.0553	48.2211	346.89	554.19
	Post-dose	16	434.662	420.135	12.1173	48.4692	381.00	555.00
placebo	Pre-dose	16	411.494	403.390	10.0700	40.2800	346.34	503.30
	Post-dose	16	440.088	428.150	13.1643	52.6571	376.69	613.17

Summary Statistics of CDR Data

Digit Vigilance - Coefficient of Variance (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	16.385	15.164	1.2517	5.0070	8.97	24.36
	Post-dose	16	16.697	14.755	1.3764	5.5056	9.84	27.51
ATDATyrD	Pre-dose	16	15.819	14.313	1.1208	4.4832	8.97	25.35
	Post-dose	16	16.693	16.470	0.9996	3.9986	9.85	22.39
ATyrD	Pre-dose	16	16.362	17.297	0.8459	3.3836	9.89	20.59
	Post-dose	16	15.840	14.979	1.1290	4.5160	8.59	23.74
placebo	Pre-dose	16	15.076	14.491	1.0761	4.3045	9.75	24.82
	Post-dose	16	17.701	17.520	0.9483	3.7933	12.81	24.55

Summary Statistics of CDR Data

Digit Vigilance - False Alarms (#)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	1.125	1.000	0.3400	1.3601	0.00	5.00
	Post-dose	16	0.938	1.000	0.1700	0.6801	0.00	2.00
ATDATyrD	Pre-dose	16	1.250	1.000	0.3708	1.4832	0.00	5.00
	Post-dose	16	1.000	1.000	0.2415	0.9661	0.00	4.00
ATyrD	Pre-dose	16	0.625	0.500	0.1797	0.7188	0.00	2.00
	Post-dose	16	0.938	1.000	0.2809	1.1236	0.00	4.00
placebo	Pre-dose	16	0.688	1.000	0.1760	0.7042	0.00	2.00
	Post-dose	16	1.313	1.000	0.3733	1.4930	0.00	5.00

Summary Statistics of CDR Data

Choice Reaction Time (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	433.408	398.115	24.5621	98.2483	352.89	750.17
	Post-dose	16	429.405	403.495	19.7912	79.1649	345.76	573.27
ATDATyrD	Pre-dose	16	433.133	405.995	20.8706	83.4826	353.67	636.81
	Post-dose	16	435.136	409.110	22.6782	90.7129	342.82	663.55
ATyrD	Pre-dose	16	422.959	407.715	16.7381	66.9522	340.56	569.47
	Post-dose	16	428.391	390.305	23.7272	94.9089	338.45	711.06
placebo	Pre-dose	16	427.178	400.040	18.7747	75.0987	358.76	624.21
	Post-dose	16	413.571	399.325	12.7007	50.8027	335.92	546.28

Summary Statistics of CDR Data

Choice Reaction Time - Coefficient of Variance (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	19.064	15.926	2.0281	8.1126	12.38	44.19
	Post-dose	16	20.444	18.757	1.8353	7.3414	13.46	43.15
ATDATyrD	Pre-dose	16	18.811	16.377	1.3721	5.4884	14.16	35.07
	Post-dose	16	18.686	16.358	1.6845	6.7380	11.93	37.02
ATyrD	Pre-dose	16	18.023	18.018	1.1740	4.6960	11.16	28.26
	Post-dose	16	19.881	17.635	1.7766	7.1065	13.57	42.59
placebo	Pre-dose	16	18.194	17.916	1.4062	5.6247	11.47	30.65
	Post-dose	16	20.693	19.562	1.5124	6.0496	13.94	37.28

Summary Statistics of CDR Data

Choice Reaction Time - Accuracy (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	96.750	98.000	0.6021	2.4083	92.00	100.00
	Post-dose	16	95.625	96.000	0.8004	3.2016	90.00	100.00
ATDATyrD	Pre-dose	16	95.625	95.000	0.8004	3.2016	92.00	100.00
	Post-dose	16	94.750	96.000	0.9811	3.9243	86.00	100.00
ATyrD	Pre-dose	16	95.375	96.000	0.7465	2.9861	90.00	100.00
	Post-dose	16	96.000	97.000	0.7958	3.1833	88.00	100.00
placebo	Pre-dose	16	96.250	96.000	0.6551	2.6204	90.00	100.00
	Post-dose	16	95.375	96.000	1.1506	4.6025	84.00	100.00

Summary Statistics of CDR Data

Spatial Working Memory - Sensitivity Index (SI)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	0.932	0.952	0.0247	0.0989	0.66	1.00
	Post-dose	16	0.896	0.899	0.0201	0.0802	0.72	1.00
ATDATyrD	Pre-dose	16	0.921	0.925	0.0175	0.0698	0.78	1.00
	Post-dose	16	0.945	0.952	0.0142	0.0570	0.84	1.00
ATyrD	Pre-dose	16	0.931	0.941	0.0139	0.0557	0.83	1.00
	Post-dose	16	0.956	1.000	0.0162	0.0649	0.83	1.00
placebo	Pre-dose	16	0.954	0.952	0.0127	0.0510	0.84	1.00
	Post-dose	16	0.929	0.941	0.0155	0.0621	0.83	1.00

Summary Statistics of CDR Data

Spatial Working Memory - Speed (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	584.204	555.905	32.3136	129.2543	439.44	923.20
	Post-dose	16	547.318	512.550	26.6749	106.6996	413.06	739.83
ATDATyrD	Pre-dose	16	604.953	555.140	36.6595	146.6380	446.29	896.86
	Post-dose	16	526.348	475.130	35.3334	141.3336	417.48	1001.97
ATyrD	Pre-dose	16	576.374	543.100	34.7254	138.9016	443.38	1022.77
	Post-dose	16	526.561	487.375	25.7615	103.0461	418.91	815.57
placebo	Pre-dose	16	596.899	538.870	36.4240	145.6959	453.24	977.42
	Post-dose	16	540.504	499.155	28.2392	112.9569	408.74	879.09

Summary Statistics of CDR Data

Spatial Working Memory - Coefficient of Variance (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	22.008	19.811	1.9892	7.9569	12.65	39.49
	Post-dose	16	23.830	22.335	1.9227	7.6907	14.39	40.38
ATDATyrD	Pre-dose	16	30.694	19.762	7.9308	31.7231	15.63	146.68
	Post-dose	16	22.942	19.416	2.5692	10.2767	13.27	57.47
ATyrD	Pre-dose	16	21.007	18.525	2.0171	8.0685	11.67	43.76
	Post-dose	16	20.380	18.470	1.8178	7.2714	11.28	37.87
placebo	Pre-dose	16	29.206	19.995	7.6232	30.4926	10.91	136.71
	Post-dose	16	22.161	18.823	2.2618	9.0472	11.56	46.81

Summary Statistics of CDR Data

Spatial Working Memory Original Stimuli - Accuracy (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	96.484	100.000	1.3939	5.5756	81.25	100.00
	Post-dose	16	93.750	93.750	1.6137	6.4550	81.25	100.00
ATDATyrD	Pre-dose	16	94.922	93.750	1.4228	5.6912	81.25	100.00
	Post-dose	16	98.438	100.000	0.6988	2.7951	93.75	100.00
ATyrD	Pre-dose	16	95.313	93.750	1.2103	4.8412	87.50	100.00
	Post-dose	16	98.438	100.000	0.9021	3.6084	87.50	100.00
placebo	Pre-dose	16	97.656	100.000	0.9674	3.8696	87.50	100.00
	Post-dose	16	96.094	96.875	1.1231	4.4925	87.50	100.00

Summary Statistics of CDR Data

Spatial Working Memory New Stimuli - Accuracy (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	96.563	100.000	1.2681	5.0724	85.00	100.00
	Post-dose	16	95.000	97.500	1.7678	7.0711	75.00	100.00
ATDATyrD	Pre-dose	16	96.875	97.500	0.8985	3.5940	90.00	100.00
	Post-dose	16	95.938	95.000	0.9375	3.7500	90.00	100.00
ATyrD	Pre-dose	16	97.500	100.000	0.7906	3.1623	90.00	100.00
	Post-dose	16	96.875	100.000	1.1968	4.7871	85.00	100.00
placebo	Pre-dose	16	97.500	100.000	0.9129	3.6515	90.00	100.00
	Post-dose	16	96.563	97.500	0.9915	3.9660	90.00	100.00

Summary Statistics of CDR Data

Spatial Working Memory Original Stimuli - Speed (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	555.518	519.685	31.8626	127.4505	420.44	855.75
	Post-dose	16	534.127	496.660	31.4699	125.8795	402.33	818.64
ATDATyrD	Pre-dose	16	585.424	519.970	43.8594	175.4376	398.40	1093.19
	Post-dose	16	509.295	461.155	38.2332	152.9328	393.80	1032.36
ATyrD	Pre-dose	16	557.604	518.530	36.0047	144.0186	434.00	1005.19
	Post-dose	16	515.706	477.440	24.0710	96.2838	403.88	756.47
placebo	Pre-dose	16	575.092	528.410	36.0811	144.3244	430.47	925.50
	Post-dose	16	530.318	495.400	31.0823	124.3294	416.87	918.07

Summary Statistics of CDR Data

Spatial Working Memory New Stimuli - Speed (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	606.933	579.105	34.7572	139.0289	448.90	980.00
	Post-dose	16	559.764	516.870	26.4947	105.9787	405.95	747.90
ATDATyrD	Pre-dose	16	619.773	569.940	35.6386	142.5544	461.47	890.65
	Post-dose	16	540.048	503.875	33.7864	135.1457	422.50	978.33
ATyrD	Pre-dose	16	590.918	556.020	34.5971	138.3883	442.30	1037.58
	Post-dose	16	535.418	501.335	28.3430	113.3722	418.32	859.90
placebo	Pre-dose	16	613.420	552.200	40.3423	161.3691	460.78	1096.35
	Post-dose	16	549.358	517.200	28.6759	114.7034	402.32	851.80

Summary Statistics of CDR Data

Power of Attention (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	1129.226	1081.930	40.1395	160.5579	955.09	1629.84
	Post-dose	16	1141.991	1097.795	43.9803	175.9212	967.53	1588.35
ATDATyrD	Pre-dose	16	1140.810	1097.335	39.9685	159.8742	995.89	1611.59
	Post-dose	16	1158.263	1115.895	40.5244	162.0975	985.80	1517.04
ATyrD	Pre-dose	16	1117.429	1092.325	31.9461	127.7845	963.82	1471.58
	Post-dose	16	1148.456	1101.280	45.4998	181.9992	1001.47	1733.82
placebo	Pre-dose	16	1114.983	1078.655	37.3420	149.3679	960.93	1569.73
	Post-dose	16	1136.124	1112.465	31.9455	127.7820	990.62	1554.43

Summary Statistics of CDR Data

Variability of Attention (%)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	17.132	15.766	1.3145	5.2580	9.88	29.49
	Post-dose	16	18.225	18.445	1.3187	5.2749	11.70	28.77
ATDATyrD	Pre-dose	16	16.977	16.530	0.9569	3.8276	12.29	27.92
	Post-dose	16	17.228	16.086	1.0697	4.2789	11.08	26.72
ATyrD	Pre-dose	16	16.450	16.400	0.7781	3.1124	10.04	22.76
	Post-dose	16	17.933	15.875	1.9451	7.7805	10.76	43.83
placebo	Pre-dose	16	16.578	14.801	1.4307	5.7226	11.17	33.96
	Post-dose	16	19.269	18.182	1.3455	5.3818	13.00	36.77

Summary Statistics of CDR Data

Contiuity of Attention (#)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	90.626	91.002	1.0200	4.0801	78.00	95.00
	Post-dose	16	89.563	90.502	1.1690	4.6759	75.00	95.00
ATDATyrD	Pre-dose	16	90.126	90.001	0.6448	2.5791	85.00	94.00
	Post-dose	16	89.438	90.002	0.9573	3.8290	79.00	94.00
ATyrD	Pre-dose	16	90.750	91.001	1.0062	4.0249	77.00	95.00
	Post-dose	16	90.250	91.001	1.0858	4.3431	79.00	95.00
placebo	Pre-dose	16	91.125	91.501	0.7899	3.1597	82.00	95.00
	Post-dose	16	89.063	91.001	1.1529	4.6114	81.00	94.00

Summary Statistics of CDR Data

Overall Coefficient of Variance (msec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	18.351	17.676	1.3010	5.2042	12.18	31.99
	Post-dose	16	19.627	19.775	1.3575	5.4300	12.37	30.74
ATDATyrD	Pre-dose	16	20.406	18.329	2.1269	8.5075	13.44	47.97
	Post-dose	16	18.656	18.753	1.3131	5.2525	12.35	34.41
ATyrD	Pre-dose	16	17.589	16.863	0.9366	3.7462	10.45	28.01
	Post-dose	16	18.545	16.831	1.7021	6.8086	10.89	38.83
placebo	Pre-dose	16	19.735	16.812	2.3065	9.2261	11.10	48.66
	Post-dose	16	19.992	19.627	1.4650	5.8600	12.64	39.28

Summary Statistics of CDR Data

Stroop Interference (sec)

Treatment	Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
ATD	Pre-dose	16	157.203	153.330	5.1388	20.5550	135.12	214.62
	Post-dose	16	152.684	148.896	3.6809	14.7237	127.35	176.98
ATDATyrD	Pre-dose	16	155.903	153.749	4.6990	18.7960	125.61	193.36
	Post-dose	16	157.241	157.004	4.2887	17.1547	130.67	190.30
ATyrD	Pre-dose	16	157.237	155.095	4.4971	17.9886	119.52	188.68
	Post-dose	16	151.206	144.920	5.3476	21.3902	111.39	192.88
placebo	Pre-dose	16	159.678	157.858	5.3333	21.3334	122.68	202.49
	Post-dose	16	158.265	150.229	5.2145	20.8581	123.73	201.57

20 Appendix 3: Summary Statistics for Chapter 9

Simple Reaction Time (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	240.002	5.0624	20.2496	236.785	214.570	275.610
	Day 1	2 hours	16	242.956	5.1319	20.5276	238.605	208.230	288.330
	Day 1	4 hours	16	252.838	8.5284	34.1136	244.750	212.200	317.200
	Day 16	Pre-dose	16	236.756	5.3918	21.5672	237.150	204.940	272.690
	Day 16	2 hours	16	242.607	6.5531	26.2123	238.815	209.260	304.100
	Day 16	4 hours	16	250.002	7.9402	31.7607	245.705	214.220	327.840
sertra	Day 1	Pre-dose	16	248.461	4.5698	18.2792	242.245	214.020	284.780
	Day 1	2 hours	16	259.513	5.3283	21.3131	261.430	217.330	303.370
	Day 1	4 hours	16	254.506	6.5614	26.2457	253.735	216.760	307.430
	Day 16	Pre-dose	16	253.175	6.2068	24.8271	247.820	229.220	334.160
	Day 16	2 hours	16	264.376	11.4484	45.7934	254.240	227.590	424.300
	Day 16	4 hours	16	263.671	11.5351	46.1406	254.400	212.870	417.600

CDR tests, unadjusted scores

Choice Reaction Time - Accuracy (%)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	94.375	0.8410	3.3640	94.000	88.000	100.000
	Day 1	2 hours	16	94.500	0.9220	3.6878	96.000	86.000	98.000
	Day 1	4 hours	16	94.375	1.0036	4.0146	94.000	86.000	100.000
	Day 16	Pre-dose	16	96.125	0.4990	1.9958	96.000	92.000	98.000
	Day 16	2 hours	16	96.375	0.7122	2.8490	98.000	90.000	100.000
	Day 16	4 hours	16	96.500	0.6708	2.6833	97.000	92.000	100.000
sertra	Day 1	Pre-dose	16	96.375	0.6115	2.4461	96.000	92.000	100.000
	Day 1	2 hours	16	96.375	0.7122	2.8490	97.000	92.000	100.000
	Day 1	4 hours	16	96.500	0.4655	1.8619	96.000	94.000	100.000
	Day 16	Pre-dose	16	96.625	0.9077	3.6309	98.000	88.000	100.000
	Day 16	2 hours	16	96.875	0.6047	2.4187	98.000	92.000	100.000
	Day 16	4 hours	16	96.750	0.7932	3.1728	97.000	90.000	100.000

CDR tests, unadjusted scores

Choice Reaction Time - (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	380.652	11.3360	45.3439	374.125	312.330	450.790
	Day 1	2 hours	16	382.889	9.9366	39.7465	370.115	332.210	468.750
	Day 1	4 hours	16	373.814	10.9295	43.7181	361.715	317.040	466.660
	Day 16	Pre-dose	16	386.558	12.4006	49.6024	374.900	316.230	485.170
	Day 16	2 hours	16	382.669	7.9687	31.8749	387.595	323.620	430.590
	Day 16	4 hours	16	387.119	9.5764	38.3054	379.080	325.890	462.420
sertra	Day 1	Pre-dose	16	398.411	6.4870	25.9479	397.920	353.540	451.330
	Day 1	2 hours	16	395.001	6.6646	26.6584	390.395	354.360	448.220
	Day 1	4 hours	16	392.133	5.2701	21.0804	396.035	357.980	432.780
	Day 16	Pre-dose	16	398.201	10.5902	42.3607	387.925	350.260	508.450
	Day 16	2 hours	16	399.961	9.4061	37.6244	398.090	326.850	481.480
	Day 16	4 hours	16	389.531	11.8733	47.4931	382.330	329.820	523.330

CDR tests, unadjusted scores

Digit Vigilance - Targets Detected (%)

Treatment	Study Day Visit		N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	98.196	0.6483	2.5931	100.000	91.110	100.000
	Day 1	2 hours	16	96.668	1.6357	6.5430	97.780	73.330	100.000
	Day 1	4 hours	16	95.003	2.3702	9.4807	97.780	60.000	100.000
	Day 16	Pre-dose	16	97.501	0.9486	3.7944	98.890	86.670	100.000
	Day 16	2 hours	16	98.195	0.9561	3.8243	100.000	86.670	100.000
	Day 16	4 hours	16	96.668	2.1757	8.7029	98.890	64.440	100.000
sertra	Day 1	Pre-dose	16	98.889	0.4537	1.8147	100.000	93.330	100.000
	Day 1	2 hours	16	98.751	0.4522	1.8089	100.000	93.330	100.000
	Day 1	4 hours	16	99.306	0.3908	1.5632	100.000	95.560	100.000
	Day 16	Pre-dose	16	99.168	0.2775	1.1100	100.000	97.780	100.000
	Day 16	2 hours	16	99.306	0.3342	1.3366	100.000	95.560	100.000
	Day 16	4 hours	16	98.751	0.5725	2.2899	100.000	93.330	100.000

CDR tests, unadjusted scores

Digit Vigilance - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	393.829	7.6191	30.4764	392.140	342.510	436.270
	Day 1	2 hours	16	391.437	6.9366	27.7466	392.825	353.800	455.180
	Day 1	4 hours	16	397.098	7.4318	29.7271	401.120	347.020	449.110
	Day 16	Pre-dose	16	390.278	6.9934	27.9737	397.750	331.310	429.040
	Day 16	2 hours	16	395.457	9.2851	37.1403	388.110	345.690	494.770
	Day 16	4 hours	16	411.910	9.4643	37.8574	399.800	370.780	501.140
sertra	Day 1	Pre-dose	16	385.166	6.5887	26.3549	381.715	339.840	437.900
	Day 1	2 hours	16	396.320	4.6125	18.4500	396.815	364.020	424.820
	Day 1	4 hours	16	397.072	6.2938	25.1752	401.440	346.310	447.810
	Day 16	Pre-dose	16	393.697	8.2930	33.1719	386.260	357.440	479.910
	Day 16	2 hours	16	405.149	9.1365	36.5458	399.230	358.820	500.950
	Day 16	4 hours	16	400.371	7.1086	28.4344	404.020	351.110	458.840

CDR tests, unadjusted scores

Digit Vigilance - False Alarms (#)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.625	0.2562	1.0247	0.000	0.000	4.000
	Day 1	2 hours	16	0.938	0.2135	0.8539	1.000	0.000	3.000
	Day 1	4 hours	16	0.625	0.2562	1.0247	0.000	0.000	4.000
	Day 16	Pre-dose	16	1.063	0.2495	0.9979	1.000	0.000	3.000
	Day 16	2 hours	16	0.250	0.1443	0.5774	0.000	0.000	2.000
	Day 16	4 hours	16	0.375	0.1548	0.6191	0.000	0.000	2.000
sertra	Day 1	Pre-dose	16	0.313	0.1505	0.6021	0.000	0.000	2.000
	Day 1	2 hours	16	0.688	0.2536	1.0145	0.000	0.000	3.000
	Day 1	4 hours	16	0.188	0.1360	0.5439	0.000	0.000	2.000
	Day 16	Pre-dose	16	0.625	0.2213	0.8851	0.000	0.000	3.000
	Day 16	2 hours	16	0.438	0.1819	0.7274	0.000	0.000	2.000
	Day 16	4 hours	16	0.688	0.2536	1.0145	0.000	0.000	3.000

CDR tests, unadjusted scores

Spatial Working Memory - Sensitivity Index (SI)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.910	0.0216	0.0863	0.941	0.718	1.000
	Day 1	2 hours	16	0.931	0.0172	0.0686	0.941	0.777	1.000
	Day 1	4 hours	16	0.858	0.0444	0.1776	0.925	0.381	1.000
	Day 16	Pre-dose	16	0.945	0.0239	0.0955	1.000	0.663	1.000
	Day 16	2 hours	16	0.884	0.0352	0.1409	0.941	0.519	1.000
	Day 16	4 hours	16	0.916	0.0215	0.0859	0.925	0.679	1.000
sertra	Day 1	Pre-dose	16	0.933	0.0139	0.0554	0.941	0.830	1.000
	Day 1	2 hours	16	0.928	0.0328	0.1312	0.952	0.451	1.000
	Day 1	4 hours	16	0.823	0.0586	0.2343	0.889	0.214	1.000
	Day 16	Pre-dose	16	0.956	0.0169	0.0675	1.000	0.777	1.000
	Day 16	2 hours	16	0.884	0.0535	0.2138	0.947	0.208	1.000
	Day 16	4 hours	16	0.952	0.0135	0.0541	0.952	0.830	1.000

CDR tests, unadjusted scores

Spatial Working Memory - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	567.429	30.3057	121.2229	540.585	385.280	843.060
	Day 1	2 hours	16	550.385	27.3343	109.3371	523.110	397.160	740.330
	Day 1	4 hours	16	630.843	53.2797	213.1190	536.665	405.070	1163.030
	Day 16	Pre-dose	16	546.485	25.5341	102.1365	503.280	413.090	731.540
	Day 16	2 hours	16	569.112	32.1044	128.4177	517.650	431.030	909.030
	Day 16	4 hours	16	503.437	24.7917	99.1666	454.015	413.760	704.160
sertra	Day 1	Pre-dose	16	579.931	19.2799	77.1197	564.030	480.080	775.940
	Day 1	2 hours	16	539.784	37.7994	151.1976	502.530	410.030	1055.960
	Day 1	4 hours	16	587.079	35.7719	143.0878	557.600	459.440	1055.380
	Day 16	Pre-dose	16	551.281	24.1232	96.4927	549.845	409.190	731.390
	Day 16	2 hours	16	567.286	28.8288	115.3152	526.155	412.170	866.550
	Day 16	4 hours	16	482.816	25.7333	102.9330	458.475	394.560	752.310

CDR tests, unadjusted scores

Numeric Working Memory - Sensitivity Index (SI)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.862	0.0204	0.0817	0.888	0.720	0.957
	Day 1	2 hours	16	0.905	0.0162	0.0648	0.912	0.800	1.000
	Day 1	4 hours	16	0.878	0.0234	0.0934	0.903	0.690	0.978
	Day 16	Pre-dose	16	0.889	0.0185	0.0742	0.900	0.690	1.000
	Day 16	2 hours	16	0.872	0.0213	0.0850	0.857	0.735	1.000
	Day 16	4 hours	16	0.883	0.0214	0.0857	0.893	0.714	1.000
sertra	Day 1	Pre-dose	16	0.924	0.0138	0.0553	0.934	0.779	1.000
	Day 1	2 hours	16	0.911	0.0122	0.0488	0.913	0.824	0.978
	Day 1	4 hours	16	0.905	0.0204	0.0817	0.916	0.631	0.978
	Day 16	Pre-dose	16	0.913	0.0114	0.0456	0.911	0.824	1.000
	Day 16	2 hours	16	0.892	0.0226	0.0904	0.913	0.592	1.000
	Day 16	4 hours	16	0.882	0.0094	0.0374	0.874	0.829	0.957

CDR tests, unadjusted scores

Numeric Working Memory - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	586.208	33.5861	134.3442	575.155	369.940	847.880
	Day 1	2 hours	16	566.512	32.1797	128.7187	553.010	341.100	820.910
	Day 1	4 hours	16	552.870	31.8100	127.2401	550.530	374.450	784.030
	Day 16	Pre-dose	16	546.924	28.9715	115.8859	540.160	353.750	757.060
	Day 16	2 hours	16	561.296	37.3150	149.2600	518.215	390.410	910.780
	Day 16	4 hours	16	565.375	38.3435	153.3740	528.265	384.730	867.840
sertra	Day 1	Pre-dose	16	584.428	19.4593	77.8373	575.955	449.330	703.310
	Day 1	2 hours	16	562.860	17.1344	68.5374	556.270	452.070	733.920
	Day 1	4 hours	16	558.169	17.2703	69.0811	562.270	422.690	686.510
	Day 16	Pre-dose	16	569.667	21.8328	87.3310	551.110	436.040	822.830
	Day 16	2 hours	16	555.321	19.7705	79.0821	532.550	416.350	700.460
	Day 16	4 hours	16	539.234	21.0890	84.3561	528.970	428.040	771.290

CDR tests, unadjusted scores

Immediate Word Recall - Words Correctly Recalled (%)

Treatment	Study Day Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1 Pre-dose	16	43.750	3.4275	13.7100	46.667	13.333	60.000
	Day 1 2 hours	16	45.417	3.0257	12.1030	43.333	26.667	70.000
	Day 1 4 hours	16	43.958	2.6739	10.6957	40.000	26.667	73.333
	Day 16 Pre-dose	16	52.083	3.4944	13.9775	53.333	26.667	86.667
	Day 16 2 hours	16	46.458	5.3270	21.3079	48.333	13.333	83.333
	Day 16 4 hours	16	45.417	3.8112	15.2449	46.667	26.667	73.333
sertra	Day 1 Pre-dose	16	53.542	3.0500	12.2001	53.333	33.333	80.000
	Day 1 2 hours	16	43.958	3.2520	13.0082	46.667	20.000	60.000
	Day 1 4 hours	16	46.667	1.8257	7.3030	46.667	33.333	60.000
	Day 16 Pre-dose	16	50.833	3.4224	13.6897	53.333	20.000	73.333
	Day 16 2 hours	16	47.500	3.3679	13.4715	40.000	33.333	66.667
	Day 16 4 hours	16	48.542	3.0118	12.0474	46.667	26.667	66.667

CDR tests, unadjusted scores

Immediate Word Recall - Intrusions (#)

Treatment	Study Day Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 1 2 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 1 4 hours	16	0.063	0.0625	0.2500	0.000	0.000	1.000
	Day 16 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 16 2 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 16 4 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000
sertra	Day 1 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 1 2 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 1 4 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 16 Pre-dose	16	0.125	0.0854	0.3416	0.000	0.000	1.000
	Day 16 2 hours	16	0.188	0.1008	0.4031	0.000	0.000	1.000
	Day 16 4 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000

CDR tests, unadjusted scores

Immediate Word Recall - Errors (#)

Treatment	Study Day Visit		N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.313	0.1505	0.6021	0.000	0.000	2.000
	Day 1	2 hours	16	0.125	0.0854	0.3416	0.000	0.000	1.000
	Day 1	4 hours	16	0.188	0.1008	0.4031	0.000	0.000	1.000
	Day 16	Pre-dose	16	0.250	0.1443	0.5774	0.000	0.000	2.000
	Day 16	2 hours	16	0.438	0.2230	0.8921	0.000	0.000	3.000
	Day 16	4 hours	16	0.313	0.1505	0.6021	0.000	0.000	2.000
sertra	Day 1	Pre-dose	16	0.250	0.1443	0.5774	0.000	0.000	2.000
	Day 1	2 hours	16	0.313	0.2536	1.0145	0.000	0.000	4.000
	Day 1	4 hours	16	0.125	0.0854	0.3416	0.000	0.000	1.000
	Day 16	Pre-dose	16	0.375	0.1548	0.6191	0.000	0.000	2.000
	Day 16	2 hours	16	0.125	0.0854	0.3416	0.000	0.000	1.000
	Day 16	4 hours	16	0.000	0.0000	0.0000	0.000	0.000	0.000

CDR tests, unadjusted scores

Delayed Word Recall - Words Correctly Recalled (%)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	29.375	3.7911	15.1642	26.667	6.667	60.000
	Day 1	2 hours	16	27.083	3.8595	15.4380	25.000	6.667	53.333
	Day 1	4 hours	16	25.833	3.9382	15.7527	26.667	0.000	60.000
	Day 16	Pre-dose	16	41.042	3.6352	14.5408	38.333	20.000	70.000
	Day 16	2 hours	16	25.000	4.5031	18.0123	23.333	0.000	63.333
	Day 16	4 hours	16	29.167	3.7945	15.1780	33.333	0.000	53.333
sertra	Day 1	Pre-dose	16	33.750	3.0104	12.0416	33.333	6.667	60.000
	Day 1	2 hours	16	21.250	3.2329	12.9314	21.667	0.000	43.333
	Day 1	4 hours	16	22.917	3.8595	15.4380	20.000	0.000	60.000
	Day 16	Pre-dose	16	35.625	3.6224	14.4898	36.667	13.333	66.667
	Day 16	2 hours	16	27.083	4.2260	16.9039	25.000	6.667	63.333
	Day 16	4 hours	16	24.583	4.5427	18.1710	20.000	0.000	73.333

CDR tests, unadjusted scores

Delayed Word Recall - Intrusions (#)

Treatment	Study Day Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1 Pre-dose	16	0.063	0.0625	0.2500	0.000	0.000	1.000
	Day 1 2 hours	16	0.375	0.1548	0.6191	0.000	0.000	2.000
	Day 1 4 hours	16	0.500	0.2415	0.9661	0.000	0.000	3.000
	Day 16 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 16 2 hours	16	0.188	0.1360	0.5439	0.000	0.000	2.000
	Day 16 4 hours	16	0.375	0.1250	0.5000	0.000	0.000	1.000
sertra	Day 1 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 1 2 hours	16	0.375	0.2016	0.8062	0.000	0.000	3.000
	Day 1 4 hours	16	0.438	0.2230	0.8921	0.000	0.000	3.000
	Day 16 Pre-dose	16	0.000	0.0000	0.0000	0.000	0.000	0.000
	Day 16 2 hours	16	0.375	0.1548	0.6191	0.000	0.000	2.000
	Day 16 4 hours	16	0.375	0.2016	0.8062	0.000	0.000	3.000

CDR tests, unadjusted scores

Delayed Word Recall - Errors (#)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.313	0.1197	0.4787	0.000	0.000	1.000
	Day 1	2 hours	16	0.500	0.1826	0.7303	0.000	0.000	2.000
	Day 1	4 hours	16	0.375	0.1548	0.6191	0.000	0.000	2.000
	Day 16	Pre-dose	16	0.250	0.1443	0.5774	0.000	0.000	2.000
	Day 16	2 hours	16	0.375	0.1548	0.6191	0.000	0.000	2.000
	Day 16	4 hours	16	0.563	0.2410	0.9639	0.000	0.000	3.000
sertra	Day 1	Pre-dose	16	0.375	0.2720	1.0878	0.000	0.000	4.000
	Day 1	2 hours	16	0.500	0.3162	1.2649	0.000	0.000	5.000
	Day 1	4 hours	16	0.750	0.2958	1.1832	0.000	0.000	4.000
	Day 16	Pre-dose	16	0.500	0.3291	1.3166	0.000	0.000	5.000
	Day 16	2 hours	16	0.563	0.3412	1.3647	0.000	0.000	4.000
	Day 16	4 hours	16	0.750	0.3476	1.3904	0.000	0.000	5.000

CDR tests, unadjusted scores

Word Recognition - Sensitivity Index (SI)

Treatment	Study Day Visit		N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.596	0.0471	0.1884	0.646	0.278	0.882
	Day 1	2 hours	16	0.437	0.0717	0.2866	0.506	-0.397	0.833
	Day 1	4 hours	16	0.579	0.0561	0.2243	0.578	0.170	0.938
	Day 16	Pre-dose	16	0.618	0.0502	0.2008	0.629	0.339	0.938
	Day 16	2 hours	16	0.556	0.0670	0.2680	0.606	0.000	0.938
	Day 16	4 hours	16	0.558	0.0452	0.1808	0.556	0.208	0.882
sertra	Day 1	Pre-dose	16	0.699	0.0524	0.2096	0.747	0.150	0.938
	Day 1	2 hours	16	0.646	0.0444	0.1774	0.658	0.204	0.882
	Day 1	4 hours	16	0.670	0.0351	0.1405	0.690	0.402	0.867
	Day 16	Pre-dose	16	0.704	0.0454	0.1814	0.768	0.268	0.938
	Day 16	2 hours	16	0.670	0.0326	0.1302	0.698	0.467	0.882
	Day 16	4 hours	16	0.644	0.0538	0.2151	0.731	0.268	0.882

CDR tests, unadjusted scores

Word Recognition - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	711.407	51.6866	206.7463	730.835	365.210	1203.850
	Day 1	2 hours	16	665.772	37.6390	150.5562	654.285	415.400	940.500
	Day 1	4 hours	16	680.023	40.5616	162.2466	694.545	409.280	1019.390
	Day 16	Pre-dose	16	676.589	25.1473	100.5894	683.200	538.050	871.770
	Day 16	2 hours	16	670.871	36.6059	146.4234	641.505	468.330	1069.200
	Day 16	4 hours	16	659.824	31.9007	127.6027	679.070	362.300	859.850
sertra	Day 1	Pre-dose	16	686.696	16.8331	67.3324	694.325	552.810	780.810
	Day 1	2 hours	16	702.818	27.2685	109.0741	669.280	586.600	941.470
	Day 1	4 hours	16	678.888	25.2403	100.9611	654.100	546.400	886.520
	Day 16	Pre-dose	16	671.871	25.6935	102.7739	638.140	538.920	929.730
	Day 16	2 hours	16	691.517	26.4471	105.7883	671.520	547.000	881.130
	Day 16	4 hours	16	655.256	26.7371	106.9484	636.785	505.830	882.960

CDR tests, unadjusted scores

Picture Recognition - Sensitivity Index (SI)

Treatment	Study Day Visit		N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	0.687	0.0565	0.2259	0.665	0.110	1.000
	Day 1	2 hours	16	0.696	0.0525	0.2102	0.750	0.256	0.952
	Day 1	4 hours	16	0.656	0.0555	0.2222	0.683	0.104	0.952
	Day 16	Pre-dose	16	0.767	0.0437	0.1746	0.804	0.358	0.952
	Day 16	2 hours	16	0.651	0.0575	0.2301	0.697	0.200	0.952
	Day 16	4 hours	16	0.646	0.0521	0.2084	0.652	0.267	0.909
sertra	Day 1	Pre-dose	16	0.810	0.0248	0.0991	0.804	0.606	1.000
	Day 1	2 hours	16	0.779	0.0236	0.0944	0.800	0.600	0.909
	Day 1	4 hours	16	0.780	0.0334	0.1335	0.804	0.400	1.000
	Day 16	Pre-dose	16	0.811	0.0252	0.1010	0.826	0.625	1.000
	Day 16	2 hours	16	0.757	0.0338	0.1350	0.804	0.502	0.952
	Day 16	4 hours	16	0.729	0.0312	0.1246	0.718	0.500	0.952

CDR tests, unadjusted scores

Picture Recognition - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	761.891	36.6252	146.5009	772.945	487.360	1034.330
	Day 1	2 hours	16	763.203	28.2368	112.9471	748.065	561.670	952.970
	Day 1	4 hours	16	744.459	32.1546	128.6184	730.565	489.230	958.690
	Day 16	Pre-dose	16	769.756	32.0226	128.0905	757.805	620.000	1075.210
	Day 16	2 hours	16	750.960	22.2757	89.1026	777.635	574.650	906.480
	Day 16	4 hours	16	748.738	29.5917	118.3666	730.370	515.090	1006.470
sertra	Day 1	Pre-dose	16	809.461	26.1452	104.5807	810.800	692.490	1046.970
	Day 1	2 hours	16	828.059	42.7776	171.1105	763.625	643.500	1257.660
	Day 1	4 hours	16	772.620	24.4261	97.7043	755.695	632.970	999.530
	Day 16	Pre-dose	16	786.024	35.3043	141.2173	750.175	601.390	1081.000
	Day 16	2 hours	16	774.013	33.5193	134.0771	750.595	561.650	1039.030
	Day 16	4 hours	16	726.073	27.8238	111.2952	697.025	582.410	984.740

CDR tests, unadjusted scores

Power of Attention (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	1014.483	20.3183	81.2732	996.940	898.210	1153.440
	Day 1	2 hours	16	1017.281	19.0085	76.0341	985.690	904.280	1212.260
	Day 1	4 hours	16	1023.749	23.5708	94.2831	1011.185	886.700	1228.630
	Day 16	Pre-dose	16	1013.591	21.1301	84.5206	1001.255	874.530	1158.940
	Day 16	2 hours	16	1020.733	19.9440	79.7761	1015.170	878.570	1214.090
	Day 16	4 hours	16	1049.031	23.4144	93.6575	1029.225	925.050	1254.260
sertra	Day 1	Pre-dose	16	1032.038	13.2763	53.1053	1021.620	950.160	1128.900
	Day 1	2 hours	16	1050.834	12.3102	49.2409	1036.375	982.380	1146.660
	Day 1	4 hours	16	1043.710	13.5651	54.2604	1046.270	938.630	1130.600
	Day 16	Pre-dose	16	1045.073	21.6761	86.7042	1021.865	968.240	1322.520
	Day 16	2 hours	16	1069.486	27.2833	109.1334	1063.400	953.000	1406.730
	Day 16	4 hours	16	1053.574	28.1139	112.4556	1033.255	918.420	1399.770

CDR tests, unadjusted scores

Continuity of Attention (#)

Treatment	Study Day Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1 Pre-dose	16	90.751	0.6225	2.4899	91.002	86.000	95.000
	Day 1 2 hours	16	89.813	0.9497	3.7989	90.501	77.999	94.000
	Day 1 4 hours	16	89.314	1.2965	5.1861	90.501	74.000	95.000
	Day 16 Pre-dose	16	90.876	0.6183	2.4730	91.000	86.000	94.000
	Day 16 2 hours	16	92.125	0.7004	2.8015	93.001	84.002	95.000
	Day 16 4 hours	16	91.375	1.1794	4.7175	92.501	74.998	95.000
sertra	Day 1 Pre-dose	16	92.375	0.4269	1.7078	92.501	89.001	95.000
	Day 1 2 hours	16	91.938	0.5662	2.2646	92.501	88.000	95.000
	Day 1 4 hours	16	92.750	0.2958	1.1830	93.000	91.000	95.000
	Day 16 Pre-dose	16	92.313	0.4977	1.9907	92.501	89.000	95.000
	Day 16 2 hours	16	92.688	0.4054	1.6214	93.001	89.000	95.000
	Day 16 4 hours	16	92.125	0.6045	2.4182	93.000	88.002	95.000

CDR tests, unadjusted scores

Quality of Working Memory (#)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	1.771	0.0333	0.1333	1.792	1.497	1.957
	Day 1	2 hours	16	1.835	0.0283	0.1132	1.834	1.581	2.000
	Day 1	4 hours	16	1.736	0.0555	0.2220	1.822	1.214	1.918
	Day 16	Pre-dose	16	1.833	0.0336	0.1343	1.868	1.523	2.000
	Day 16	2 hours	16	1.756	0.0430	0.1718	1.766	1.329	2.000
	Day 16	4 hours	16	1.799	0.0310	0.1239	1.809	1.602	2.000
sertra	Day 1	Pre-dose	16	1.858	0.0226	0.0904	1.888	1.609	1.956
	Day 1	2 hours	16	1.839	0.0398	0.1591	1.854	1.296	1.978
	Day 1	4 hours	16	1.728	0.0623	0.2490	1.816	1.103	1.978
	Day 16	Pre-dose	16	1.869	0.0235	0.0938	1.897	1.688	2.000
	Day 16	2 hours	16	1.776	0.0539	0.2155	1.842	1.121	1.941
	Day 16	4 hours	16	1.835	0.0155	0.0621	1.848	1.707	1.918

CDR tests, unadjusted scores

Quality of Episodic Secondary Memory (#)

Treatment	Study Day Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1 Pre-dose	16	192.603	15.0546	60.2184	180.832	76.670	306.660
	Day 1 2 hours	16	174.479	15.2955	61.1821	160.003	24.993	283.333
	Day 1 4 hours	16	181.562	15.7370	62.9481	175.002	63.333	275.000
	Day 16 Pre-dose	16	223.229	14.8838	59.5354	234.170	108.330	339.997
	Day 16 2 hours	16	177.185	17.3312	69.3247	178.332	73.330	334.997
	Day 16 4 hours	16	180.938	13.6699	54.6796	185.832	66.667	283.333
sertra	Day 1 Pre-dose	16	230.624	13.0128	52.0513	242.498	93.330	326.670
	Day 1 2 hours	16	193.229	13.7777	55.1107	211.670	51.667	263.337
	Day 1 4 hours	16	200.416	9.4641	37.8565	200.833	115.000	271.667
	Day 16 Pre-dose	16	226.041	13.6481	54.5923	243.333	101.670	323.330
	Day 16 2 hours	16	201.666	11.9609	47.8436	196.663	128.330	295.003
	Day 16 4 hours	16	197.292	14.5913	58.3652	186.667	108.337	316.670

CDR tests, unadjusted scores

Speed of Memory (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Pre-dose	16	2626.934	132.2090	528.8359	2667.925	1682.000	3419.640
	Day 1	2 hours	16	2545.871	110.2117	440.8469	2518.710	1810.340	3257.720
	Day 1	4 hours	16	2608.194	134.4813	537.9253	2537.380	1688.960	3546.710
	Day 16	Pre-dose	16	2539.754	101.3939	405.5758	2463.065	1924.890	3247.440
	Day 16	2 hours	16	2552.238	112.8495	451.3982	2445.960	1965.660	3533.690
	Day 16	4 hours	16	2477.374	105.5287	422.1149	2439.980	1692.080	3231.340
sertra	Day 1	Pre-dose	16	2660.516	59.1692	236.6769	2610.025	2332.660	3279.620
	Day 1	2 hours	16	2633.521	105.4393	421.7573	2456.885	2277.570	3989.010
	Day 1	4 hours	16	2596.756	74.5479	298.1914	2532.100	2245.940	3448.770
	Day 16	Pre-dose	16	2578.844	85.7703	343.0811	2487.410	2192.020	3564.950
	Day 16	2 hours	16	2588.138	84.0306	336.1224	2519.415	2125.520	3264.460
	Day 16	4 hours	16	2403.378	85.7213	342.8850	2289.830	2032.340	3296.870

CDR tests, unadjusted scores

Mackworth Clock - Accuracy (%)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Predose	16	72.917	6.1229	24.4915	80.000	20.000	96.670
	Day 16	2 hours	16	66.874	6.4294	25.7175	70.000	6.670	100.000
sertra	Day 1	Predose	16	84.374	3.0385	12.1541	90.000	53.330	96.670
	Day 16	2 hours	16	77.293	2.9848	11.9392	76.670	56.670	96.670

CDR tests, unadjusted scores

Mackworth Clock - Speed (msec)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Predose	16	693.023	36.3784	145.5136	676.360	390.560	1018.500
	Day 16	2 hours	16	633.762	54.6519	218.6076	532.215	481.260	1307.000
sertra	Day 1	Predose	16	654.603	27.0610	108.2441	665.550	429.820	901.380
	Day 16	2 hours	16	684.051	28.3780	113.5120	695.745	442.720	897.000

CDR tests, unadjusted scores

Mackworth Clock - False Alarms (#)

Treatment	Study Day	Visit	N	Mean	Standard Error	Standard Deviation	Median	Minimum	Maximum
escita	Day 1	Predose	16	2.375	0.6945	2.7779	1.000	0.000	9.000
	Day 16	2 hours	16	1.938	1.0020	4.0078	0.500	0.000	16.000
sertra	Day 1	Predose	16	1.813	0.6722	2.6887	1.000	0.000	11.000
	Day 16	2 hours	16	1.313	0.4351	1.7405	1.000	0.000	5.000

21 Appendix 4: Summary Statistics for Chapter 10

Summary Statistics of CDR Data

Simple Reaction Time (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	261.935	252.905	8.3069	37.1498	213.62	348.17
	0.5 Hour Post-dose	20	277.652	263.110	9.9392	44.4494	215.26	369.77
	1.5 Hour Post-dose	20	285.136	286.160	10.3010	46.0674	230.98	409.39
	2.5 Hour Post-dose	20	285.336	268.060	12.9841	58.0666	211.45	437.56
	4 Hour Post-dose	20	275.129	268.110	9.2405	41.3246	213.14	347.78
	6 Hour Post-dose	20	270.018	259.170	10.1938	45.5880	218.24	400.17
	8 Hour Post-dose	20	267.597	263.755	10.5037	46.9739	196.44	375.26
	12 Hour Post-dose	20	263.634	255.590	8.7353	39.0654	215.70	363.19
	24 Hour Post-dose	20	271.353	264.300	9.6838	43.3073	208.19	355.08
00mg+Placebo	Pre-dose	20	268.137	257.360	8.8513	39.5840	206.24	354.77
	0.5 Hour Post-dose	20	283.314	264.780	12.0510	53.8938	216.35	400.42
	1.5 Hour Post-dose	20	277.254	276.470	10.6580	47.6639	203.37	395.74
	2.5 Hour Post-dose	20	288.418	268.055	11.9409	53.4015	213.79	397.50
	4 Hour Post-dose	20	286.401	263.190	12.6765	56.6912	214.17	403.22
	6 Hour Post-dose	19	276.778	255.850	12.2291	53.3056	222.92	377.90
	8 Hour Post-dose	20	276.959	268.415	11.0653	49.4854	209.24	373.50
	12 Hour Post-dose	20	265.013	254.025	10.1845	45.5465	205.84	384.52
	24 Hour Post-dose	20	274.880	258.355	10.6348	47.5603	213.72	390.53

60mg+Ethanol	Pre-dose	20	258.906	268.680	6.3917	28.5845	214.02	297.84
	0.5 Hour Post-dose	20	268.780	264.135	7.3250	32.7585	218.54	311.48
	1.5 Hour Post-dose	20	286.392	275.235	10.3069	46.0936	219.35	370.91
	2.5 Hour Post-dose	20	281.848	281.260	10.7252	47.9645	217.20	376.90
	4 Hour Post-dose	20	271.574	271.050	9.2774	41.4897	211.33	364.55
	6 Hour Post-dose	20	266.114	255.900	10.1280	45.2936	209.55	371.63
	8 Hour Post-dose	20	264.355	252.180	8.2870	37.0605	214.76	366.88
	12 Hour Post-dose	20	256.992	247.475	7.2225	32.2998	209.58	323.08
	24 Hour Post-dose	20	266.706	255.450	9.2718	41.4646	210.46	347.91
60mg+Placebo	Pre-dose	20	269.076	262.200	7.9424	35.5195	212.35	338.77
	0.5 Hour Post-dose	19	276.433	272.120	9.3595	40.7971	214.54	366.90
	1.5 Hour Post-dose	20	276.699	267.015	8.9966	40.2340	229.29	355.57
	2.5 Hour Post-dose	20	279.976	268.515	10.8986	48.7399	209.85	381.71
	4 Hour Post-dose	20	278.032	263.650	9.3867	41.9786	224.98	369.68
	6 Hour Post-dose	20	277.621	260.760	10.4056	46.5354	211.56	356.20
	8 Hour Post-dose	20	267.045	251.810	9.5370	42.6507	207.39	376.76
	12 Hour Post-dose	20	267.376	254.265	7.3505	32.8724	215.90	315.75
	24 Hour Post-dose	20	278.225	264.825	10.2381	45.7862	205.20	352.98

Summary Statistics of CDR Data

Choice Reaction Time - Accuracy (%)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	95.700	96.000	0.7578	3.3888	88.00	100.00
	0.5 Hour Post-dose	20	94.900	96.000	0.8395	3.7543	86.00	100.00
	1.5 Hour Post-dose	20	92.900	94.000	0.8763	3.9189	86.00	98.00
	2.5 Hour Post-dose	20	94.600	95.000	0.7691	3.4397	84.00	98.00
	4 Hour Post-dose	20	94.700	96.000	1.0185	4.5549	80.00	100.00
	6 Hour Post-dose	20	94.000	96.000	1.1145	4.9842	84.00	100.00
	8 Hour Post-dose	20	94.100	96.000	1.0610	4.7451	82.00	100.00
	12 Hour Post-dose	20	94.500	96.000	0.8569	3.8320	86.00	100.00
	24 Hour Post-dose	20	94.500	96.000	1.0748	4.8068	80.00	100.00
00mg+Placebo	Pre-dose	20	94.600	96.000	1.0964	4.9033	84.00	100.00
	0.5 Hour Post-dose	20	94.500	94.000	0.9611	4.2981	86.00	100.00
	1.5 Hour Post-dose	20	94.600	96.000	1.0964	4.9033	80.00	100.00
	2.5 Hour Post-dose	20	93.300	96.000	1.4745	6.5943	70.00	100.00
	4 Hour Post-dose	20	94.800	96.000	0.7313	3.2703	88.00	98.00
	6 Hour Post-dose	19	93.368	94.000	0.9918	4.3232	78.00	98.00
	8 Hour Post-dose	20	94.700	96.000	0.8977	4.0144	86.00	100.00
	12 Hour Post-dose	20	93.200	95.000	1.2000	5.3666	82.00	100.00
	24 Hour Post-dose	20	95.500	96.000	0.9047	4.0458	86.00	100.00

60mg+Ethanol	Pre-dose	20	94.400	96.000	0.9905	4.4296	86.00	100.00
	0.5 Hour Post-dose	19	94.526	96.000	0.7775	3.3890	88.00	100.00
	1.5 Hour Post-dose	20	92.500	94.000	1.2555	5.6148	78.00	100.00
	2.5 Hour Post-dose	20	93.700	95.000	1.0689	4.7804	84.00	100.00
	4 Hour Post-dose	20	95.000	96.000	0.9570	4.2797	82.00	100.00
	6 Hour Post-dose	20	95.100	96.000	0.7030	3.1439	86.00	100.00
	8 Hour Post-dose	20	94.000	95.000	1.0954	4.8990	84.00	100.00
	12 Hour Post-dose	20	94.000	94.000	1.0052	4.4956	82.00	100.00
	24 Hour Post-dose	20	94.200	96.000	1.1689	5.2275	84.00	100.00
60mg+Placebo	Pre-dose	20	95.500	96.000	0.7522	3.3639	88.00	100.00
	0.5 Hour Post-dose	19	94.632	96.000	1.0035	4.3743	86.00	100.00
	1.5 Hour Post-dose	20	93.400	93.000	0.8473	3.7892	86.00	98.00
	2.5 Hour Post-dose	20	94.800	96.000	1.0301	4.6066	82.00	100.00
	4 Hour Post-dose	20	94.000	95.000	1.0260	4.5883	84.00	100.00
	6 Hour Post-dose	20	94.900	96.000	0.8140	3.6404	88.00	100.00
	8 Hour Post-dose	20	94.800	96.000	0.9989	4.4674	82.00	100.00
	12 Hour Post-dose	20	94.100	95.000	0.9676	4.3274	86.00	100.00
	24 Hour Post-dose	20	95.500	96.000	0.8062	3.6056	88.00	100.00

Summary Statistics of CDR Data

Choice Reaction Time - (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	417.642	404.115	10.8600	48.5672	354.35	544.87
	0.5 Hour Post-dose	20	437.760	425.310	12.4282	55.5807	361.57	586.74
	1.5 Hour Post-dose	20	429.195	418.000	10.1921	45.5806	359.67	525.39
	2.5 Hour Post-dose	20	430.789	411.005	14.3319	64.0940	351.67	554.55
	4 Hour Post-dose	20	418.075	396.175	11.3362	50.6969	355.15	539.39
	6 Hour Post-dose	20	410.975	400.235	11.1549	49.8862	362.32	578.62
	8 Hour Post-dose	20	413.358	399.715	9.8956	44.2543	361.96	515.97
	12 Hour Post-dose	20	401.042	380.230	11.7590	52.5880	343.13	551.35
	24 Hour Post-dose	20	416.583	403.295	12.3021	55.0166	365.34	586.38
00mg+Placebo	Pre-dose	20	431.732	413.485	13.5415	60.5596	358.51	553.43
	0.5 Hour Post-dose	20	439.395	404.540	16.9924	75.9924	357.13	640.79
	1.5 Hour Post-dose	20	428.741	415.245	12.9481	57.9056	349.59	554.41
	2.5 Hour Post-dose	20	439.639	429.395	16.0144	71.6185	360.23	633.48
	4 Hour Post-dose	20	412.749	394.680	10.9890	49.1443	342.96	497.26
	6 Hour Post-dose	19	422.042	405.890	13.3935	58.3810	357.55	532.20
	8 Hour Post-dose	20	423.616	399.535	13.8821	62.0825	353.61	588.45
	12 Hour Post-dose	20	419.650	395.895	15.8543	70.9027	335.15	606.27
	24 Hour Post-dose	20	413.370	398.655	13.7222	61.3676	340.52	588.47

60mg+Ethanol	Pre-dose	20	421.743	414.095	8.4313	37.7059	363.60	496.27
	0.5 Hour Post-dose	19	434.302	418.150	11.6828	50.9243	349.20	548.69
	1.5 Hour Post-dose	20	433.708	422.110	12.8154	57.3121	352.70	540.17
	2.5 Hour Post-dose	20	420.398	421.170	10.2574	45.8724	338.20	507.81
	4 Hour Post-dose	20	415.415	403.125	10.1202	45.2589	351.24	535.37
	6 Hour Post-dose	20	408.295	390.465	9.0612	40.5231	352.33	487.60
	8 Hour Post-dose	20	411.063	400.385	8.8404	39.5353	361.45	509.08
	12 Hour Post-dose	20	411.445	396.955	11.9975	53.6546	345.91	571.65
	24 Hour Post-dose	20	411.522	397.130	13.9976	62.5993	349.67	600.38
60mg+Placebo	Pre-dose	20	422.109	411.355	10.3394	46.2390	362.26	548.33
	0.5 Hour Post-dose	19	436.618	426.430	13.9915	60.9874	361.23	599.51
	1.5 Hour Post-dose	20	431.270	421.070	11.4302	51.1175	368.77	553.84
	2.5 Hour Post-dose	20	432.431	414.235	13.1980	59.0232	355.65	550.13
	4 Hour Post-dose	20	430.742	417.650	11.4059	51.0087	363.34	557.06
	6 Hour Post-dose	20	415.953	402.475	10.1153	45.2372	351.76	496.37
	8 Hour Post-dose	20	417.518	395.485	11.3689	50.8432	352.88	507.60
	12 Hour Post-dose	20	412.749	402.525	10.2547	45.8605	359.62	511.41
	24 Hour Post-dose	20	421.087	409.705	12.2336	54.7103	356.92	542.91

Summary Statistics of CDR Data

Digit Vigilance - Targets Detected (%)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	98.112	100.000	0.7941	3.5514	86.67	100.00
	0.5 Hour Post-dose	20	95.445	100.000	1.8696	8.3612	71.11	100.00
	1.5 Hour Post-dose	20	93.112	97.780	3.2439	14.5071	35.56	100.00
	2.5 Hour Post-dose	20	96.000	98.890	1.3354	5.9722	80.00	100.00
	4 Hour Post-dose	20	96.223	100.000	1.8388	8.2234	68.89	100.00
	6 Hour Post-dose	20	97.112	98.890	0.8392	3.7529	86.67	100.00
	8 Hour Post-dose	20	96.668	97.780	1.0752	4.8086	86.67	100.00
	12 Hour Post-dose	20	96.779	100.000	1.0629	4.7532	86.67	100.00
	24 Hour Post-dose	20	94.335	96.670	1.6478	7.3693	77.78	100.00
00mg+Placebo	Pre-dose	20	95.779	98.890	1.4229	6.3633	77.78	100.00
	0.5 Hour Post-dose	20	95.001	98.890	2.0062	8.9722	71.11	100.00
	1.5 Hour Post-dose	20	94.223	95.560	1.5581	6.9681	77.78	100.00
	2.5 Hour Post-dose	20	94.001	97.780	2.1697	9.7031	62.22	100.00
	4 Hour Post-dose	20	95.557	97.780	1.5548	6.9532	71.11	100.00
	6 Hour Post-dose	19	94.738	97.780	1.5963	6.9583	73.33	100.00
	8 Hour Post-dose	20	96.223	97.780	1.0828	4.8424	84.44	100.00
	12 Hour Post-dose	20	96.001	97.780	1.4563	6.5127	75.56	100.00
	24 Hour Post-dose	20	94.445	97.780	1.7917	8.0129	73.33	100.00

60mg+Ethanol	Pre-dose	20	97.334	98.890	0.8623	3.8565	84.44	100.00
	0.5 Hour Post-dose	20	96.667	100.000	1.1570	5.1743	82.22	100.00
	1.5 Hour Post-dose	20	94.333	97.780	1.9909	8.9036	62.22	100.00
	2.5 Hour Post-dose	20	93.779	97.780	2.3175	10.3641	62.22	100.00
	4 Hour Post-dose	20	96.224	97.780	1.3105	5.8607	75.56	100.00
	6 Hour Post-dose	20	95.779	97.780	1.2785	5.7178	77.78	100.00
	8 Hour Post-dose	20	95.224	97.780	1.4976	6.6973	80.00	100.00
	12 Hour Post-dose	20	97.112	97.780	0.7407	3.3126	91.11	100.00
	24 Hour Post-dose	20	96.889	100.000	1.1448	5.1198	84.44	100.00
60mg+Placebo	Pre-dose	20	95.446	96.670	1.2115	5.4181	77.78	100.00
	0.5 Hour Post-dose	19	96.258	97.780	1.1657	5.0813	84.44	100.00
	1.5 Hour Post-dose	20	94.890	97.780	1.7223	7.7022	73.33	100.00
	2.5 Hour Post-dose	20	95.779	97.780	1.0921	4.8842	86.67	100.00
	4 Hour Post-dose	20	94.556	97.780	1.3440	6.0107	82.22	100.00
	6 Hour Post-dose	20	95.890	97.780	1.2722	5.6895	80.00	100.00
	8 Hour Post-dose	20	95.556	97.780	1.2798	5.7235	84.44	100.00
	12 Hour Post-dose	20	95.556	97.780	1.2696	5.6777	82.22	100.00
	24 Hour Post-dose	20	95.556	100.000	1.7952	8.0285	68.89	100.00

Summary Statistics of CDR Data

Digit Vigilance - Speed (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	415.722	417.725	9.3928	42.0057	340.13	506.41
	0.5 Hour Post-dose	20	425.979	419.025	11.5469	51.6391	337.11	554.92
	1.5 Hour Post-dose	20	446.647	436.485	14.1404	63.2378	332.69	621.95
	2.5 Hour Post-dose	20	430.549	421.525	11.6937	52.2956	348.51	571.49
	4 Hour Post-dose	20	432.676	422.530	12.7860	57.1808	333.18	551.25
	6 Hour Post-dose	20	412.670	408.975	10.9563	48.9982	348.16	569.46
	8 Hour Post-dose	20	421.120	418.035	12.6434	56.5429	304.02	563.54
	12 Hour Post-dose	20	414.838	403.640	10.5194	47.0442	327.09	550.21
	24 Hour Post-dose	20	418.985	409.085	11.3533	50.7736	346.60	548.80
00mg+Placebo	Pre-dose	20	423.558	422.880	14.9136	66.6958	311.78	579.51
	0.5 Hour Post-dose	20	437.504	418.425	15.1170	67.6052	348.91	629.41
	1.5 Hour Post-dose	20	436.559	425.125	14.5745	65.1793	344.91	600.64
	2.5 Hour Post-dose	20	448.358	434.560	15.4496	69.0925	334.07	639.68
	4 Hour Post-dose	20	428.940	420.465	13.3016	59.4863	347.13	584.94
	6 Hour Post-dose	19	434.634	431.500	14.3869	62.7110	334.87	550.79
	8 Hour Post-dose	20	432.284	428.255	11.8790	53.1247	345.33	544.36
	12 Hour Post-dose	20	422.227	411.040	12.8893	57.6429	337.71	561.60
	24 Hour Post-dose	20	429.005	413.035	13.8154	61.7841	342.04	594.97

60mg+Ethanol	Pre-dose	20	408.570	402.145	8.5692	38.3228	335.78	491.92
	0.5 Hour Post-dose	20	427.309	428.100	10.1198	45.2571	343.91	544.70
	1.5 Hour Post-dose	20	443.622	444.170	12.3623	55.2859	351.53	589.00
	2.5 Hour Post-dose	20	441.364	429.300	11.8136	52.8321	348.49	556.71
	4 Hour Post-dose	20	423.685	432.835	11.2788	50.4403	343.73	515.02
	6 Hour Post-dose	20	414.643	412.010	11.1557	49.8898	318.56	539.63
	8 Hour Post-dose	20	418.884	410.910	11.1194	49.7276	340.93	524.69
	12 Hour Post-dose	20	414.294	401.615	11.9145	53.2833	332.16	519.86
	24 Hour Post-dose	20	418.727	406.365	12.3304	55.1431	338.18	510.29
60mg+Placebo	Pre-dose	20	427.446	423.345	10.5617	47.2332	355.33	507.83
	0.5 Hour Post-dose	19	438.579	428.160	11.1270	48.5015	353.60	551.16
	1.5 Hour Post-dose	20	441.576	429.110	11.9216	53.3150	354.42	554.29
	2.5 Hour Post-dose	20	434.503	416.320	11.5438	51.6254	349.89	507.88
	4 Hour Post-dose	20	440.473	427.345	12.3324	55.1520	349.98	548.89
	6 Hour Post-dose	20	420.605	421.190	11.3608	50.8070	330.75	503.70
	8 Hour Post-dose	20	428.306	423.035	13.2061	59.0593	343.98	552.74
	12 Hour Post-dose	20	432.936	434.235	11.0278	49.3178	338.02	512.33
	24 Hour Post-dose	20	426.232	431.800	13.3925	59.8929	335.51	535.47

Summary Statistics of CDR Data

Digit Vigilance - False Alarms (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	1.000	1.000	0.2294	1.0260	0.00	3.00
	0.5 Hour Post-dose	20	0.850	0.500	0.2436	1.0894	0.00	4.00
	1.5 Hour Post-dose	20	0.950	0.000	0.3118	1.3945	0.00	5.00
	2.5 Hour Post-dose	20	1.300	1.000	0.4110	1.8382	0.00	6.00
	4 Hour Post-dose	20	1.150	1.000	0.2927	1.3089	0.00	5.00
	6 Hour Post-dose	20	0.700	1.000	0.1792	0.8013	0.00	3.00
	8 Hour Post-dose	20	1.050	1.000	0.2233	0.9987	0.00	4.00
	12 Hour Post-dose	20	0.900	0.000	0.3154	1.4105	0.00	5.00
	24 Hour Post-dose	20	1.150	1.000	0.2325	1.0400	0.00	3.00
00mg+Placebo	Pre-dose	20	0.450	0.000	0.1535	0.6863	0.00	2.00
	0.5 Hour Post-dose	20	0.550	0.000	0.1846	0.8256	0.00	2.00
	1.5 Hour Post-dose	20	0.650	0.000	0.1817	0.8127	0.00	2.00
	2.5 Hour Post-dose	20	0.950	1.000	0.2112	0.9445	0.00	3.00
	4 Hour Post-dose	20	1.150	1.000	0.3267	1.4609	0.00	5.00
	6 Hour Post-dose	19	1.053	1.000	0.2226	0.9703	0.00	3.00
	8 Hour Post-dose	20	0.750	1.000	0.1758	0.7864	0.00	2.00
	12 Hour Post-dose	20	1.350	1.000	0.2436	1.0894	0.00	3.00
	24 Hour Post-dose	20	1.600	1.000	0.3509	1.5694	0.00	5.00

60mg+Ethanol	Pre-dose	20	1.300	1.000	0.2188	0.9787	0.00	3.00
	0.5 Hour Post-dose	20	0.850	0.500	0.2741	1.2258	0.00	5.00
	1.5 Hour Post-dose	20	1.450	1.000	0.3362	1.5035	0.00	5.00
	2.5 Hour Post-dose	20	1.650	1.000	0.4369	1.9541	0.00	8.00
	4 Hour Post-dose	20	1.150	1.000	0.2325	1.0400	0.00	3.00
	6 Hour Post-dose	20	1.000	1.000	0.2714	1.2140	0.00	4.00
	8 Hour Post-dose	20	1.300	1.000	0.3487	1.5594	0.00	5.00
	12 Hour Post-dose	20	0.800	1.000	0.2000	0.8944	0.00	3.00
	24 Hour Post-dose	20	1.050	1.000	0.2348	1.0501	0.00	3.00
60mg+Placebo	Pre-dose	20	0.850	1.000	0.1957	0.8751	0.00	3.00
	0.5 Hour Post-dose	19	0.579	0.000	0.1922	0.8377	0.00	2.00
	1.5 Hour Post-dose	20	1.200	1.000	0.2675	1.1965	0.00	4.00
	2.5 Hour Post-dose	20	0.900	1.000	0.2283	1.0208	0.00	3.00
	4 Hour Post-dose	20	1.300	1.000	0.2724	1.2183	0.00	4.00
	6 Hour Post-dose	20	1.000	1.000	0.2406	1.0761	0.00	4.00
	8 Hour Post-dose	20	1.000	0.500	0.3162	1.4142	0.00	4.00
	12 Hour Post-dose	20	0.950	1.000	0.2348	1.0501	0.00	4.00
	24 Hour Post-dose	20	0.900	0.000	0.2705	1.2096	0.00	4.00

Summary Statistics of CDR Data

Numeric Working Memory - Sensitivity Index (SI)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.870	0.891	0.0206	0.0923	0.54	0.96
	0.5 Hour Post-dose	20	0.891	0.902	0.0130	0.0583	0.76	0.96
	1.5 Hour Post-dose	20	0.854	0.878	0.0270	0.1206	0.53	0.98
	2.5 Hour Post-dose	20	0.821	0.891	0.0457	0.2044	0.19	1.00
	4 Hour Post-dose	20	0.892	0.934	0.0250	0.1120	0.57	1.00
	6 Hour Post-dose	20	0.861	0.911	0.0297	0.1326	0.47	0.98
	8 Hour Post-dose	20	0.856	0.891	0.0298	0.1331	0.47	1.00
	12 Hour Post-dose	20	0.838	0.897	0.0425	0.1901	0.20	0.96
	24 Hour Post-dose	20	0.865	0.903	0.0238	0.1064	0.57	0.98
00mg+Placebo	Pre-dose	20	0.871	0.912	0.0282	0.1259	0.43	1.00
	0.5 Hour Post-dose	20	0.872	0.893	0.0246	0.1098	0.57	1.00
	1.5 Hour Post-dose	20	0.878	0.913	0.0325	0.1455	0.31	1.00
	2.5 Hour Post-dose	20	0.889	0.907	0.0225	0.1006	0.59	1.00
	4 Hour Post-dose	20	0.838	0.875	0.0333	0.1491	0.29	1.00
	6 Hour Post-dose	19	0.831	0.868	0.0305	0.1328	0.45	0.98
	8 Hour Post-dose	20	0.837	0.895	0.0346	0.1548	0.45	1.00
	12 Hour Post-dose	20	0.840	0.861	0.0291	0.1302	0.40	1.00
	24 Hour Post-dose	20	0.844	0.924	0.0354	0.1585	0.32	0.96

60mg+Ethanol	Pre-dose	20	0.879	0.886	0.0146	0.0655	0.73	0.98
	0.5 Hour Post-dose	19	0.829	0.874	0.0407	0.1772	0.33	1.00
	1.5 Hour Post-dose	20	0.844	0.874	0.0252	0.1129	0.63	1.00
	2.5 Hour Post-dose	20	0.853	0.889	0.0224	0.1004	0.56	0.96
	4 Hour Post-dose	20	0.847	0.913	0.0370	0.1655	0.36	1.00
	6 Hour Post-dose	20	0.883	0.916	0.0230	0.1030	0.53	0.98
	8 Hour Post-dose	20	0.862	0.889	0.0229	0.1023	0.58	0.96
	12 Hour Post-dose	20	0.834	0.912	0.0390	0.1744	0.38	0.98
	24 Hour Post-dose	20	0.887	0.903	0.0186	0.0830	0.65	0.98
60mg+Placebo	Pre-dose	20	0.880	0.900	0.0207	0.0926	0.66	0.98
	0.5 Hour Post-dose	19	0.867	0.893	0.0232	0.1010	0.56	1.00
	1.5 Hour Post-dose	20	0.862	0.906	0.0249	0.1112	0.55	1.00
	2.5 Hour Post-dose	20	0.829	0.858	0.0255	0.1140	0.59	0.98
	4 Hour Post-dose	20	0.893	0.926	0.0191	0.0852	0.65	0.98
	6 Hour Post-dose	20	0.832	0.871	0.0272	0.1216	0.60	0.98
	8 Hour Post-dose	20	0.835	0.889	0.0298	0.1333	0.49	0.98
	12 Hour Post-dose	20	0.850	0.891	0.0267	0.1194	0.60	1.00
	24 Hour Post-dose	20	0.889	0.916	0.0234	0.1045	0.57	1.00

Summary Statistics of CDR Data

Numeric Working Memory - Speed (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	573.576	560.290	16.2913	72.8568	424.63	692.40
	0.5 Hour Post-dose	20	564.181	560.835	21.1740	94.6929	406.71	845.99
	1.5 Hour Post-dose	20	541.912	545.015	12.7605	57.0667	412.81	652.05
	2.5 Hour Post-dose	20	543.241	536.455	15.6218	69.8629	384.21	643.74
	4 Hour Post-dose	20	545.912	529.030	17.3428	77.5596	407.78	724.92
	6 Hour Post-dose	20	556.269	564.150	17.1321	76.6169	394.86	752.22
	8 Hour Post-dose	20	560.887	552.720	20.5896	92.0793	422.82	850.26
	12 Hour Post-dose	20	536.875	542.850	16.6445	74.4364	389.31	677.43
	24 Hour Post-dose	20	540.479	527.835	15.1853	67.9108	395.76	669.47
00mg+Placebo	Pre-dose	20	559.567	559.915	16.9191	75.6644	370.20	681.14
	0.5 Hour Post-dose	20	574.232	566.995	23.1543	103.5491	386.59	837.23
	1.5 Hour Post-dose	20	532.644	536.175	20.2538	90.5776	373.69	816.87
	2.5 Hour Post-dose	20	535.421	531.495	16.6280	74.3625	362.23	679.88
	4 Hour Post-dose	20	540.755	554.295	17.2137	76.9820	340.54	717.52
	6 Hour Post-dose	19	564.148	562.570	20.0028	87.1901	359.32	730.93
	8 Hour Post-dose	20	540.760	545.785	15.3440	68.6205	358.87	632.47
	12 Hour Post-dose	20	534.319	535.950	17.9904	80.4555	338.79	716.67
	24 Hour Post-dose	20	527.149	532.600	16.2621	72.7262	346.04	653.57

60mg+Ethanol	Pre-dose	20	578.863	563.070	21.2098	94.8532	391.38	809.05
	0.5 Hour Post-dose	19	585.782	548.340	34.6815	151.1733	385.66	1074.19
	1.5 Hour Post-dose	20	548.181	533.305	21.5023	96.1610	376.00	805.12
	2.5 Hour Post-dose	20	562.094	552.260	21.3551	95.5029	404.60	849.21
	4 Hour Post-dose	20	551.456	535.980	19.7406	88.2825	413.63	783.28
	6 Hour Post-dose	20	553.688	547.425	19.9007	88.9985	391.35	753.88
	8 Hour Post-dose	20	558.835	545.115	23.9019	106.8928	385.11	855.52
	12 Hour Post-dose	20	540.912	535.815	18.1739	81.2763	377.53	745.20
	24 Hour Post-dose	20	528.937	533.115	15.5563	69.5700	369.16	668.41
60mg+Placebo	Pre-dose	20	560.871	543.990	19.1057	85.4434	375.65	741.67
	0.5 Hour Post-dose	19	543.747	548.180	15.0972	65.8071	369.67	676.62
	1.5 Hour Post-dose	20	542.605	531.030	17.5887	78.6590	365.64	702.60
	2.5 Hour Post-dose	20	548.579	551.675	18.3972	82.2749	371.44	735.58
	4 Hour Post-dose	20	539.642	546.490	16.5636	74.0748	360.00	690.91
	6 Hour Post-dose	20	564.246	577.050	17.4233	77.9195	393.45	755.56
	8 Hour Post-dose	20	553.343	556.490	17.7049	79.1788	367.88	694.85
	12 Hour Post-dose	20	546.323	557.650	17.7561	79.4079	374.72	691.73
	24 Hour Post-dose	20	531.647	539.175	15.4950	69.2956	360.98	696.67

Summary Statistics of CDR Data

Spatial Working Memory - Sensitivity Index (SI)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.897	0.941	0.0329	0.1471	0.33	1.00
	0.5 Hour Post-dose	20	0.846	0.889	0.0306	0.1370	0.49	1.00
	1.5 Hour Post-dose	20	0.873	0.941	0.0318	0.1423	0.49	1.00
	2.5 Hour Post-dose	20	0.844	0.889	0.0357	0.1595	0.34	1.00
	4 Hour Post-dose	20	0.873	0.925	0.0407	0.1819	0.15	1.00
	6 Hour Post-dose	20	0.880	0.941	0.0271	0.1211	0.61	1.00
	8 Hour Post-dose	20	0.885	0.941	0.0332	0.1484	0.44	1.00
	12 Hour Post-dose	20	0.841	0.888	0.0345	0.1545	0.36	1.00
	24 Hour Post-dose	20	0.878	0.941	0.0417	0.1866	0.27	1.00
00mg+Placebo	Pre-dose	20	0.935	0.941	0.0126	0.0564	0.83	1.00
	0.5 Hour Post-dose	20	0.888	0.941	0.0255	0.1141	0.55	1.00
	1.5 Hour Post-dose	20	0.832	0.941	0.0553	0.2471	0.00	1.00
	2.5 Hour Post-dose	20	0.867	0.888	0.0202	0.0904	0.66	1.00
	4 Hour Post-dose	20	0.793	0.888	0.0656	0.2935	-0.15	1.00
	6 Hour Post-dose	19	0.862	0.909	0.0330	0.1437	0.44	1.00
	8 Hour Post-dose	20	0.849	0.889	0.0351	0.1571	0.49	1.00
	12 Hour Post-dose	20	0.817	0.889	0.0553	0.2474	0.10	1.00
	24 Hour Post-dose	20	0.855	0.889	0.0322	0.1440	0.55	1.00

60mg+Ethanol	Pre-dose	20	0.932	0.941	0.0156	0.0697	0.75	1.00
	0.5 Hour Post-dose	19	0.871	0.888	0.0257	0.1121	0.61	1.00
	1.5 Hour Post-dose	20	0.884	0.889	0.0235	0.1051	0.66	1.00
	2.5 Hour Post-dose	20	0.872	0.941	0.0397	0.1774	0.21	1.00
	4 Hour Post-dose	20	0.805	0.889	0.0594	0.2657	-0.09	1.00
	6 Hour Post-dose	20	0.890	0.941	0.0273	0.1222	0.61	1.00
	8 Hour Post-dose	20	0.903	0.941	0.0190	0.0851	0.72	1.00
	12 Hour Post-dose	20	0.850	0.888	0.0342	0.1531	0.44	1.00
	24 Hour Post-dose	20	0.922	0.941	0.0122	0.0545	0.83	1.00
60mg+Placebo	Pre-dose	20	0.905	0.941	0.0226	0.1012	0.55	1.00
	0.5 Hour Post-dose	19	0.884	0.941	0.0341	0.1488	0.45	1.00
	1.5 Hour Post-dose	20	0.860	0.879	0.0326	0.1457	0.50	1.00
	2.5 Hour Post-dose	20	0.861	0.889	0.0339	0.1515	0.38	1.00
	4 Hour Post-dose	20	0.793	0.865	0.0574	0.2568	-0.01	1.00
	6 Hour Post-dose	20	0.857	0.915	0.0360	0.1611	0.38	1.00
	8 Hour Post-dose	20	0.857	0.941	0.0347	0.1550	0.38	1.00
	12 Hour Post-dose	20	0.878	0.941	0.0429	0.1916	0.23	1.00
	24 Hour Post-dose	20	0.897	0.941	0.0191	0.0856	0.72	1.00

Summary Statistics of CDR Data

Spatial Working Memory - Speed (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	568.514	506.650	36.0516	161.2278	445.38	1106.59
	0.5 Hour Post-dose	20	545.538	490.405	30.7551	137.5411	399.70	816.93
	1.5 Hour Post-dose	20	539.980	490.260	32.7709	146.5561	396.24	993.59
	2.5 Hour Post-dose	20	541.271	477.570	35.3047	157.8873	395.48	1052.78
	4 Hour Post-dose	20	539.969	500.435	24.7728	110.7872	402.33	747.42
	6 Hour Post-dose	20	529.481	503.535	29.3181	131.1145	387.75	897.34
	8 Hour Post-dose	20	535.083	473.735	32.5525	145.5794	411.44	923.80
	12 Hour Post-dose	20	527.341	474.105	25.6704	114.8015	388.17	790.91
	24 Hour Post-dose	20	529.040	471.270	30.0240	134.2715	389.46	846.03
00mg+Placebo	Pre-dose	20	549.286	533.505	24.5758	109.9065	376.67	778.06
	0.5 Hour Post-dose	20	543.576	507.875	33.6458	150.4685	363.06	907.97
	1.5 Hour Post-dose	20	555.551	469.865	42.8864	191.7938	389.31	1034.53
	2.5 Hour Post-dose	20	565.179	526.600	32.6606	146.0625	387.47	850.84
	4 Hour Post-dose	20	518.699	502.315	25.3519	113.3769	338.39	781.55
	6 Hour Post-dose	19	539.592	501.790	32.7264	142.6510	360.25	844.80
	8 Hour Post-dose	20	543.071	478.380	32.6545	146.0353	392.29	800.73
	12 Hour Post-dose	20	501.392	474.485	25.9445	116.0274	309.47	800.13
	24 Hour Post-dose	20	532.082	475.175	34.9966	156.5097	342.27	990.61

60mg+Ethanol	Pre-dose	20	565.358	491.840	33.0931	147.9970	386.97	883.18
	0.5 Hour Post-dose	19	564.711	476.780	38.3014	166.9520	424.00	958.49
	1.5 Hour Post-dose	20	550.822	505.275	35.0398	156.7025	383.37	987.85
	2.5 Hour Post-dose	20	540.402	463.885	33.9614	151.8801	414.14	954.59
	4 Hour Post-dose	20	566.529	507.550	46.8449	209.4967	368.11	1245.80
	6 Hour Post-dose	20	543.167	525.675	24.0739	107.6619	392.38	805.31
	8 Hour Post-dose	20	534.507	465.120	34.9721	156.3998	380.97	893.78
	12 Hour Post-dose	20	525.461	481.930	28.1203	125.7578	406.00	826.33
	24 Hour Post-dose	20	507.887	492.655	20.5360	91.8399	391.50	747.53
60mg+Placebo	Pre-dose	20	537.113	504.225	24.5418	109.7542	411.47	859.68
	0.5 Hour Post-dose	19	543.838	483.190	30.2551	131.8791	427.29	859.23
	1.5 Hour Post-dose	20	547.567	485.475	34.9719	156.3993	359.64	985.45
	2.5 Hour Post-dose	20	528.503	464.385	36.7233	164.2317	383.72	1100.86
	4 Hour Post-dose	20	519.198	463.635	27.5551	123.2303	409.66	819.91
	6 Hour Post-dose	20	543.002	534.410	19.4629	87.0409	431.94	759.29
	8 Hour Post-dose	20	542.834	487.430	41.1084	183.8422	366.76	1107.00
	12 Hour Post-dose	20	502.642	494.375	18.4315	82.4284	400.76	741.77
	24 Hour Post-dose	20	498.189	473.085	18.6970	83.6157	377.10	695.97

Summary Statistics of CDR Data

Immediate Word Recall - Words Correctly Recalled (%)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	45.667	46.670	2.5602	11.4495	26.67	60.00
	0.5 Hour Post-dose	20	42.000	40.000	2.3869	10.6743	20.00	60.00
	1.5 Hour Post-dose	20	35.833	33.330	2.1746	9.7252	20.00	53.33
	2.5 Hour Post-dose	20	42.001	40.000	2.8755	12.8598	20.00	70.00
	4 Hour Post-dose	20	39.166	40.000	2.5233	11.2847	20.00	63.33
	6 Hour Post-dose	20	45.168	46.670	3.2576	14.5683	20.00	73.33
	8 Hour Post-dose	20	43.166	40.000	2.1961	9.8214	30.00	63.33
	12 Hour Post-dose	20	44.668	43.335	2.9362	13.1310	23.33	66.67
	24 Hour Post-dose	20	41.167	40.000	2.4933	11.1502	23.33	76.67
00mg+Placebo	Pre-dose	20	43.833	40.000	2.8845	12.9001	20.00	66.67
	0.5 Hour Post-dose	20	43.000	40.000	2.5468	11.3895	26.67	66.67
	1.5 Hour Post-dose	20	40.999	40.000	2.2952	10.2646	26.67	60.00
	2.5 Hour Post-dose	20	41.167	40.000	2.5850	11.5604	20.00	60.00
	4 Hour Post-dose	20	42.334	40.000	2.6167	11.7021	26.67	73.33
	6 Hour Post-dose	19	41.929	40.000	2.8536	12.4385	20.00	66.67
	8 Hour Post-dose	20	43.167	40.000	2.9067	12.9992	20.00	66.67
	12 Hour Post-dose	20	42.167	40.000	3.8487	17.2117	13.33	93.33
	24 Hour Post-dose	20	48.834	46.670	2.6299	11.7614	33.33	73.33

60mg+Ethanol	Pre-dose	20	46.334	46.670	2.7774	12.4210	20.00	60.00
	0.5 Hour Post-dose	20	44.999	46.665	2.8357	12.6816	26.67	66.67
	1.5 Hour Post-dose	20	34.667	33.330	2.9260	13.0853	13.33	60.00
	2.5 Hour Post-dose	20	40.333	38.335	2.8602	12.7912	13.33	63.33
	4 Hour Post-dose	20	36.667	35.000	2.6927	12.0421	20.00	56.67
	6 Hour Post-dose	20	37.999	40.000	2.5179	11.2605	20.00	60.00
	8 Hour Post-dose	20	44.833	46.670	3.5747	15.9866	20.00	66.67
	12 Hour Post-dose	20	46.334	50.000	2.9903	13.3729	20.00	66.67
	24 Hour Post-dose	20	42.334	40.000	2.2827	10.2085	26.67	60.00
60mg+Placebo	Pre-dose	20	46.832	48.330	3.4833	15.5779	13.33	73.33
	0.5 Hour Post-dose	20	41.334	40.000	3.6064	16.1281	16.67	73.33
	1.5 Hour Post-dose	20	39.667	40.000	2.6035	11.6432	16.67	66.67
	2.5 Hour Post-dose	20	41.835	46.670	2.8966	12.9542	16.67	66.67
	4 Hour Post-dose	20	39.500	40.000	2.5165	11.2542	13.33	56.67
	6 Hour Post-dose	20	42.333	40.000	2.8620	12.7992	26.67	63.33
	8 Hour Post-dose	20	45.166	43.330	2.8968	12.9548	30.00	80.00
	12 Hour Post-dose	20	43.334	40.000	2.3694	10.5963	20.00	66.67
	24 Hour Post-dose	20	46.168	46.670	3.0231	13.5199	26.67	73.33

Summary Statistics of CDR Data

Immediate Word Recall - Intrusions (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	0.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	1.5 Hour Post-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	2.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	4 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	6 Hour Post-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	8 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	12 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	24 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
00mg+Placebo	Pre-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	0.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	1.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	2.5 Hour Post-dose	20	0.200	0.000	0.0918	0.4104	0.00	1.00
	4 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	6 Hour Post-dose	19	0.158	0.000	0.1150	0.5015	0.00	2.00
	8 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
	12 Hour Post-dose	20	0.250	0.000	0.1230	0.5501	0.00	2.00
	24 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00

60mg+Ethanol	Pre-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	0.5 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	1.5 Hour Post-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	2.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	4 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
	6 Hour Post-dose	20	0.250	0.000	0.1428	0.6387	0.00	2.00
	8 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	12 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
	24 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
60mg+Placebo	Pre-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	0.5 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	1.5 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	2.5 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
	4 Hour Post-dose	20	0.150	0.000	0.0819	0.3663	0.00	1.00
	6 Hour Post-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	8 Hour Post-dose	20	0.100	0.000	0.1000	0.4472	0.00	2.00
	12 Hour Post-dose	20	0.100	0.000	0.1000	0.4472	0.00	2.00
	24 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00

Summary Statistics of CDR Data

Immediate Word Recall - Errors (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.350	0.000	0.1313	0.5871	0.00	2.00
	0.5 Hour Post-dose	20	0.250	0.000	0.1602	0.7164	0.00	3.00
	1.5 Hour Post-dose	20	0.450	0.000	0.1698	0.7592	0.00	2.00
	2.5 Hour Post-dose	20	0.300	0.000	0.1469	0.6569	0.00	2.00
	4 Hour Post-dose	20	0.500	0.000	0.1987	0.8885	0.00	3.00
	6 Hour Post-dose	20	0.200	0.000	0.1170	0.5231	0.00	2.00
	8 Hour Post-dose	20	0.400	0.000	0.1338	0.5982	0.00	2.00
	12 Hour Post-dose	20	0.550	0.000	0.2348	1.0501	0.00	4.00
	24 Hour Post-dose	20	0.500	0.000	0.1987	0.8885	0.00	3.00
00mg+Placebo	Pre-dose	20	0.300	0.000	0.1277	0.5712	0.00	2.00
	0.5 Hour Post-dose	20	0.450	0.000	0.1352	0.6048	0.00	2.00
	1.5 Hour Post-dose	20	0.450	0.000	0.1535	0.6863	0.00	2.00
	2.5 Hour Post-dose	20	0.300	0.000	0.1277	0.5712	0.00	2.00
	4 Hour Post-dose	20	0.200	0.000	0.0918	0.4104	0.00	1.00
	6 Hour Post-dose	19	0.316	0.000	0.1719	0.7493	0.00	3.00
	8 Hour Post-dose	20	0.450	0.000	0.1352	0.6048	0.00	2.00
	12 Hour Post-dose	20	0.400	0.000	0.1686	0.7539	0.00	2.00
	24 Hour Post-dose	20	0.400	0.000	0.1522	0.6806	0.00	2.00

60mg+Ethanol	Pre-dose	20	0.500	0.000	0.1701	0.7609	0.00	2.00
	0.5 Hour Post-dose	20	0.450	0.000	0.1352	0.6048	0.00	2.00
	1.5 Hour Post-dose	20	0.500	0.000	0.1701	0.7609	0.00	2.00
	2.5 Hour Post-dose	20	0.550	0.000	0.2348	1.0501	0.00	3.00
	4 Hour Post-dose	20	0.800	0.000	0.2956	1.3219	0.00	4.00
	6 Hour Post-dose	20	0.450	0.000	0.2233	0.9987	0.00	4.00
	8 Hour Post-dose	20	0.500	0.000	0.1987	0.8885	0.00	3.00
	12 Hour Post-dose	20	0.350	0.000	0.1817	0.8127	0.00	3.00
	24 Hour Post-dose	20	0.500	0.000	0.1987	0.8885	0.00	3.00
60mg+Placebo	Pre-dose	20	0.500	0.000	0.2236	1.0000	0.00	3.00
	0.5 Hour Post-dose	20	0.500	0.000	0.2236	1.0000	0.00	4.00
	1.5 Hour Post-dose	20	0.450	0.000	0.2458	1.0990	0.00	4.00
	2.5 Hour Post-dose	20	0.300	0.000	0.1277	0.5712	0.00	2.00
	4 Hour Post-dose	20	0.550	0.000	0.2233	0.9987	0.00	3.00
	6 Hour Post-dose	20	0.650	0.000	0.2741	1.2258	0.00	5.00
	8 Hour Post-dose	20	0.300	0.000	0.1277	0.5712	0.00	2.00
	12 Hour Post-dose	20	0.500	0.000	0.1701	0.7609	0.00	2.00
	24 Hour Post-dose	20	0.200	0.000	0.1556	0.6959	0.00	3.00

Summary Statistics of CDR Data

Delayed Word Recall - Words Correctly Recalled (%)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	29.168	26.670	3.2521	14.5437	0.00	53.33
	0.5 Hour Post-dose	20	18.000	13.330	3.2026	14.3224	0.00	43.33
	1.5 Hour Post-dose	20	10.667	8.335	1.6857	7.5385	0.00	26.67
	2.5 Hour Post-dose	20	21.000	20.000	3.4036	15.2213	0.00	63.33
	4 Hour Post-dose	20	18.500	13.330	3.3635	15.0421	0.00	53.33
	6 Hour Post-dose	20	20.167	18.335	2.8965	12.9535	0.00	50.00
	8 Hour Post-dose	20	21.168	21.665	2.1394	9.5678	6.67	43.33
	12 Hour Post-dose	20	21.834	23.335	2.7729	12.4007	0.00	46.67
	24 Hour Post-dose	20	22.167	20.000	2.0700	9.2574	6.67	40.00
00mg+Placebo	Pre-dose	20	31.167	31.665	2.1531	9.6289	13.33	53.33
	0.5 Hour Post-dose	20	23.834	23.335	3.0519	13.6484	0.00	53.33
	1.5 Hour Post-dose	20	20.001	18.335	3.2353	14.4688	0.00	53.33
	2.5 Hour Post-dose	20	13.000	13.330	3.1240	13.9711	0.00	53.33
	4 Hour Post-dose	20	19.833	20.000	2.5187	11.2640	0.00	40.00
	6 Hour Post-dose	19	20.000	20.000	2.5489	11.1103	0.00	40.00
	8 Hour Post-dose	20	14.333	13.330	2.8828	12.8922	0.00	46.67
	12 Hour Post-dose	20	20.834	20.000	3.4527	15.4411	0.00	60.00
	24 Hour Post-dose	20	26.166	26.670	1.9387	8.6701	13.33	40.00

60mg+Ethanol	Pre-dose	20	28.001	26.670	1.7506	7.8288	13.33	46.67
	0.5 Hour Post-dose	19	15.263	13.330	2.7612	12.0359	0.00	46.67
	1.5 Hour Post-dose	20	6.834	0.000	2.1421	9.5798	0.00	33.33
	2.5 Hour Post-dose	20	9.667	6.670	2.0789	9.2971	0.00	26.67
	4 Hour Post-dose	20	13.501	13.330	1.5759	7.0476	0.00	26.67
	6 Hour Post-dose	20	15.835	16.665	2.3556	10.5344	0.00	36.67
	8 Hour Post-dose	20	20.333	20.000	2.6589	11.8908	0.00	40.00
	12 Hour Post-dose	20	18.834	20.000	3.1741	14.1952	0.00	40.00
	24 Hour Post-dose	20	22.667	20.000	3.2225	14.4116	0.00	63.33
60mg+Placebo	Pre-dose	20	30.500	33.330	2.3357	10.4458	13.33	46.67
	0.5 Hour Post-dose	19	23.333	20.000	3.2545	14.1859	0.00	60.00
	1.5 Hour Post-dose	20	10.167	6.670	2.6211	11.7221	0.00	46.67
	2.5 Hour Post-dose	20	12.166	13.330	2.4335	10.8828	0.00	33.33
	4 Hour Post-dose	20	22.166	20.000	3.1182	13.9450	0.00	50.00
	6 Hour Post-dose	20	19.334	16.665	3.2769	14.6546	0.00	46.67
	8 Hour Post-dose	20	12.000	13.330	2.1276	9.5147	0.00	30.00
	12 Hour Post-dose	20	20.334	20.000	2.9608	13.2409	0.00	46.67
	24 Hour Post-dose	20	27.167	23.335	3.3791	15.1118	6.67	60.00

Summary Statistics of CDR Data

Delayed Word Recall - Intrusions (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.000	0.000	0.0000	0.0000	0.00	0.00
	0.5 Hour Post-dose	20	0.350	0.000	0.1817	0.8127	0.00	3.00
	1.5 Hour Post-dose	20	0.650	0.000	0.2209	0.9881	0.00	3.00
	2.5 Hour Post-dose	20	0.650	0.000	0.2436	1.0894	0.00	3.00
	4 Hour Post-dose	20	0.550	0.000	0.2112	0.9445	0.00	3.00
	6 Hour Post-dose	20	0.450	0.000	0.2233	0.9987	0.00	4.00
	8 Hour Post-dose	20	0.300	0.000	0.1638	0.7327	0.00	3.00
	12 Hour Post-dose	20	0.200	0.000	0.0918	0.4104	0.00	1.00
	24 Hour Post-dose	20	0.300	0.000	0.1051	0.4702	0.00	1.00
00mg+Placebo	Pre-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00
	0.5 Hour Post-dose	20	0.300	0.000	0.2065	0.9234	0.00	4.00
	1.5 Hour Post-dose	20	0.500	0.000	0.2856	1.2773	0.00	5.00
	2.5 Hour Post-dose	20	0.550	0.000	0.1846	0.8256	0.00	2.00
	4 Hour Post-dose	20	0.550	0.000	0.1983	0.8870	0.00	3.00
	6 Hour Post-dose	19	0.789	0.000	0.2920	1.2727	0.00	5.00
	8 Hour Post-dose	20	0.550	0.000	0.2233	0.9987	0.00	4.00
	12 Hour Post-dose	20	0.550	0.000	0.2562	1.1459	0.00	4.00
	24 Hour Post-dose	20	0.250	0.000	0.1230	0.5501	0.00	2.00

60mg+Ethanol	Pre-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	0.5 Hour Post-dose	19	0.368	0.000	0.1906	0.8307	0.00	3.00
	1.5 Hour Post-dose	20	1.050	0.000	0.3662	1.6376	0.00	6.00
	2.5 Hour Post-dose	20	0.550	0.000	0.2458	1.0990	0.00	4.00
	4 Hour Post-dose	20	0.500	0.000	0.1850	0.8272	0.00	3.00
	6 Hour Post-dose	20	0.750	0.000	0.3152	1.4096	0.00	5.00
	8 Hour Post-dose	20	0.450	0.000	0.1698	0.7592	0.00	3.00
	12 Hour Post-dose	20	0.600	0.000	0.2449	1.0954	0.00	4.00
	24 Hour Post-dose	20	0.200	0.000	0.1376	0.6156	0.00	2.00
60mg+Placebo	Pre-dose	20	0.100	0.000	0.0688	0.3078	0.00	1.00
	0.5 Hour Post-dose	19	0.474	0.000	0.1598	0.6967	0.00	2.00
	1.5 Hour Post-dose	20	0.750	0.000	0.2702	1.2085	0.00	4.00
	2.5 Hour Post-dose	20	0.600	0.000	0.1974	0.8826	0.00	3.00
	4 Hour Post-dose	20	0.450	0.000	0.1698	0.7592	0.00	2.00
	6 Hour Post-dose	20	0.550	0.000	0.2348	1.0501	0.00	3.00
	8 Hour Post-dose	20	0.600	0.000	0.1974	0.8826	0.00	3.00
	12 Hour Post-dose	20	0.400	0.000	0.1835	0.8208	0.00	3.00
	24 Hour Post-dose	20	0.050	0.000	0.0500	0.2236	0.00	1.00

Summary Statistics of CDR Data

Delayed Word Recall - Errors (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.700	0.000	0.1933	0.8645	0.00	2.00
	0.5 Hour Post-dose	20	0.550	0.000	0.2562	1.1459	0.00	4.00
	1.5 Hour Post-dose	20	0.500	0.000	0.1850	0.8272	0.00	3.00
	2.5 Hour Post-dose	20	1.000	1.000	0.2176	0.9733	0.00	3.00
	4 Hour Post-dose	20	0.750	0.000	0.2392	1.0699	0.00	4.00
	6 Hour Post-dose	20	0.350	0.000	0.1500	0.6708	0.00	2.00
	8 Hour Post-dose	20	1.050	1.000	0.2233	0.9987	0.00	3.00
	12 Hour Post-dose	20	0.650	0.000	0.2741	1.2258	0.00	4.00
	24 Hour Post-dose	20	0.600	0.000	0.2224	0.9947	0.00	3.00
00mg+Placebo	Pre-dose	20	0.650	0.000	0.2325	1.0400	0.00	3.00
	0.5 Hour Post-dose	20	0.500	0.000	0.1850	0.8272	0.00	3.00
	1.5 Hour Post-dose	20	0.450	0.000	0.1698	0.7592	0.00	2.00
	2.5 Hour Post-dose	20	0.650	0.000	0.2325	1.0400	0.00	3.00
	4 Hour Post-dose	20	0.900	0.000	0.2982	1.3338	0.00	5.00
	6 Hour Post-dose	19	0.474	0.000	0.1930	0.8412	0.00	3.00
	8 Hour Post-dose	20	1.050	0.500	0.2945	1.3169	0.00	4.00
	12 Hour Post-dose	20	0.900	0.000	0.2503	1.1192	0.00	3.00
	24 Hour Post-dose	20	0.700	0.000	0.2306	1.0311	0.00	3.00

60mg+Ethanol	Pre-dose	20	0.750	0.500	0.2280	1.0195	0.00	4.00
	0.5 Hour Post-dose	19	0.368	0.000	0.2191	0.9551	0.00	4.00
	1.5 Hour Post-dose	20	0.400	0.000	0.1522	0.6806	0.00	2.00
	2.5 Hour Post-dose	20	0.650	0.000	0.2542	1.1367	0.00	4.00
	4 Hour Post-dose	20	1.050	0.500	0.3033	1.3563	0.00	4.00
	6 Hour Post-dose	20	0.850	0.000	0.2542	1.1367	0.00	3.00
	8 Hour Post-dose	20	0.900	0.000	0.2606	1.1653	0.00	3.00
	12 Hour Post-dose	20	0.400	0.000	0.1686	0.7539	0.00	2.00
	24 Hour Post-dose	20	0.800	0.500	0.2248	1.0052	0.00	3.00
60mg+Placebo	Pre-dose	20	0.600	0.000	0.2555	1.1425	0.00	4.00
	0.5 Hour Post-dose	19	0.316	0.000	0.1336	0.5824	0.00	2.00
	1.5 Hour Post-dose	20	0.950	0.000	0.3439	1.5381	0.00	5.00
	2.5 Hour Post-dose	20	0.600	0.000	0.2340	1.0463	0.00	3.00
	4 Hour Post-dose	20	1.100	0.500	0.3472	1.5526	0.00	6.00
	6 Hour Post-dose	20	0.550	0.000	0.2458	1.0990	0.00	4.00
	8 Hour Post-dose	20	0.750	0.000	0.2798	1.2513	0.00	5.00
	12 Hour Post-dose	20	0.750	0.000	0.2280	1.0195	0.00	3.00
	24 Hour Post-dose	20	0.750	0.000	0.2280	1.0195	0.00	3.00

Summary Statistics of CDR Data

Word Recognition - Sensitivity Index (SI)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	0.543	0.600	0.0533	0.2383	0.00	0.88
	0.5 Hour Post-dose	20	0.521	0.546	0.0418	0.1869	0.20	0.83
	1.5 Hour Post-dose	20	0.525	0.584	0.0534	0.2386	-0.07	0.88
	2.5 Hour Post-dose	20	0.450	0.442	0.0511	0.2287	0.00	0.79
	4 Hour Post-dose	20	0.465	0.471	0.0525	0.2349	-0.24	0.88
	6 Hour Post-dose	20	0.465	0.410	0.0450	0.2015	0.14	0.79
	8 Hour Post-dose	20	0.498	0.519	0.0521	0.2332	0.00	0.80
	12 Hour Post-dose	20	0.456	0.471	0.0584	0.2613	-0.07	0.94
	24 Hour Post-dose	20	0.431	0.434	0.0635	0.2840	-0.42	0.75
00mg+Placebo	Pre-dose	20	0.531	0.600	0.0463	0.2069	0.22	0.88
	0.5 Hour Post-dose	20	0.447	0.489	0.0482	0.2154	0.00	0.75
	1.5 Hour Post-dose	20	0.420	0.439	0.0468	0.2092	0.00	0.69
	2.5 Hour Post-dose	20	0.454	0.507	0.0556	0.2486	0.00	0.80
	4 Hour Post-dose	20	0.431	0.368	0.0470	0.2100	0.13	0.79
	6 Hour Post-dose	19	0.452	0.475	0.0484	0.2110	0.00	0.75
	8 Hour Post-dose	20	0.426	0.417	0.0513	0.2294	0.00	0.88
	12 Hour Post-dose	20	0.378	0.410	0.0538	0.2407	0.00	0.83
	24 Hour Post-dose	20	0.514	0.475	0.0487	0.2178	0.13	0.94

60mg+Ethanol	Pre-dose	20	0.515	0.471	0.0620	0.2771	0.00	1.00
	0.5 Hour Post-dose	19	0.478	0.511	0.0742	0.3235	-0.42	0.88
	1.5 Hour Post-dose	20	0.412	0.434	0.0568	0.2539	-0.14	0.88
	2.5 Hour Post-dose	20	0.423	0.442	0.0466	0.2086	0.00	0.80
	4 Hour Post-dose	20	0.531	0.546	0.0433	0.1936	0.20	0.94
	6 Hour Post-dose	20	0.470	0.471	0.0462	0.2067	0.00	0.80
	8 Hour Post-dose	20	0.448	0.476	0.0548	0.2449	0.09	0.88
	12 Hour Post-dose	20	0.464	0.489	0.0678	0.3034	-0.17	0.88
	24 Hour Post-dose	20	0.475	0.477	0.0512	0.2292	0.00	0.94
60mg+Placebo	Pre-dose	20	0.576	0.556	0.0366	0.1639	0.27	0.83
	0.5 Hour Post-dose	19	0.482	0.536	0.0641	0.2794	-0.17	0.94
	1.5 Hour Post-dose	20	0.364	0.375	0.0637	0.2851	-0.17	0.79
	2.5 Hour Post-dose	20	0.446	0.446	0.0448	0.2004	0.07	0.83
	4 Hour Post-dose	20	0.398	0.400	0.0524	0.2343	0.00	0.83
	6 Hour Post-dose	20	0.467	0.568	0.0624	0.2789	0.00	0.83
	8 Hour Post-dose	20	0.449	0.471	0.0693	0.3101	-0.24	0.88
	12 Hour Post-dose	20	0.439	0.450	0.0612	0.2736	0.07	0.80
	24 Hour Post-dose	20	0.529	0.578	0.0409	0.1829	0.00	0.79

Summary Statistics of CDR Data

Word Recognition - Speed (msec)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	688.734	685.745	26.4341	118.2167	487.74	1002.22
	0.5 Hour Post-dose	20	688.865	681.035	23.4252	104.7608	542.00	974.17
	1.5 Hour Post-dose	20	667.033	643.870	20.8196	93.1079	549.37	866.88
	2.5 Hour Post-dose	20	698.646	687.325	26.5425	118.7015	518.60	955.83
	4 Hour Post-dose	20	689.369	673.715	23.1858	103.6901	507.18	892.70
	6 Hour Post-dose	20	680.792	635.380	26.4440	118.2610	494.46	928.17
	8 Hour Post-dose	20	680.250	656.380	21.0371	94.0806	548.69	916.58
	12 Hour Post-dose	20	681.150	672.885	23.5470	105.3054	531.41	930.33
	24 Hour Post-dose	20	655.620	646.800	25.7648	115.2236	489.43	941.43
00mg+Placebo	Pre-dose	20	696.126	709.440	27.6311	123.5702	447.11	933.40
	0.5 Hour Post-dose	20	715.074	727.940	28.7090	128.3907	435.65	914.00
	1.5 Hour Post-dose	20	683.142	703.395	29.7792	133.1768	413.29	1033.73
	2.5 Hour Post-dose	20	669.888	702.750	25.9449	116.0291	440.77	805.95
	4 Hour Post-dose	20	650.185	629.670	21.0422	94.1037	499.32	789.82
	6 Hour Post-dose	19	697.877	731.880	29.3235	127.8181	385.58	878.47
	8 Hour Post-dose	20	657.412	673.380	22.8178	102.0442	479.87	842.63
	12 Hour Post-dose	20	653.351	651.085	29.2469	130.7963	424.87	889.35
	24 Hour Post-dose	20	635.768	639.690	20.6937	92.5450	388.30	817.95

60mg+Ethanol	Pre-dose	20	690.148	652.335	26.6817	119.3243	518.05	883.95
	0.5 Hour Post-dose	19	685.599	652.700	26.5199	115.5977	535.24	931.76
	1.5 Hour Post-dose	20	691.149	704.990	26.0891	116.6742	472.45	982.00
	2.5 Hour Post-dose	20	711.856	700.440	31.4756	140.7632	508.05	1077.23
	4 Hour Post-dose	20	685.707	672.750	23.6244	105.6517	495.77	858.00
	6 Hour Post-dose	20	691.546	704.935	25.2207	112.7904	483.87	933.00
	8 Hour Post-dose	20	664.774	670.195	26.8543	120.0959	466.22	835.20
	12 Hour Post-dose	20	678.474	642.840	35.2515	157.6495	416.61	1041.29
	24 Hour Post-dose	20	659.403	644.170	22.7689	101.8254	473.94	882.76
60mg+Placebo	Pre-dose	20	686.355	677.425	25.8123	115.4359	474.00	950.14
	0.5 Hour Post-dose	19	672.407	678.420	21.1695	92.2756	419.06	782.68
	1.5 Hour Post-dose	20	674.572	676.035	23.2932	104.1703	394.31	865.41
	2.5 Hour Post-dose	20	677.901	676.110	22.5376	100.7912	491.29	906.24
	4 Hour Post-dose	20	668.234	669.295	25.6499	114.7097	413.27	848.62
	6 Hour Post-dose	20	693.176	693.525	18.2361	81.5545	527.00	860.19
	8 Hour Post-dose	20	684.574	688.570	22.6790	101.4234	474.69	862.68
	12 Hour Post-dose	20	658.047	659.410	20.9324	93.6126	495.69	829.96
	24 Hour Post-dose	20	655.404	642.615	21.9351	98.0966	504.89	824.85

Summary Statistics of CDR Data

Tracking - Average Distance From Target (mm)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	16.297	16.325	0.4303	1.9243	13.24	21.79
	0.5 Hour Post-dose	20	16.904	16.695	0.6447	2.8831	12.98	25.69
	1.5 Hour Post-dose	20	16.889	16.675	0.5055	2.2607	13.74	21.28
	2.5 Hour Post-dose	20	17.413	16.745	0.5756	2.5741	13.52	22.79
	4 Hour Post-dose	20	16.436	16.050	0.5188	2.3204	13.33	21.03
	6 Hour Post-dose	20	17.198	17.490	0.4996	2.2344	12.26	20.76
	8 Hour Post-dose	20	16.746	16.325	0.5212	2.3309	13.83	20.97
	12 Hour Post-dose	20	16.910	16.355	0.5972	2.6708	12.79	22.26
	24 Hour Post-dose	20	16.074	15.525	0.5750	2.5717	12.51	23.47
00mg+Placebo	Pre-dose	20	17.090	16.510	0.5360	2.3972	13.41	21.34
	0.5 Hour Post-dose	20	17.118	16.940	0.5756	2.5740	13.78	22.95
	1.5 Hour Post-dose	20	17.234	16.610	0.7032	3.1450	13.22	25.93
	2.5 Hour Post-dose	20	17.389	16.880	0.5719	2.5578	13.79	22.83
	4 Hour Post-dose	20	16.738	16.655	0.3925	1.7554	13.60	20.53
	6 Hour Post-dose	19	17.310	16.440	0.7111	3.0997	13.68	27.50
	8 Hour Post-dose	20	17.247	16.580	0.7945	3.5531	12.81	28.66
	12 Hour Post-dose	20	16.847	16.175	0.5300	2.3700	13.52	22.14
	24 Hour Post-dose	20	16.743	16.360	0.3758	1.6806	14.36	20.84

60mg+Ethanol	Pre-dose	20	16.533	16.240	0.6422	2.8721	12.31	25.86
	0.5 Hour Post-dose	19	16.819	17.280	0.4226	1.8421	13.79	19.93
	1.5 Hour Post-dose	20	18.009	17.835	0.6433	2.8770	14.55	24.78
	2.5 Hour Post-dose	20	17.400	17.515	0.5516	2.4669	12.95	22.47
	4 Hour Post-dose	20	16.738	16.250	0.5429	2.4281	13.53	23.53
	6 Hour Post-dose	20	16.717	16.485	0.4889	2.1863	12.47	21.09
	8 Hour Post-dose	20	16.750	16.575	0.5256	2.3506	13.14	20.59
	12 Hour Post-dose	20	17.011	16.370	0.6568	2.9375	13.38	25.80
	24 Hour Post-dose	20	16.207	16.525	0.4191	1.8744	12.72	20.00
60mg+Placebo	Pre-dose	20	16.500	15.940	0.4602	2.0579	12.38	20.04
	0.5 Hour Post-dose	19	16.857	16.830	0.4748	2.0697	14.28	22.24
	1.5 Hour Post-dose	20	17.027	16.805	0.4654	2.0814	13.50	22.09
	2.5 Hour Post-dose	20	16.996	16.890	0.4123	1.8440	14.03	20.26
	4 Hour Post-dose	20	16.599	16.340	0.4895	2.1893	12.21	20.95
	6 Hour Post-dose	20	16.633	16.430	0.4561	2.0399	13.47	20.45
	8 Hour Post-dose	20	16.585	16.550	0.5347	2.3913	13.28	22.26
	12 Hour Post-dose	20	16.843	16.050	0.5380	2.4059	12.62	22.05
	24 Hour Post-dose	20	16.452	16.190	0.4610	2.0615	13.45	20.16

Summary Statistics of CDR Data

DSST Score - Number Correct (#)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	71.900	72.500	2.9009	12.9733	44.00	100.00
	0.5 Hour Post-dose	20	74.000	75.000	2.9110	13.0182	47.00	100.00
	1.5 Hour Post-dose	20	74.400	73.500	2.9221	13.0682	50.00	100.00
	2.5 Hour Post-dose	20	75.050	74.500	2.6031	11.6415	52.00	98.00
	4 Hour Post-dose	20	75.350	76.000	2.9391	13.1440	48.00	100.00
	6 Hour Post-dose	20	74.850	74.500	2.5806	11.5407	50.00	100.00
	8 Hour Post-dose	20	75.750	76.500	2.6387	11.8004	54.00	100.00
	12 Hour Post-dose	20	74.450	75.000	2.4788	11.0857	54.00	100.00
	24 Hour Post-dose	20	76.650	77.000	2.5734	11.5087	49.00	100.00
00mg+Placebo	Pre-dose	20	72.500	71.500	2.5397	11.3578	49.00	100.00
	0.5 Hour Post-dose	20	73.100	74.000	2.8069	12.5526	49.00	100.00
	1.5 Hour Post-dose	20	75.100	73.500	2.5078	11.2151	55.00	100.00
	2.5 Hour Post-dose	20	75.250	74.500	2.8181	12.6027	52.00	100.00
	4 Hour Post-dose	20	75.050	76.000	2.4596	10.9999	56.00	100.00
	6 Hour Post-dose	19	75.105	73.000	2.4755	10.7904	56.00	100.00
	8 Hour Post-dose	20	75.350	75.000	2.7637	12.3598	53.00	100.00
	12 Hour Post-dose	20	75.650	74.500	2.6420	11.8156	51.00	100.00
	24 Hour Post-dose	20	77.750	74.500	2.5648	11.4702	56.00	100.00

60mg+Ethanol	Pre-dose	20	71.850	74.000	3.0438	13.6122	48.00	96.00
	0.5 Hour Post-dose	19	73.105	69.000	2.8438	12.3958	50.00	92.00
	1.5 Hour Post-dose	20	72.350	71.000	3.0195	13.5035	46.00	93.00
	2.5 Hour Post-dose	20	72.600	71.000	2.6954	12.0543	55.00	96.00
	4 Hour Post-dose	20	74.300	72.000	2.7425	12.2651	57.00	95.00
	6 Hour Post-dose	20	73.300	72.500	2.8145	12.5870	51.00	98.00
	8 Hour Post-dose	20	74.100	73.500	2.7643	12.3625	55.00	94.00
	12 Hour Post-dose	20	73.850	75.500	2.8006	12.5248	54.00	91.00
	24 Hour Post-dose	20	76.900	75.500	2.9063	12.9976	52.00	100.00
60mg+Placebo	Pre-dose	20	72.550	71.000	2.8933	12.9390	52.00	95.00
	0.5 Hour Post-dose	19	73.684	73.000	2.7231	11.8699	56.00	93.00
	1.5 Hour Post-dose	20	74.050	70.500	2.5469	11.3901	58.00	96.00
	2.5 Hour Post-dose	20	74.450	74.000	2.6011	11.6324	59.00	96.00
	4 Hour Post-dose	20	72.950	72.000	2.5459	11.3855	53.00	95.00
	6 Hour Post-dose	20	72.400	71.500	2.7071	12.1065	56.00	96.00
	8 Hour Post-dose	20	74.800	73.000	2.6917	12.0377	56.00	96.00
	12 Hour Post-dose	20	74.850	74.500	2.7070	12.1060	59.00	100.00
	24 Hour Post-dose	20	77.000	78.000	2.9299	13.1028	51.00	100.00

Summary Statistics of CDR Data

Self-Rated Alertness (mm)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	57.106	54.780	3.4165	15.2789	30.56	90.33
	0.5 Hour Post-dose	20	53.433	51.945	3.4222	15.3047	25.33	88.56
	1.5 Hour Post-dose	20	45.684	44.665	2.2797	10.1951	23.00	59.89
	2.5 Hour Post-dose	20	46.289	46.110	3.1194	13.9503	16.56	70.44
	4 Hour Post-dose	20	50.395	49.275	3.4542	15.4478	24.33	81.11
	6 Hour Post-dose	20	51.183	51.890	2.6691	11.9368	31.11	79.22
	8 Hour Post-dose	20	52.809	50.165	2.5909	11.5870	38.44	85.00
	12 Hour Post-dose	20	53.833	53.445	2.7893	12.4740	30.11	82.89
	24 Hour Post-dose	20	60.245	59.055	3.1120	13.9171	25.67	94.33
00mg+Placebo	Pre-dose	20	59.995	61.500	2.8094	12.5640	31.78	78.56
	0.5 Hour Post-dose	20	52.484	52.170	2.9931	13.3857	29.11	76.22
	1.5 Hour Post-dose	20	52.990	50.670	2.9929	13.3845	29.78	80.00
	2.5 Hour Post-dose	20	50.784	49.555	3.0795	13.7717	30.11	77.67
	4 Hour Post-dose	20	52.944	51.610	3.0004	13.4184	26.78	81.33
	6 Hour Post-dose	19	51.720	53.000	3.3676	14.6792	17.22	80.67
	8 Hour Post-dose	20	52.205	52.610	2.5409	11.3633	27.22	72.89
	12 Hour Post-dose	20	53.084	51.115	2.5928	11.5952	37.56	76.78
	24 Hour Post-dose	20	57.884	58.055	2.8923	12.9350	38.56	83.00

60mg+Ethanol	Pre-dose	20	60.700	57.390	2.6208	11.7204	42.00	91.22
	0.5 Hour Post-dose	19	48.929	45.890	4.0529	17.6663	12.78	78.78
	1.5 Hour Post-dose	20	40.051	40.835	2.4703	11.0473	15.22	64.67
	2.5 Hour Post-dose	20	42.975	42.225	2.9882	13.3636	19.33	68.33
	4 Hour Post-dose	20	47.011	48.500	2.7677	12.3773	25.22	81.78
	6 Hour Post-dose	19	49.274	50.780	2.8816	12.5606	25.22	68.89
	8 Hour Post-dose	20	52.186	50.000	2.7269	12.1951	24.38	76.56
	12 Hour Post-dose	20	51.317	50.165	3.1948	14.2876	24.56	82.22
	24 Hour Post-dose	20	62.662	60.945	2.6151	11.6951	39.56	85.78
60mg+Placebo	Pre-dose	20	57.950	57.445	2.7916	12.4846	33.44	83.00
	0.5 Hour Post-dose	20	51.185	50.500	3.2254	14.4245	26.78	82.22
	1.5 Hour Post-dose	20	40.128	40.000	2.6667	11.9257	18.22	68.89
	2.5 Hour Post-dose	20	44.923	43.890	2.9989	13.4115	23.56	71.44
	4 Hour Post-dose	20	50.484	48.500	3.0697	13.7282	20.78	81.89
	6 Hour Post-dose	20	51.834	52.775	3.0882	13.8107	26.22	84.00
	8 Hour Post-dose	20	50.767	53.610	2.9003	12.9704	29.22	79.67
	12 Hour Post-dose	20	49.794	50.280	3.5834	16.0255	14.33	84.78
	24 Hour Post-dose	20	59.122	58.225	3.1532	14.1015	20.44	85.11

Summary Statistics of CDR Data

Self-Rated Contentment (mm)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	64.240	62.700	2.4684	11.0388	46.60	87.60
	0.5 Hour Post-dose	20	64.090	62.100	2.7045	12.0950	52.00	90.20
	1.5 Hour Post-dose	20	67.720	67.700	2.5892	11.5792	49.60	87.80
	2.5 Hour Post-dose	20	65.280	64.200	2.5426	11.3710	48.40	83.80
	4 Hour Post-dose	20	63.383	58.925	2.6007	11.6307	48.40	83.80
	6 Hour Post-dose	20	65.210	62.300	2.5043	11.1995	50.20	86.60
	8 Hour Post-dose	20	66.540	63.400	2.4928	11.1480	50.80	86.80
	12 Hour Post-dose	20	65.730	63.800	2.2952	10.2644	49.80	88.20
	24 Hour Post-dose	20	67.390	64.900	2.6403	11.8077	49.40	97.20
00mg+Placebo	Pre-dose	20	67.320	66.600	2.5771	11.5251	49.40	89.40
	0.5 Hour Post-dose	20	64.280	61.600	2.7569	12.3292	49.80	90.60
	1.5 Hour Post-dose	20	65.240	61.600	2.6175	11.7060	48.20	90.20
	2.5 Hour Post-dose	20	65.970	64.000	2.5393	11.3563	51.40	89.20
	4 Hour Post-dose	20	66.460	64.500	2.9826	13.3385	49.80	88.20
	6 Hour Post-dose	19	64.632	59.000	2.7137	11.8288	49.80	87.60
	8 Hour Post-dose	20	64.550	64.400	2.1399	9.5701	48.80	83.20
	12 Hour Post-dose	20	67.180	67.300	2.4510	10.9613	49.60	88.80
	24 Hour Post-dose	20	68.790	67.700	2.8405	12.7032	49.20	88.60

60mg+Ethanol	Pre-dose	20	67.620	66.500	2.6445	11.8266	48.60	94.00
	0.5 Hour Post-dose	19	64.097	62.000	3.3845	14.7526	43.80	90.20
	1.5 Hour Post-dose	20	63.660	60.600	3.6279	16.2246	33.20	91.80
	2.5 Hour Post-dose	20	64.230	59.900	3.3081	14.7941	45.60	97.00
	4 Hour Post-dose	20	64.420	63.300	2.8231	12.6253	49.80	93.60
	6 Hour Post-dose	19	65.579	61.600	2.5298	11.0270	52.80	88.00
	8 Hour Post-dose	20	65.060	61.600	2.4918	11.1437	50.40	85.20
	12 Hour Post-dose	20	64.630	63.000	3.0846	13.7948	35.40	87.20
	24 Hour Post-dose	20	68.270	66.300	2.4656	11.0267	51.60	92.60
60mg+Placebo	Pre-dose	20	66.180	66.400	2.6413	11.8122	45.80	85.60
	0.5 Hour Post-dose	20	62.070	60.500	2.8910	12.9290	43.00	89.00
	1.5 Hour Post-dose	20	58.480	56.600	2.5908	11.5863	31.40	81.00
	2.5 Hour Post-dose	20	60.360	61.000	2.7004	12.0764	28.80	79.40
	4 Hour Post-dose	20	64.190	62.500	2.8800	12.8796	37.00	87.40
	6 Hour Post-dose	20	62.820	62.200	2.5071	11.2120	44.00	85.20
	8 Hour Post-dose	20	63.560	62.900	2.6436	11.8225	37.40	86.80
	12 Hour Post-dose	20	62.760	62.300	2.7980	12.5128	36.60	87.40
	24 Hour Post-dose	20	67.100	65.800	2.4366	10.8967	49.80	87.60

Summary Statistics of CDR Data

Self-Rated Calmness (mm)

Dosage	Time of Assessment	N	Mean	Median	S.E. Mean	Standard Deviation	Minimum	Maximum
00mg+Ethanol	Pre-dose	20	66.450	66.500	2.8011	12.5267	46.00	98.00
	0.5 Hour Post-dose	20	62.750	60.000	3.4659	15.5000	28.50	95.00
	1.5 Hour Post-dose	20	68.425	64.250	3.2655	14.6038	46.00	96.50
	2.5 Hour Post-dose	20	68.850	67.250	2.9087	13.0082	45.00	98.00
	4 Hour Post-dose	20	70.650	70.750	2.7566	12.3279	52.00	94.50
	6 Hour Post-dose	20	67.550	64.750	2.9346	13.1238	48.50	93.00
	8 Hour Post-dose	20	68.575	68.250	3.0349	13.5727	46.50	93.50
	12 Hour Post-dose	20	68.025	65.250	2.2122	9.8935	54.50	93.00
	24 Hour Post-dose	20	62.175	60.000	2.7761	12.4153	36.50	95.00
00mg+Placebo	Pre-dose	20	67.025	66.500	2.9889	13.3668	43.50	97.50
	0.5 Hour Post-dose	20	65.675	65.500	2.8645	12.8106	49.50	97.50
	1.5 Hour Post-dose	20	66.575	63.000	2.6324	11.7723	52.00	99.50
	2.5 Hour Post-dose	20	67.775	64.250	2.8825	12.8907	48.00	99.00
	4 Hour Post-dose	20	67.900	64.250	2.7624	12.3540	47.50	98.00
	6 Hour Post-dose	19	68.132	64.500	2.6416	11.5143	50.00	97.50
	8 Hour Post-dose	20	64.575	63.000	3.1802	14.2222	33.50	98.50
	12 Hour Post-dose	20	69.525	67.750	2.5586	11.4426	51.50	98.00
	24 Hour Post-dose	20	65.625	63.250	3.7810	16.9091	14.00	99.50

60mg+Ethanol	Pre-dose	20	64.450	64.750	2.9762	13.3100	42.00	90.50
	0.5 Hour Post-dose	19	63.395	65.500	3.1474	13.7190	39.00	95.00
	1.5 Hour Post-dose	20	68.475	70.250	3.8778	17.3421	30.50	98.00
	2.5 Hour Post-dose	20	72.175	69.750	3.0466	13.6249	56.50	100.00
	4 Hour Post-dose	20	67.050	61.750	3.2564	14.5628	48.50	94.50
	6 Hour Post-dose	19	67.711	63.500	2.9403	12.8163	50.50	95.50
	8 Hour Post-dose	20	66.650	64.500	2.6943	12.0494	52.50	98.50
	12 Hour Post-dose	20	67.300	66.750	3.3932	15.1748	28.50	98.50
	24 Hour Post-dose	20	64.925	64.000	3.2772	14.6559	41.00	99.00
60mg+Placebo	Pre-dose	20	65.800	66.250	2.1644	9.6796	47.50	88.50
	0.5 Hour Post-dose	20	65.300	65.500	2.8206	12.6141	47.50	95.50
	1.5 Hour Post-dose	20	67.125	64.250	3.3445	14.9568	43.00	97.50
	2.5 Hour Post-dose	20	64.875	60.750	3.3441	14.9551	43.50	100.00
	4 Hour Post-dose	20	63.050	61.000	2.9696	13.2803	38.50	97.50
	6 Hour Post-dose	20	64.975	63.750	2.9068	12.9995	43.00	95.50
	8 Hour Post-dose	20	67.100	64.500	2.5832	11.5526	54.00	98.50
	12 Hour Post-dose	20	68.125	69.500	2.6170	11.7034	50.00	98.50
	24 Hour Post-dose	20	60.725	60.500	3.0991	13.8597	38.00	99.50

22 Appendix 4: Summary Statistics for Chapter 11

22.1 Part 1 – Physostimine

Analysis Variable : SIMPLE REACTION TIME

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	223.2	12	6.855	23.74
	2	12	228.9	12	6.229	21.58
	3	12	269	12	10.67	36.97
	4.5	12	275.2	12	17.76	61.51
Placebo	-1	12	228.6	12	7.587	26.28
	2	12	295.3	12	21.14	73.22
	3	12	281.3	12	17.09	59.2
	4.5	12	261.4	12	12.85	44.5

Analysis Variable : CHOICE REACTION TIME

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	413.2	12	9.833	34.06
	2	12	416.8	12	13.01	45.08
	3	12	434.6	12	15.01	51.99
	4.5	12	441.2	12	15.7	54.37
Placebo	-1	12	420.5	12	10.24	35.47
	2	12	458.8	12	20.22	70.04
	3	12	455.9	12	19.2	66.53
	4.5	12	460.8	12	19.26	66.72

Analysis Variable : CHOICE REACTION TIME - ACCURACY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	96.5	12	0.657	2.276
	2	12	95.33	12	0.791	2.741
	3	12	93.5	12	0.657	2.276
	4.5	12	93.5	12	0.557	1.931
Placebo	-1	12	95.83	12	0.575	1.992
	2	12	92.17	12	1.058	3.664
	3	12	93.67	12	0.98	3.393
	4.5	12	94	12	1.101	3.814

Analysis Variable : DIGIT VIGILANCE - CORRECT DETECTIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	99.45	12	0.29	1.004
	2	12	98.33	12	0.73	2.529
	3	12	96.85	12	1.353	4.688
	4.5	12	95.37	12	1.143	3.96
Placebo	-1	12	98.52	12	0.741	2.566
	2	12	93.33	12	2.337	8.096
	3	12	93.7	12	1.443	5
	4.5	12	92.78	12	1.575	5.456

Analysis Variable : DIGIT VIGILANCE - SPEED OF DETECTIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	388.2	12	8.461	29.31
	2	12	399.7	12	10.7	37.07
	3	12	410.5	12	12.98	44.97
	4.5	12	424.9	12	13.37	46.33
Placebo	-1	12	396.8	12	15.17	52.54
	2	12	438.6	12	19.03	65.91
	3	12	434.3	12	12.19	42.21
	4.5	12	441	12	16.66	57.72

Analysis Variable : DIGIT VIGILANCE - FALSE ALARMS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.75	12	0.25	0.866
	2	12	1.083	12	0.336	1.165
	3	12	1.333	12	0.376	1.303
	4.5	12	2	12	0.522	1.809
Placebo	-1	12	1	12	0.275	0.953
	2	12	2.5	12	0.557	1.931
	3	12	2.417	12	0.723	2.503
	4.5	12	2	12	0.389	1.348

Analysis Variable : NUMERIC WORKING MEMORY - SENSITIVITY INDEX

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.943	12	0.012	0.041
	2	12	0.887	12	0.02	0.069
	3	12	0.819	12	0.023	0.08
	4.5	12	0.869	12	0.031	0.108
Placebo	-1	12	0.918	12	0.017	0.06
	2	12	0.815	12	0.028	0.098
	3	12	0.806	12	0.038	0.132
	4.5	12	0.852	12	0.023	0.081

Analysis Variable : NUMERIC WORKING MEMORY - SPEED

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	592.3	12	20.83	72.17
	2	12	588	12	26.87	93.07
	3	12	650.6	12	34.65	120
	4.5	12	660.1	12	36.05	124.9
Placebo	-1	12	596.5	12	22.73	78.73
	2	12	689.3	12	32.13	111.3
	3	12	710.3	12	45.8	158.7
	4.5	12	711.6	12	50.56	175.1

Analysis Variable : IMMEDIATE WORD RECALL - ACCURACY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	43.06	12	2.511	8.699
	2	12	40	12	3.204	11.1
	3	12	39.44	12	3.596	12.46
	4.5	12	32.78	12	3.596	12.46
Placebo	-1	12	41.67	12	2.857	9.898
	2	12	29.17	12	2.962	10.26
	3	12	28.89	12	2.439	8.447
	4.5	12	39.44	12	3.995	13.84

Analysis Variable : IMMEDIATE WORD RECALL - INTRUSIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0	12	0	0
	2	12	0	12	0	0
	3	12	0.083	12	0.083	0.289
	4.5	12	0	12	0	0
Placebo	-1	12	0	12	0	0
	2	12	0.083	12	0.083	0.289
	3	12	0.167	12	0.112	0.389
	4.5	12	0	12	0	0

Analysis Variable : IMMEDIATE WORD RECALL - ERRORS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.333	12	0.142	0.492
	2	12	0.5	12	0.23	0.798
	3	12	0.25	12	0.131	0.452
	4.5	12	0.5	12	0.289	1
Placebo	-1	12	0.167	12	0.112	0.389
	2	12	0.667	12	0.188	0.651
	3	12	0.333	12	0.142	0.492
	4.5	12	0.167	12	0.112	0.389

Analysis Variable : DELAYED WORD RECALL - ACCURACY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	32.22	12	2.54	8.799
	2	12	17.78	12	3.316	11.49
	3	12	13.06	12	2.189	7.582
	4.5	12	9.167	12	2.397	8.302
Placebo	-1	12	30.56	12	4.221	14.62
	2	12	10.56	12	3.329	11.53
	3	12	8.612	12	2.477	8.582
	4.5	12	15.28	12	3.444	11.93

Analysis Variable : DELAYED WORD RECALL - INTRUSIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0	12	0	0
	2	12	0.333	12	0.188	0.651
	3	12	0.917	12	0.358	1.24
	4.5	12	0.75	12	0.329	1.138
Placebo	-1	12	0	12	0	0
	2	12	0.5	12	0.261	0.905
	3	12	0.833	12	0.322	1.115
	4.5	12	0.583	12	0.193	0.669

Analysis Variable : DELAYED WORD RECALL - ERRORS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.167	12	0.112	0.389
	2	12	0.417	12	0.193	0.669
	3	12	0.417	12	0.149	0.515
	4.5	12	0.5	12	0.151	0.522
Placebo	-1	12	0.083	12	0.083	0.289
	2	12	0.25	12	0.131	0.452
	3	12	0.083	12	0.083	0.289
	4.5	12	0.417	12	0.193	0.669

Analysis Variable : WORD RECOGNITION - SENSITIVITY INDEX

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.557	12	0.065	0.225
	2	12	0.559	12	0.059	0.206
	3	12	0.481	12	0.046	0.159
	4.5	12	0.566	12	0.05	0.174
Placebo	-1	12	0.61	12	0.056	0.192
	2	12	0.44	12	0.051	0.175
	3	12	0.513	12	0.066	0.228
	4.5	12	0.51	12	0.056	0.194

Analysis Variable : WORD RECOGNITION - SPEED

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	696.1	12	34.24	118.6
	2	12	675.8	12	28.83	99.86
	3	12	708.5	12	37.6	130.3
	4.5	12	777.6	12	44.41	153.8
Placebo	-1	12	677	12	34.65	120
	2	12	773.6	12	35.89	124.3
	3	12	746.4	12	35.8	124
	4.5	12	707.7	12	18.88	65.39

Analysis Variable : PICTURE RECOGNITION - SENSITIVITY INDEX

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	0.796	12	0.047	0.164
	2	12	0.694	12	0.05	0.172
	3	12	0.69	12	0.052	0.18
	4.5	12	0.676	12	0.061	0.212
Placebo	-1	12	0.76	12	0.054	0.187
	2	12	0.643	12	0.044	0.152
	3	12	0.71	12	0.053	0.183
	4.5	12	0.679	12	0.029	0.102

Analysis Variable : PICTURE RECOGNITION - SPEED

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	755.8	12	30.86	106.9
	2	12	711.5	12	21.83	75.61
	3	12	784.6	12	34.28	118.8
	4.5	12	810.7	12	38.9	134.8
Placebo	-1	12	724.8	12	22.08	76.5
	2	12	850.5	12	45.34	157.1
	3	12	827.8	12	48.62	168.4
	4.5	12	819.6	12	64.1	222

Analysis Variable : CONTINUITY OF ATTENTION

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	92.25	12	0.351	1.216
	2	12	90.83	12	0.52	1.801
	3	12	89	12	0.779	2.698
	4.5	12	87.67	12	0.856	2.965
Placebo	-1	12	91.25	12	0.664	2.301
	2	12	85.58	12	1.617	5.6
	3	12	86.58	12	1.221	4.231
	4.5	12	86.75	12	0.88	3.049

Analysis Variable : POWER OF ATTENTION

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	1025	12	20.45	70.85
	2	12	1045	12	27.01	93.55
	3	12	1114	12	34.01	117.8
	4.5	12	1141	12	40.24	139.4
Placebo	-1	12	1046	12	29.63	102.7
	2	12	1193	12	58.52	202.7
	3	12	1172	12	44.37	153.7
	4.5	12	1163	12	45.65	158.1

Analysis Variable : QUALITY OF SECONDARY MEMORY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	201.7	12	11.47	39.74
	2	12	166.9	12	12.36	42.8
	3	12	151.1	12	10.15	35.16
	4.5	12	148.3	12	11.38	39.43
Placebo	-1	12	199.3	12	10.95	37.95
	2	12	132.1	12	12	41.55
	3	12	143.9	12	12.02	41.64
	4.5	12	157.1	12	11.56	40.03

Analysis Variable : SPEED OF MEMORY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	2044	12	74.53	258.2
	2	12	1975	12	65.32	226.3
	3	12	2144	12	93.14	322.7
	4.5	12	2248	12	111	384.6
Placebo	-1	12	1998	12	72.32	250.5
	2	12	2313	12	97.26	336.9
	3	12	2284	12	116.9	404.8
	4.5	12	2239	12	120.3	416.8

Analysis Variable : SELF-RATED ALERTNESS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	64.42	12	5.296	18.35
	2	12	52.95	12	4.58	15.87
	3	12	48.83	12	3.5	12.13
	4.5	12	47.41	12	3.274	11.34
Placebo	-1	12	60.18	12	4.03	13.96
	2	12	39.15	12	4.073	14.11
	3	12	42.46	12	4.157	14.4
	4.5	12	48.72	12	4.136	14.33

Analysis Variable : SELF-RATED CALMNESS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	65.13	12	5.082	17.6
	2	12	68.08	12	3.995	13.84
	3	12	60.79	12	5.91	20.47
	4.5	12	65.38	12	4.368	15.13
Placebo	-1	12	68.29	12	4.975	17.23
	2	12	66.63	12	5.64	19.54
	3	12	61.46	12	5.902	20.45
	4.5	12	65.21	12	4.866	16.86

Analysis Variable : SELF-RATED CONTENTMENT

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Phys 1.5mg	-1	12	65.82	12	5.025	17.41
	2	12	64.62	12	4.309	14.93
	3	12	60.8	12	3.627	12.57
	4.5	12	63.63	12	3.42	11.85
Placebo	-1	12	69.4	12	3.459	11.98
	2	12	63.75	12	3.271	11.33
	3	12	60.85	12	4.068	14.09
	4.5	12	65.92	12	3.582	12.41

22.2 Part 2A - Active 75 mg

Analysis Variable : SIMPLE REACTION TIME

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	242.7	18	7.898	33.51
		1.5	18	319.6	18	14.1	59.81
		2.5	18	335.4	18	19.03	80.74
		4	18	317.1	18	18.16	77.06
		6	18	287.7	18	12.82	54.38
Active	75mg	-1	18	234.1	18	6.713	28.48
		1.5	18	314.2	18	15.02	63.73
		2.5	18	322.7	18	13.25	56.22
		4	18	323.4	18	21.05	89.31
		6	18	294.2	18	15.91	67.49

Analysis Variable : CHOICE REACTION TIME

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	443.2	18	18.95	80.41
		1.5	18	495.7	18	20.21	85.73
		2.5	18	527.9	18	29.52	125.2
		4	18	499.8	18	23.81	101
		6	18	467.7	18	20.6	87.39
Active	75mg	-1	18	428.8	18	12.36	52.43
		1.5	18	502.1	18	20.67	87.68
		2.5	18	494.5	18	20.17	85.58
		4	18	493.5	18	23.67	100.4
		6	18	473.6	18	20.5	86.96

Analysis Variable : CHOICE REACTION TIME - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	95.78	18	0.703	2.981
		1.5	18	94.22	18	0.684	2.901
		2.5	18	93.89	18	0.863	3.66
		4	18	93.33	18	0.97	4.116
		6	18	93.33	18	0.808	3.43
Active	75mg	-1	18	96.22	18	0.79	3.353
		1.5	18	92.22	18	0.969	4.11
		2.5	18	94	18	0.808	3.43
		4	18	92.67	18	1.21	5.134
		6	18	93.67	18	0.672	2.849

Analysis Variable : DIGIT VIGILANCE - CORRECT DETECTIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	95.68	18	1.07	4.54
		1.5	18	88.4	18	2.284	9.688
		2.5	18	85.31	18	2.87	12.18
		4	18	85.68	18	2.842	12.06
		6	18	89.14	18	2.272	9.639
Active	75mg	-1	18	96.3	18	0.968	4.106
		1.5	18	87.41	18	2.049	8.692
		2.5	18	85.93	18	2.823	11.98
		4	18	86.17	18	2.71	11.5
		6	18	88.64	18	2.534	10.75

Analysis Variable : DIGIT VIGILANCE - SPEED OF DETECTIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	406.3	18	10.33	43.83
		1.5	18	452	18	11.3	47.95
		2.5	18	453.2	18	14.09	59.79
		4	18	440.4	18	12.66	53.72
		6	18	427.2	18	10.2	43.28
Active	75mg	-1	18	406.2	18	11.46	48.63
		1.5	18	442.6	18	13.41	56.87
		2.5	18	446.3	18	12.72	53.96
		4	18	438.5	18	12.43	52.74
		6	18	421.3	18	11.45	48.57

Analysis Variable : DIGIT VIGILANCE - FALSE ALARMS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	1.111	18	0.29	1.231
		1.5	18	1.722	18	0.419	1.776
		2.5	18	2.611	18	0.444	1.883
		4	18	1.667	18	0.352	1.495
		6	18	1.944	18	0.439	1.862
Active	75mg	-1	18	0.778	18	0.207	0.878
		1.5	18	1.889	18	0.312	1.323
		2.5	18	2.889	18	0.723	3.066
		4	18	2.444	18	1.039	4.409
		6	18	1.556	18	0.326	1.381

Analysis Variable : NUMERIC WORKING MEMORY - SENSITIVITY INDEX

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.913	18	0.019	0.079
		1.5	18	0.754	18	0.046	0.194
		2.5	18	0.77	18	0.041	0.175
		4	18	0.825	18	0.043	0.183
		6	18	0.864	18	0.025	0.104
Active	75mg	-1	18	0.888	18	0.022	0.093
		1.5	18	0.771	18	0.052	0.219
		2.5	18	0.778	18	0.037	0.157
		4	18	0.838	18	0.036	0.153
		6	18	0.853	18	0.033	0.139

Analysis Variable : NUMERIC WORKING MEMORY - SPEED

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	590.2	18	24.83	105.4
		1.5	18	697.6	18	28.8	122.2
		2.5	18	704.9	18	31.68	134.4
		4	18	739.7	18	50.72	215.2
		6	18	664.8	18	27.6	117.1
Active	75mg	-1	18	580.9	18	22.7	96.32
		1.5	18	718.4	18	31.12	132
		2.5	18	739	18	41.64	176.7
		4	18	694.8	18	32.54	138.1
		6	18	695.2	18	41.7	176.9

Analysis Variable : IMMEDIATE WORD RECALL - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	42.96	18	3.429	14.55
		1.5	18	27.78	18	2.222	9.428
		2.5	18	28.15	18	2.258	9.58
		4	18	31.11	18	2.952	12.52
		6	18	37.41	18	1.858	7.884
Active	75mg	-1	18	42.22	18	2.585	10.97
		1.5	18	32.41	18	1.995	8.464
		2.5	18	32.59	18	2.688	11.41
		4	18	32.04	18	1.686	7.152
		6	18	30	18	2.286	9.7

Analysis Variable : IMMEDIATE WORD RECALL - INTRUSIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0	18	0	0
		1.5	18	0.167	18	0.121	0.514
		2.5	18	0.056	18	0.056	0.236
		4	18	0.167	18	0.121	0.514
		6	18	0	18	0	0
Active	75mg	-1	18	0	18	0	0
		1.5	18	0.111	18	0.076	0.323
		2.5	18	0.111	18	0.076	0.323
		4	18	0	18	0	0
		6	18	0.111	18	0.076	0.323

Analysis Variable : IMMEDIATE WORD RECALL - ERRORS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.389	18	0.164	0.698
		1.5	18	0.222	18	0.129	0.548
		2.5	18	0.833	18	0.218	0.924
		4	18	0.778	18	0.191	0.808
		6	18	0.611	18	0.216	0.916
Active	75mg	-1	18	0.167	18	0.121	0.514
		1.5	18	0.278	18	0.158	0.669
		2.5	18	0.5	18	0.146	0.618
		4	18	0.444	18	0.166	0.705
		6	18	0.611	18	0.183	0.778

Analysis Variable : DELAYED WORD RECALL - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	29.63	18	3.365	14.27
		1.5	18	8.889	18	1.639	6.954
		2.5	18	7.037	18	1.825	7.745
		4	18	10.37	18	2.541	10.78
		6	18	11.11	18	1.924	8.164
Active	75mg	-1	18	34.26	18	3.046	12.93
		1.5	18	10.19	18	1.671	7.091
		2.5	18	12.78	18	2.226	9.446
		4	18	9.444	18	2.143	9.092
		6	18	9.814	18	2.31	9.8

Analysis Variable : DELAYED WORD RECALL - INTRUSIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.056	18	0.056	0.236
		1.5	18	1.111	18	0.332	1.41
		2.5	18	1.278	18	0.394	1.674
		4	18	1.333	18	0.464	1.97
		6	18	0.778	18	0.319	1.353
Active	75mg	-1	18	0	18	0	0
		1.5	18	1.167	18	0.316	1.339
		2.5	18	0.889	18	0.403	1.711
		4	18	1.389	18	0.572	2.429
		6	18	1	18	0.45	1.91

Analysis Variable : DELAYED WORD RECALL - ERRORS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.444	18	0.185	0.784
		1.5	18	0.611	18	0.216	0.916
		2.5	18	0.889	18	0.361	1.53
		4	18	0.667	18	0.256	1.085
		6	18	0.778	18	0.25	1.06
Active	75mg	-1	18	0.222	18	0.129	0.548
		1.5	18	0.556	18	0.166	0.705
		2.5	18	1	18	0.268	1.138
		4	18	0.944	18	0.249	1.056
		6	18	0.889	18	0.254	1.079

Analysis Variable : WORD RECOGNITION - SENSITIVITY INDEX

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.589	18	0.053	0.226
		1.5	18	0.394	18	0.048	0.202
		2.5	18	0.419	18	0.066	0.278
		4	18	0.419	18	0.045	0.189
		6	18	0.479	18	0.044	0.187
Active	75mg	-1	18	0.549	18	0.039	0.166
		1.5	18	0.384	18	0.053	0.224
		2.5	18	0.451	18	0.057	0.242
		4	18	0.415	18	0.056	0.237
		6	18	0.485	18	0.047	0.199

Analysis Variable : WORD RECOGNITION - SPEED

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	680.4	18	22.27	94.48
		1.5	18	781.6	18	32.77	139
		2.5	18	794.9	18	36.73	155.8
		4	18	776	18	44.93	190.6
		6	18	724.1	18	25.78	109.4
Active	75mg	-1	18	678.5	18	30.85	130.9
		1.5	18	738.4	18	27.83	118.1
		2.5	18	758.7	18	23.83	101.1
		4	18	723	18	24.1	102.2
		6	18	731.5	18	25.51	108.2

Analysis Variable : PICTURE RECOGNITION - SENSITIVITY INDEX

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.665	18	0.051	0.216
		1.5	18	0.578	18	0.045	0.189
		2.5	18	0.634	18	0.048	0.205
		4	18	0.618	18	0.05	0.213
		6	18	0.607	18	0.048	0.202
Active	75mg	-1	18	0.666	18	0.048	0.203
		1.5	18	0.584	18	0.058	0.245
		2.5	18	0.581	18	0.065	0.275
		4	18	0.537	18	0.062	0.261
		6	18	0.543	18	0.053	0.227

Analysis Variable : PICTURE RECOGNITION - SPEED

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	787.5	18	27.01	114.6
		1.5	18	864.6	18	34.91	148.1
		2.5	18	896.7	18	37.86	160.6
		4	18	910.6	18	42.13	178.7
		6	18	817.4	18	22.74	96.49
Active	75mg	-1	18	786.3	18	18.67	79.21
		1.5	18	852.6	18	32.4	137.5
		2.5	18	846.7	18	29.25	124.1
		4	18	828.9	18	31.91	135.4
		6	18	834	18	36.44	154.6

Analysis Variable : CONTINUITY OF ATTENTION

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	89.83	18	0.742	3.148
		1.5	18	85.17	18	1.435	6.09
		2.5	18	82.72	18	1.728	7.332
		4	18	83.56	18	1.41	5.983
		6	18	84.83	18	1.332	5.649
Active	75mg	-1	18	90.67	18	0.657	2.787
		1.5	18	83.56	18	1.269	5.382
		2.5	18	82.78	18	1.802	7.643
		4	18	82.67	18	2.124	9.01
		6	18	85.17	18	1.299	5.513

Analysis Variable : POWER OF ATTENTION

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	1092	18	31.98	135.7
		1.5	18	1267	18	39.43	167.3
		2.5	18	1316	18	56.3	238.9
		4	18	1257	18	48.66	206.4
		6	18	1183	18	38.49	163.3
Active	75mg	-1	18	1069	18	27.14	115.1
		1.5	18	1259	18	44.17	187.4
		2.5	18	1263	18	42.14	178.8
		4	18	1255	18	47.84	203
		6	18	1189	18	37.47	159

Analysis Variable : QUALITY OF SECONDARY MEMORY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	184.2	18	15.83	67.15
		1.5	18	113.3	18	6.59	27.96
		2.5	18	113.5	18	12.03	51.04
		4	18	118.9	18	12.02	51
		6	18	139.3	18	10.52	44.63
Active	75mg	-1	18	187.2	18	10.78	45.75
		1.5	18	122.9	18	10.9	46.25
		2.5	18	129.4	18	12.18	51.69
		4	18	112.7	18	11.42	48.44
		6	18	118.7	18	10.99	46.62

Analysis Variable : SPEED OF MEMORY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	2058	18	69.31	294.1
		1.5	18	2344	18	88.43	375.2
		2.5	18	2397	18	96.94	411.3
		4	18	2426	18	128.8	546.5
		6	18	2206	18	64.69	274.5
Active	75mg	-1	18	2046	18	65.48	277.8
		1.5	18	2309	18	77.3	328
		2.5	18	2344	18	80.03	339.6
		4	18	2247	18	77.39	328.3
		6	18	2261	18	82.34	349.3

Analysis Variable : SELF-RATED ALERTNESS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	76.58	18	3.162	13.41
		1.5	18	41.93	18	3.088	13.1
		2.5	18	46.39	18	3.611	15.32
		4	18	53.6	18	4.097	17.38
		6	18	61.18	18	4.354	18.47
Active	75mg	-1	18	79.17	18	3.34	14.17
		1.5	18	41.81	18	3.063	13
		2.5	18	48.49	18	3.848	16.33
		4	18	56.84	18	3.917	16.62
		6	18	60.97	18	3.255	13.81

Analysis Variable : SELF-RATED CALMNESS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	77.47	18	3.334	14.15
		1.5	18	71.28	18	4.483	19.02
		2.5	18	71.17	18	3.992	16.94
		4	18	68.56	18	3.436	14.58
		6	18	72.36	18	3.426	14.53
Active	75mg	-1	18	80.03	18	3.196	13.56
		1.5	18	73.33	18	5.114	21.7
		2.5	18	69.08	18	5.287	22.43
		4	18	69.94	18	3.712	15.75
		6	18	65.22	18	5.01	21.26

Analysis Variable : SELF-RATED CONTENTMENT

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	76.42	18	3.146	13.35
		1.5	18	64.04	18	4.71	19.98
		2.5	18	63.59	18	4.251	18.03
		4	18	68.26	18	3.419	14.51
		6	18	68.97	18	4.089	17.35
Active	75mg	-1	18	76.23	18	3.4	14.43
		1.5	18	66.5	18	2.999	12.72
		2.5	18	63.72	18	4.513	19.15
		4	18	64.67	18	3.77	15.99
		6	18	66.78	18	4.005	16.99

22.3 Part 2B - Active 300 mg

Analysis Variable : SIMPLE REACTION TIME

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	235.7	18	7.915	33.58
	1.5	18	304.1	18	14.67	62.25
	2.5	18	299.9	18	10.28	43.61
	4	18	282.7	18	10.44	44.31
	6	18	260.8	18	7.176	30.44
Active 300m	-1	18	235.1	18	7.75	32.88
	1.5	18	299.2	18	13.61	57.75
	2.5	18	308.2	18	14	59.39
	4	18	296.2	18	12.33	52.31
	6	18	266.6	18	9.35	39.67

Analysis Variable : CHOICE REACTION TIME

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	418	18	9.603	40.74
		1.5	18	487.8	18	15.96	67.7
		2.5	18	477.5	18	14.53	61.63
		4	18	470.7	18	18.39	78.01
		6	18	453.6	18	17.83	75.66
Active	300m	-1	18	421.4	18	9.727	41.27
		1.5	18	484.6	18	15.53	65.88
		2.5	18	490.3	18	20.59	87.34
		4	18	478.8	18	16.81	71.33
		6	18	452.1	18	14.97	63.53

Analysis Variable : CHOICE REACTION TIME - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	96.56	18	0.532	2.255
		1.5	18	94.78	18	0.917	3.889
		2.5	18	94.89	18	0.844	3.579
		4	18	95.56	18	0.573	2.431
		6	18	93.89	18	0.749	3.179
Active	300m	-1	18	96	18	0.758	3.218
		1.5	18	94.89	18	0.671	2.847
		2.5	18	95.44	18	0.682	2.895
		4	18	95.44	18	0.789	3.347
		6	18	96.44	18	0.849	3.601

Analysis Variable : DIGIT VIGILANCE - CORRECT DETECTIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	97.66	18	0.813	3.449
	1.5	18	91.73	18	1.605	6.811
	2.5	18	94.2	18	1.247	5.29
	4	18	91.36	18	1.722	7.306
	6	18	94.2	18	1.153	4.89
Active 300m	-1	18	97.78	18	0.696	2.952
	1.5	18	93.33	18	1.867	7.922
	2.5	18	90.25	18	2.238	9.495
	4	18	91.98	18	1.789	7.59
	6	18	95.56	18	1.296	5.498

Analysis Variable : DIGIT VIGILANCE - SPEED OF DETECTIONS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	400.6	18	11.27	47.82
	1.5	18	459.4	18	12.91	54.77
	2.5	18	458.2	18	10.73	45.53
	4	18	441.6	18	13.03	55.27
	6	18	435.4	18	12.36	52.46
Active 300m	-1	18	399.6	18	9.777	41.48
	1.5	18	446.5	18	11.19	47.45
	2.5	18	456.3	18	13.62	57.8
	4	18	442.1	18	8.699	36.9
	6	18	423.1	18	12.94	54.89

Analysis Variable : DIGIT VIGILANCE - FALSE ALARMS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	0.444	18	0.232	0.984
	1.5	18	1.5	18	0.271	1.15
	2.5	18	0.944	18	0.189	0.802
	4	18	1.056	18	0.286	1.211
	6	18	1.056	18	0.286	1.211
Active 300m	-1	18	0.389	18	0.143	0.608
	1.5	18	1.222	18	0.263	1.114
	2.5	18	1.278	18	0.321	1.364
	4	18	1.333	18	0.291	1.237
	6	18	1.111	18	0.301	1.278

Analysis Variable : NUMERIC WORKING MEMORY - SENSITIVITY INDEX

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	0.929	18	0.012	0.052
	1.5	18	0.862	18	0.023	0.099
	2.5	18	0.885	18	0.024	0.103
	4	18	0.885	18	0.024	0.102
	6	18	0.916	18	0.011	0.045
Active 300m	-1	18	0.922	18	0.014	0.058
	1.5	18	0.866	18	0.019	0.081
	2.5	18	0.799	18	0.044	0.185
	4	18	0.869	18	0.028	0.121
	6	18	0.911	18	0.018	0.076

Analysis Variable : NUMERIC WORKING MEMORY - SPEED

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	604.5	18	34.06	144.5
		1.5	18	713.3	18	46.74	198.3
		2.5	18	705	18	43.76	185.6
		4	18	696.8	18	45.93	194.9
		6	18	698.7	18	59.45	252.2
Active	300m	-1	18	600	18	25.43	107.9
		1.5	18	715.5	18	44.55	189
		2.5	18	736.8	18	43.07	182.7
		4	18	692.3	18	39.05	165.7
		6	18	685.5	18	49.88	211.6

Analysis Variable : IMMEDIATE WORD RECALL - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	45	18	2.918	12.38
		1.5	18	31.3	18	1.889	8.014
		2.5	18	28.52	18	2.021	8.573
		4	18	32.22	18	2.528	10.72
		6	18	32.22	18	2.017	8.556
Active	300m	-1	18	44.44	18	2.052	8.707
		1.5	18	30.56	18	1.929	8.184
		2.5	18	34.07	18	2.358	10
		4	18	34.81	18	2.616	11.1
		6	18	38.15	18	1.599	6.785

Analysis Variable : IMMEDIATE WORD RECALL - INTRUSIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.056	18	0.056	0.236
		1.5	18	0.056	18	0.056	0.236
		2.5	18	0.111	18	0.076	0.323
		4	18	0	18	0	0
		6	18	0.333	18	0.14	0.594
Active	300m	-1	18	0	18	0	0
		1.5	18	0	18	0	0
		2.5	18	0.056	18	0.056	0.236
		4	18	0.111	18	0.076	0.323
		6	18	0	18	0	0

Analysis Variable : IMMEDIATE WORD RECALL - ERRORS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.056	18	0.056	0.236
		1.5	18	0.333	18	0.14	0.594
		2.5	18	0.833	18	0.246	1.043
		4	18	0.389	18	0.2	0.85
		6	18	0.278	18	0.109	0.461
Active	300m	-1	18	0.167	18	0.09	0.383
		1.5	18	0.167	18	0.09	0.383
		2.5	18	0.5	18	0.167	0.707
		4	18	0.167	18	0.09	0.383
		6	18	0.278	18	0.135	0.575

Analysis Variable : DELAYED WORD RECALL - ACCURACY

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	31.11	18	4.042	17.15
		1.5	18	7.036	18	1.592	6.752
		2.5	18	11.11	18	2.271	9.633
		4	18	9.629	18	2.409	10.22
		6	18	10.93	18	2.392	10.15
Active	300m	-1	18	28.15	18	2.842	12.06
		1.5	18	10.56	18	2.429	10.31
		2.5	18	12.22	18	2.528	10.73
		4	18	8.889	18	2.668	11.32
		6	18	17.59	18	2.496	10.59

Analysis Variable : DELAYED WORD RECALL - INTRUSIONS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.167	18	0.09	0.383
		1.5	18	0.556	18	0.202	0.856
		2.5	18	0.722	18	0.158	0.669
		4	18	0.778	18	0.222	0.943
		6	18	0.5	18	0.167	0.707
Active	300m	-1	18	0.056	18	0.056	0.236
		1.5	18	0.5	18	0.167	0.707
		2.5	18	0.278	18	0.109	0.461
		4	18	0.667	18	0.268	1.138
		6	18	0.333	18	0.198	0.84

Analysis Variable : DELAYED WORD RECALL - ERRORS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.111	18	0.076	0.323
		1.5	18	0.778	18	0.222	0.943
		2.5	18	0.556	18	0.185	0.784
		4	18	0.389	18	0.143	0.608
		6	18	0.5	18	0.185	0.786
Active	300m	-1	18	0.222	18	0.129	0.548
		1.5	18	0.167	18	0.09	0.383
		2.5	18	0.389	18	0.164	0.698
		4	18	0.167	18	0.121	0.514
		6	18	0.222	18	0.101	0.428

Analysis Variable : WORD RECOGNITION - SENSITIVITY INDEX

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	0.618	18	0.04	0.171
		1.5	18	0.451	18	0.054	0.227
		2.5	18	0.476	18	0.043	0.182
		4	18	0.52	18	0.049	0.209
		6	18	0.554	18	0.046	0.194
Active	300m	-1	18	0.619	18	0.036	0.154
		1.5	18	0.591	18	0.044	0.187
		2.5	18	0.564	18	0.048	0.202
		4	18	0.518	18	0.045	0.192
		6	18	0.505	18	0.046	0.196

Analysis Variable : WORD RECOGNITION - SPEED

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	716.1	18	31.9	135.3
	1.5	18	804.3	18	43.96	186.5
	2.5	18	860.9	18	61.5	260.9
	4	18	856	18	65.38	277.4
	6	18	810	18	47.86	203
Active 300m	-1	18	713.3	18	27.58	117
	1.5	18	809.9	18	30.52	129.5
	2.5	18	836.6	18	40.55	172
	4	18	847.6	18	45.06	191.2
	6	18	801.2	18	49.18	208.7

Analysis Variable : PICTURE RECOGNITION - SENSITIVITY INDEX

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	0.656	18	0.059	0.25
	1.5	18	0.642	18	0.043	0.184
	2.5	18	0.661	18	0.043	0.184
	4	18	0.662	18	0.054	0.231
	6	18	0.66	18	0.047	0.197
Active 300m	-1	18	0.689	18	0.041	0.172
	1.5	18	0.641	18	0.042	0.179
	2.5	18	0.743	18	0.034	0.142
	4	18	0.649	18	0.053	0.224
	6	18	0.688	18	0.043	0.182

Analysis Variable : PICTURE RECOGNITION - SPEED

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	773.5	18	24.13	102.4
		1.5	18	889.5	18	46.89	198.9
		2.5	18	912.6	18	46.28	196.3
		4	18	869.6	18	44.39	188.3
		6	18	898.8	18	58.5	248.2
Active	300m	-1	18	774.6	18	21.02	89.17
		1.5	18	900.1	18	47.56	201.8
		2.5	18	969.9	18	80.06	339.7
		4	18	939.8	18	67.84	287.8
		6	18	914	18	92.77	393.6

Analysis Variable : CONTINUITY OF ATTENTION

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	91.78	18	0.655	2.777
		1.5	18	87.17	18	1.083	4.593
		2.5	18	88.89	18	0.889	3.772
		4	18	87.83	18	0.994	4.218
		6	18	88.28	18	1	4.241
Active	300m	-1	18	91.61	18	0.444	1.883
		1.5	18	88.22	18	0.941	3.994
		2.5	18	87.06	18	1.214	5.151
		4	18	87.78	18	1.021	4.333
		6	18	90.11	18	0.97	4.115

Analysis Variable : POWER OF ATTENTION

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	1054	18	24.49	103.9
	1.5	18	1251	18	37.17	157.7
	2.5	18	1236	18	32.03	135.9
	4	18	1195	18	39.23	166.4
	6	18	1150	18	34.3	145.5
Active 300m	-1	18	1056	18	22.03	93.48
	1.5	18	1230	18	38.26	162.3
	2.5	18	1255	18	45.5	193
	4	18	1217	18	34.07	144.6
	6	18	1142	18	33.41	141.7

Analysis Variable : QUALITY OF SECONDARY MEMORY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	194.6	18	12.62	53.53
	1.5	18	128.4	18	9.62	40.81
	2.5	18	132.5	18	10.91	46.27
	4	18	143.3	18	11.54	48.97
	6	18	146.5	18	9.404	39.9
Active 300m	-1	18	192.8	18	9.164	38.88
	1.5	18	150.8	18	9.691	41.11
	2.5	18	161.9	18	9.607	40.76
	4	18	147.8	18	11.61	49.24
	6	18	164.8	18	8.799	37.33

Analysis Variable : SPEED OF MEMORY

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	2094	18	79.71	338.2
	1.5	18	2407	18	120	509.2
	2.5	18	2478	18	126	534.7
	4	18	2422	18	129.8	550.8
	6	18	2408	18	155.4	659.4
Active 300m	-1	18	2088	18	61.55	261.1
	1.5	18	2426	18	101.7	431.7
	2.5	18	2543	18	137.1	581.5
	4	18	2480	18	135.6	575.4
	6	18	2401	18	152.1	645.4

Analysis Variable : SELF-RATED ALERTNESS

COND	VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo	-1	18	66.03	18	4.031	17.1
	1.5	18	41.43	18	3.186	13.52
	2.5	18	46.41	18	2.919	12.38
	4	18	51.58	18	3.384	14.36
	6	18	57.29	18	2.725	11.56
Active 300m	-1	18	65.09	18	3.382	14.35
	1.5	18	42.81	18	3.478	14.75
	2.5	18	44.33	18	4.144	17.58
	4	18	50.61	18	3.637	15.43
	6	18	59.82	18	2.671	11.33

Analysis Variable : SELF-RATED CALMNESS

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	66.86	18	4.404	18.69
		1.5	18	68.78	18	3.536	15
		2.5	18	62.03	18	4.337	18.4
		4	18	63.75	18	3.554	15.08
		6	18	63.61	18	4.051	17.19
Active	300m	-1	18	62.56	18	3.981	16.89
		1.5	18	65.44	18	2.968	12.59
		2.5	18	64.92	18	3.939	16.71
		4	18	67.36	18	3.217	13.65
		6	18	66.89	18	3.019	12.81

Analysis Variable : SELF-RATED CONTENTMENT

COND		VISIT	N Obs	Mean	N	Std Error	Std Dev
Placebo		-1	18	68.42	18	3.603	15.29
		1.5	18	60.21	18	3.138	13.32
		2.5	18	61.92	18	2.998	12.72
		4	18	62.87	18	2.984	12.66
		6	18	65.58	18	2.749	11.66
Active	300m	-1	18	66.64	18	2.798	11.87
		1.5	18	60.28	18	3.306	14.03
		2.5	18	60.6	18	3.508	14.88
		4	18	60.77	18	3.177	13.48
		6	18	64.29	18	2.894	12.28

23 Appendix 5: Normative Data and Test-retest Reliability Chapters 12 and 13

NAME OF FORMER VARIABLE=AGE

The MEANS Procedure

Variable	Mean	N	Std Dev	Std Error	Min	Max
_1	30.36	3299	7.779	0.135	21.00	50.00
_2	30.36	3299	7.778	0.135	21.00	50.00
_3	30.36	3299	7.778	0.135	21.00	50.00
_4	30.36	3299	7.778	0.135	21.00	50.00
_5	30.36	3299	7.778	0.135	21.00	50.00

NAME OF FORMER VARIABLE=CRT

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3165	426.95376	55.32612	421.21000	282.09000	1033
_2	3165	420.28993	49.55172	415.00000	299.35000	752.29000
_3	3165	412.01095	49.10944	406.37000	296.43000	857.74000
_4	3165	414.91443	50.32830	409.53000	303.80000	763.41000
_5	3165	415.08275	47.61231	410.75000	296.35000	700.15000

Spearman Correlation Coefficients, N = 3165
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.75243	0.71681	0.68316	0.63288
		<.0001	<.0001	<.0001	<.0001
_2	0.75243	1.00000	0.77993	0.75156	0.70677
	<.0001		<.0001	<.0001	<.0001
_3	0.71681	0.77993	1.00000	0.80400	0.74131
	<.0001	<.0001		<.0001	<.0001
_4	0.68316	0.75156	0.80400	1.00000	0.75258
	<.0001	<.0001	<.0001		<.0001
_5	0.63288	0.70677	0.74131	0.75258	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=CRTACC ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3165	95.40940	3.50011	96.00000	66.67000	100.00000
_2	3165	96.16244	3.06844	96.00000	78.00000	100.00000
_3	3165	95.42627	3.52507	96.00000	74.00000	100.00000
_4	3165	96.21741	3.16751	96.00000	76.67000	100.00000
_5	3165	95.87785	3.27739	96.00000	70.00000	100.00000

Spearman Correlation Coefficients, N = 3165
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.39451	0.42570	0.35477	0.35470
		<.0001	<.0001	<.0001	<.0001
_2	0.39451	1.00000	0.45876	0.41026	0.41496
	<.0001		<.0001	<.0001	<.0001
_3	0.42570	0.45876	1.00000	0.41509	0.44945
	<.0001	<.0001		<.0001	<.0001
_4	0.35477	0.41026	0.41509	1.00000	0.43801
	<.0001	<.0001	<.0001		<.0001
_5	0.35470	0.41496	0.44945	0.43801	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=CRTL ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	422	15.19455	78.46415	0	0	525.07000
_2	422	14.54841	74.86080	0	0	506.26000
_3	422	14.97156	77.51700	0	0	513.69000
_4	422	14.42486	74.31800	0	0	495.69000
_5	422	14.77431	75.75902	0	0	476.86000

Spearman Correlation Coefficients, N = 422
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.27101	0.28440	0.17484	0.23364
		<.0001	<.0001	0.0003	<.0001
_2	0.27101	1.00000	0.17523	0.17933	0.14097
	<.0001		0.0003	0.0002	0.0037
_3	0.28440	0.17523	1.00000	0.21057	0.20695
	<.0001	0.0003		<.0001	<.0001
_4	0.17484	0.17933	0.21057	1.00000	0.15560
	0.0003	0.0002	<.0001		0.0013
_5	0.23364	0.14097	0.20695	0.15560	1.00000
	<.0001	0.0037	<.0001	0.0013	

---------- NAME OF FORMER VARIABLE=CRTM ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	185	408.47151	107.09944	419.50000	86.67000	642.00000
_2	185	395.89886	100.71160	411.50000	93.33000	637.00000
_3	185	389.38800	97.74092	402.00000	82.22000	559.50000
_4	185	385.81622	95.75192	402.00000	84.44000	596.00000
_5	185	388.30092	95.17734	406.00000	88.89000	563.00000

Spearman Correlation Coefficients, N = 185
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.79810	0.74307	0.73120	0.67601
		<.0001	<.0001	<.0001	<.0001
_2	0.79810	1.00000	0.81974	0.80928	0.73170
	<.0001		<.0001	<.0001	<.0001
_3	0.74307	0.81974	1.00000	0.82270	0.76604
	<.0001	<.0001		<.0001	<.0001
_4	0.73120	0.80928	0.82270	1.00000	0.81412
	<.0001	<.0001	<.0001		<.0001
_5	0.67601	0.73170	0.76604	0.81412	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=CRTSD ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	422	82.64730	78.35407	76.38500	0	1583
_2	422	75.26341	27.31831	73.76500	0	212.41000
_3	422	76.14972	65.90345	71.87500	0	1322
_4	422	72.37315	26.05947	70.45500	0	194.85000
_5	422	72.15955	25.33298	70.08000	0	187.94000

Spearman Correlation Coefficients, N = 422
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.62518	0.57180	0.57477	0.54582
		<.0001	<.0001	<.0001	<.0001
_2	0.62518	1.00000	0.59908	0.63202	0.56925
	<.0001		<.0001	<.0001	<.0001
_3	0.57180	0.59908	1.00000	0.65400	0.60118
	<.0001	<.0001		<.0001	<.0001
_4	0.57477	0.63202	0.65400	1.00000	0.61545
	<.0001	<.0001	<.0001		<.0001
_5	0.54582	0.56925	0.60118	0.61545	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=SRT ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3103	248.92582	34.98181	243.92000	147.27000	587.00000
_2	3103	250.43497	30.22744	246.58000	165.73000	417.88000
_3	3103	248.46536	30.16558	245.21000	173.38000	436.84000
_4	3103	250.82020	31.66865	246.55000	180.50000	427.27000
_5	3103	250.27300	29.36823	247.86000	172.37000	473.76000

Spearman Correlation Coefficients, N = 3103
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.67302	0.64288	0.57729	0.58488
		<.0001	<.0001	<.0001	<.0001
_2	0.67302	1.00000	0.74960	0.71625	0.68781
	<.0001		<.0001	<.0001	<.0001
_3	0.64288	0.74960	1.00000	0.78153	0.72911
	<.0001	<.0001		<.0001	<.0001
_4	0.57729	0.71625	0.78153	1.00000	0.73010
	<.0001	<.0001	<.0001		<.0001
_5	0.58488	0.68781	0.72911	0.73010	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=SRTL ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	375	1.36000	1.22190	1.00000	0	6.00000
_2	375	1.06667	1.14851	1.00000	0	6.00000
_3	375	1.15200	1.16584	1.00000	0	7.00000
_4	375	1.14133	1.12520	1.00000	0	5.00000
_5	375	1.18400	1.15659	1.00000	0	7.00000

Spearman Correlation Coefficients, N = 375
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.15028	0.13848	0.14539	0.05459
		0.0035	0.0072	0.0048	0.2917
_2	0.15028	1.00000	0.10526	0.07690	0.03273
	0.0035		0.0416	0.1372	0.5274
_3	0.13848	0.10526	1.00000	0.17053	-0.01810
	0.0072	0.0416		0.0009	0.7267
_4	0.14539	0.07690	0.17053	1.00000	0.07934
	0.0048	0.1372	0.0009		0.1251
_5	0.05459	0.03273	-0.01810	0.07934	1.00000
	0.2917	0.5274	0.7267	0.1251	

NAME OF FORMER VARIABLE=SRTM

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	171	251.19298	31.65890	247.50000	204.00000	458.50000
_2	171	252.54094	26.08123	248.50000	202.50000	353.00000
_3	171	249.09357	28.91697	245.50000	201.00000	436.00000
_4	171	248.47953	28.17009	244.00000	197.50000	412.50000
_5	171	248.09942	22.19152	247.00000	201.00000	339.00000

Spearman Correlation Coefficients, N = 171
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.58419	0.65812	0.57883	0.54026
		<.0001	<.0001	<.0001	<.0001
_2	0.58419	1.00000	0.71673	0.64368	0.60385
	<.0001		<.0001	<.0001	<.0001
_3	0.65812	0.71673	1.00000	0.80958	0.74309
	<.0001	<.0001		<.0001	<.0001
_4	0.57883	0.64368	0.80958	1.00000	0.76560
	<.0001	<.0001	<.0001		<.0001
_5	0.54026	0.60385	0.74309	0.76560	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=SRTSD ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	375	41.45056	20.38861	36.62000	14.13000	167.90000
_2	375	40.84285	16.66138	37.48000	12.45000	96.92000
_3	375	38.99544	16.95602	34.26000	12.31000	99.62000
_4	375	41.50376	21.37012	36.28000	12.96000	189.18000
_5	375	38.80659	17.66334	35.57000	14.37000	117.06000

Spearman Correlation Coefficients, N = 375
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.46962	0.47252	0.40771	0.42049
		<.0001	<.0001	<.0001	<.0001
_2	0.46962	1.00000	0.57220	0.49429	0.54274
	<.0001		<.0001	<.0001	<.0001
_3	0.47252	0.57220	1.00000	0.61614	0.58649
	<.0001	<.0001		<.0001	<.0001
_4	0.40771	0.49429	0.61614	1.00000	0.57104
	<.0001	<.0001	<.0001		<.0001
_5	0.42049	0.54274	0.58649	0.57104	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=VIGACC ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3103	96.91367	5.32307	97.78000	13.33000	100.00000
_2	3103	97.97999	3.92628	100.00000	11.11000	100.00000
_3	3103	97.89116	3.59293	100.00000	66.67000	100.00000
_4	3103	98.04664	3.54552	100.00000	68.89000	100.00000
_5	3103	97.65440	3.87825	100.00000	44.44000	100.00000

Spearman Correlation Coefficients, N = 3103
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.36637	0.31506	0.28314	0.29510
		<.0001	<.0001	<.0001	<.0001
_2	0.36637	1.00000	0.36859	0.33622	0.33326
	<.0001		<.0001	<.0001	<.0001
_3	0.31506	0.36859	1.00000	0.36736	0.33177
	<.0001	<.0001		<.0001	<.0001
_4	0.28314	0.33622	0.36736	1.00000	0.34408
	<.0001	<.0001	<.0001		<.0001
_5	0.29510	0.33326	0.33177	0.34408	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=VIGFA ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3103	1.45762	2.09972	1.00000	0	47.00000
_2	3103	0.87432	1.35186	1.00000	0	26.00000
_3	3103	0.99839	1.28486	1.00000	0	12.00000
_4	3103	0.77860	1.15063	0	0	22.00000
_5	3103	0.87045	1.18201	1.00000	0	14.00000

Spearman Correlation Coefficients, N = 3103
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.29794	0.29015	0.29997	0.23593
		<.0001	<.0001	<.0001	<.0001
_2	0.29794	1.00000	0.28767	0.24070	0.28609
	<.0001		<.0001	<.0001	<.0001
_3	0.29015	0.28767	1.00000	0.24663	0.28035
	<.0001	<.0001		<.0001	<.0001
_4	0.29997	0.24070	0.24663	1.00000	0.25903
	<.0001	<.0001	<.0001		<.0001
_5	0.23593	0.28609	0.28035	0.25903	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=VIGRT ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	3103	394.22974	38.08851	391.12000	297.20000	842.83000
_2	3103	394.82528	37.38356	391.81000	292.00000	541.24000
_3	3103	394.84484	37.56097	391.98000	301.27000	588.23000
_4	3103	397.73820	38.01745	394.64000	299.13000	613.13000
_5	3103	402.58675	37.94713	398.53000	292.16000	574.58000

Spearman Correlation Coefficients, N = 3103
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.77161	0.71646	0.70412	0.64096
		<.0001	<.0001	<.0001	<.0001
_2	0.77161	1.00000	0.77682	0.76916	0.71166
	<.0001		<.0001	<.0001	<.0001
_3	0.71646	0.77682	1.00000	0.79422	0.73358
	<.0001	<.0001		<.0001	<.0001
_4	0.70412	0.76916	0.79422	1.00000	0.76080
	<.0001	<.0001	<.0001		<.0001
_5	0.64096	0.71166	0.73358	0.76080	1.00000
	<.0001	<.0001	<.0001	<.0001	

---------- NAME OF FORMER VARIABLE=VIGSD ----------

The CORR Procedure

5 Variables: _1 _2 _3 _4 _5

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
_1	347	61.11271	19.06818	57.59000	25.48000	123.56000
_2	347	60.72677	20.64947	57.16000	25.73000	133.12000
_3	347	61.60484	20.43712	57.26000	25.25000	149.54000
_4	347	60.20398	20.77848	56.34000	22.66000	147.40000
_5	347	63.13902	20.87826	59.08000	26.31000	132.88000

Spearman Correlation Coefficients, N = 347
Prob > |r| under H0: Rho=0

	_1	_2	_3	_4	_5
_1	1.00000	0.39933	0.39617	0.38369	0.33627
		<.0001	<.0001	<.0001	<.0001
_2	0.39933	1.00000	0.45343	0.40421	0.46169
	<.0001		<.0001	<.0001	<.0001
_3	0.39617	0.45343	1.00000	0.47731	0.44580
	<.0001	<.0001		<.0001	<.0001
_4	0.38369	0.40421	0.47731	1.00000	0.39553
	<.0001	<.0001	<.0001		<.0001
_5	0.33627	0.46169	0.44580	0.39553	1.00000
	<.0001	<.0001	<.0001	<.0001	