The Effects of Acute Tryptophan Depletion on Movement, Mood, and Cognition in Patients with Parkinson’s Disease and Healthy Older Persons

Janet Lee Mace

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Abstract

Background  Serotonin (5-HT) function has been seldom investigated comprehensively in Parkinson’s disease (PD) and in healthy older persons. The response of these groups to such manipulation may differ from that of younger healthy individuals. Degeneration of the cholinergic and dopaminergic neurotransmitter systems is associated with impaired cognition and movement, especially in PD. 5-HT has a modulating role in the brain, inhibiting the activity of other neurotransmitters. Abnormalities in the serotonergic system are also linked with depression. It is possible manipulations of the serotonergic system influence different aspects of behaviour, which can be measured on a number of parameters.

The technique of acute tryptophan depletion (ATD) provides a safe, temporary and replicable reduction in central 5-HT levels. It involves ingestion of a 50-100 g drink of amino acids minus tryptophan. The effect is twofold. Firstly, protein synthesis is initiated such that tryptophan circulating in the blood is incorporated into proteins and is unavailable to cross the blood brain barrier. Secondly, the large neutral amino acids out-compete tryptophan for shared transport into the brain.

Objective  To investigate the effects an acute reduction in serotonin synthesis has on mood, movement and cognitive function in Parkinson’s disease and healthy older persons.

Method  Three studies having double-blind, placebo-controlled, randomised, counterbalanced, crossover designs were completed. Treatment involved administration of a tryptophan depleting drink and a placebo drink. Study A comprised a new analysis of a group of 33 healthy older persons across two previous studies that used a low and a high dose of ATD. Study B compared the effects of ATD in 20 PD patients and 35 healthy matched controls. Study C examined a group of 43 healthy older persons to compare differences across those aged 50-69 years and 70-89 years. Mood and movement were assessed at baseline and at 4 and 6 hours. A large battery of behavioural and cognitive tasks, including the Modified Mini-Mental State examination (3MS), was administered between 4 and 6 hours post treatment.

Results  The reduction of plasma free tryptophan after ATD was 69-71% across Studies B and C. There was no significant effect of treatment on mood, despite the number of participants being at risk for mood lowering effects of ATD. For PD patients, ATD was associated with impaired global cognitive status (3MS) and verbal memory, but improved visual memory and increased psychomotor speed; the effects in matched controls tended to be the reverse. In healthy ageing, increased age was associated with reduced psychomotor speed during ATD.

Conclusion  ATD does not affect mood in PD patients or healthy older persons. However, ATD improves psychomotor speed and visual memory selectively in PD. Impairment in global cognitive status during ATD occurred selectively in PD and replicates previous work in patients with Alzheimer’s disease and recovered depression. These data suggest differing effects on aspects of
neuropsychiatric function depending on the exact function being tested. Effects also depend on neuropsychiatric status, especially the degree of cholinergic deficit. The data add to evidence regarding the role of the serotonergic system in neuropsychiatric function in the elderly and in PD and may be relevant when considering potential treatments which act on the serotonergic system.
This PhD conforms to the author-date (Harvard) system for the documentation style of referencing sources in text and the American Psychological Association (A.P.A) Publication Manual (5th ed.) for bibliographic style.

The research for this PhD thesis was carried out between January 2002 and December 2007 while the PhD candidate was enrolled in the Department of Psychological Medicine, University of Otago Christchurch. The research was based at the Van der Veer Institute for Parkinson’s and Brain Research, Christchurch, New Zealand. The research was supervised by Associate Professor Richard Porter, Department of Psychological Medicine, University of Otago Christchurch and Associate Professor John Dalrymple-Alford, Department of Psychology, University of Canterbury. Financial support was provided by a University of Otago Postgraduate Scholarship and a grant from the University of Otago.

The data for one study (Study A) was collected by researchers in Newcastle-Upon-Tyne, UK, and analysed by the PhD candidate in Christchurch, NZ. The other two studies (Study B and Study C) were conducted solely at the Van der Veer Institute. Patients for Study B were recruited from neurologists in Christchurch (in particular, Professor Tim Anderson), while healthy participants for Studies B and C were recruited from (a) a volunteer database at the Van der Veer Institute, (b) club newsletters, or (c) word of mouth. Ethical approval for all studies was obtained prior to recruitment and testing. All movement, mood and cognitive tests were freely available to the research, including VOSP from the Psychology Department Neuropsychology Test Library, University of Canterbury, CANTAB from the Department of Psychological Medicine Neuropsychology Test Library, and CDR from Cognitive Drug Research Ltd. The experimental assessment of all participants for Studies B and C was administered by the PhD candidate and a research assistant. The design of the assessment battery, experimental protocols, equipment preparation and operation, data analysis, and the creation of this thesis were performed solely by the PhD candidate.

Aspects of this research have been, or will be, presented at the following: the Department of Psychological Medicine Research Meetings, Van der Veer Institute Forums, Brain Research Forums, and International College of Geriatric Psychoneuropsycharmacology conference (Hiroshima, Japan, October 2006), the International College of Geriatric Psychoneuropsycharmacology conference (Sydney 2008).

There are six papers from this research currently published, submitted, or being written by the author:


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## Abbreviations

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<th>Full Form</th>
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<tbody>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine or serotonin</td>
</tr>
<tr>
<td>3MS</td>
<td>Modified Mini Mental State examination</td>
</tr>
<tr>
<td>5-HTP</td>
<td>5-hydroxytryptophan</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>ADLs</td>
<td>activities of daily living</td>
</tr>
<tr>
<td>ATD</td>
<td>acute tryptophan depletion</td>
</tr>
<tr>
<td>APTD</td>
<td>acute phenylalanine-tyrosine depletion</td>
</tr>
<tr>
<td>CANTAB</td>
<td>CAMbridge Neuropsychological Automated Battery</td>
</tr>
<tr>
<td>CDR</td>
<td>Cognitive Drug Research Ltd</td>
</tr>
<tr>
<td>ChAT</td>
<td>choline acetyltransferase</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>COWA</td>
<td>Controlled Oral Word Association test</td>
</tr>
<tr>
<td>CRT</td>
<td>Choice Reaction Time</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DFFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DigitsB</td>
<td>Digit Span Backwards</td>
</tr>
<tr>
<td>DigitsF</td>
<td>Digit Span Forwards</td>
</tr>
<tr>
<td>DLB</td>
<td>dementia with Lewy bodies</td>
</tr>
<tr>
<td>DMS</td>
<td>Delayed Matching to Sample task</td>
</tr>
<tr>
<td>DV</td>
<td>Digit Vigilance task</td>
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<tr>
<td>DWR</td>
<td>Delayed Working Memory task</td>
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<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma amino butyric acid</td>
</tr>
<tr>
<td>GPe</td>
<td>globus pallidus externa</td>
</tr>
<tr>
<td>GPI</td>
<td>globus pallidus interna</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic pituitary adrenal</td>
</tr>
<tr>
<td>HVA</td>
<td>homovanillic acid</td>
</tr>
<tr>
<td>IWR</td>
<td>Immediate Working Memory task</td>
</tr>
<tr>
<td>LNAA</td>
<td>large neutral amino acid</td>
</tr>
<tr>
<td>MAO</td>
<td>monoaminoxidase</td>
</tr>
<tr>
<td>MAO-A</td>
<td>monoaminoxidase-A</td>
</tr>
<tr>
<td>MAO-B</td>
<td>monoaminoxidase-B</td>
</tr>
<tr>
<td>MAOI</td>
<td>monoaminoxidase inhibitor</td>
</tr>
<tr>
<td>mCPP</td>
<td>metachlorophenylpiperazine</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini Mental State examination</td>
</tr>
<tr>
<td>MOT</td>
<td>Motor Screening Test</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NA</td>
<td>noradrenaline</td>
</tr>
<tr>
<td>OFC</td>
<td>orbitofrontal cortex</td>
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<tr>
<td>PD</td>
<td>Parkinson's disease</td>
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<tr>
<td>PDD</td>
<td>Parkinson's disease and dementia</td>
</tr>
<tr>
<td>PDND</td>
<td>Parkinson's disease no dementia</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PRM</td>
<td>Pattern Recognition Memory task</td>
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<tr>
<td>RAVLT</td>
<td>Rey Auditory Verbal Learning Test</td>
</tr>
<tr>
<td>RVDLT</td>
<td>Rey Visual Design Learning Test</td>
</tr>
<tr>
<td>SDAT</td>
<td>senile dementia of the Alzheimer's type</td>
</tr>
<tr>
<td>SERT</td>
<td>serotonin transporter</td>
</tr>
<tr>
<td>SMRT</td>
<td>Simultaneous Matching to Sample task</td>
</tr>
<tr>
<td>SNpc</td>
<td>substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNpr</td>
<td>substantia nigra pars reticular</td>
</tr>
<tr>
<td>SNRI</td>
<td>serotonin-norepinephrine reuptake inhibitor</td>
</tr>
<tr>
<td>SRM</td>
<td>Spatial Recognition Memory task</td>
</tr>
<tr>
<td>SRT</td>
<td>Simple Reaction Time task</td>
</tr>
<tr>
<td>SSP</td>
<td>Spatial Span</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>SWM</td>
<td>Spatial Working Memory task</td>
</tr>
<tr>
<td>TCA</td>
<td>tricylic antidepressant</td>
</tr>
<tr>
<td>TRP</td>
<td>tryptophan</td>
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</table>
I dedicate this thesis to my beautiful mother Val Gardiner, to my loving children Hamish, Jack, Oliver, and Sophie, and to my darling husband James.
1 INTRODUCTION

The research presented in this thesis provides insights into the functional integrity of the central serotonergic system in healthy older persons and patients with Parkinson’s disease.

Biochemical studies have shown changes in the serotonergic system as people age, changes which are juxtaposed with those in other neurotransmitter systems to potentially limit the everyday life of older persons. However, studies on the functional integrity of the serotonergic system in older persons are severely lacking. This is interesting because older adults regularly complain of cognitive deficits (Cohen & Burke, 1993; Craik et al., 1995) and serotonin has been shown to have an elemental role in learning and memory (McEntee & Crook, 1991). Moreover, older age is associated with depression, and, irrespective of whether this depression has endogenous or psychosocial aetiology, depressed patients are usually treated with serotonergic acting drugs. Older age is also associated with physical disablement, yet movement has never been examined during serotonin (5-hydroxytryptamine, 5-HT) challenge studies. It is fundamental that the functional serotonergic system be examined in healthy older persons if generalisations are to be made about behaviour in human adults.

The serotonergic system changes with age and, along with the cholinergic, noradrenergic, and dopaminergic systems, is dysregulated in neurodegenerative disorders like senile dementia of the Alzheimer’s type (SDAT), Parkinson’s disease (PD), and dementia with Lewy bodies (DLB). It is often the complaints attributable to dysfunctions in these neurotransmitter systems that have the greatest impact on a person’s quality of life. For example, in PD, the strongest predictor of life quality, surprisingly, is not motor disability but the presence of depression (Schrag et al., 2001). Furthermore, in all these disorders a severely compromised cholinergic system is likely to most impact cognitive function. A comorbid dysregulation of several neurotransmitter systems may mean this group of patients will respond differently to an artificially induced serotonergic reduction than either healthy persons of younger or similar age, or younger patient groups.

Much of the information about the serotonergic system in PD is generated from post-mortem assays, presumably, using the brains of late-in-the-course patients. Animal models provide some clues about the status of 5-HT in patients with mild to moderate symptoms and there is an emerging body of data coming from neuroimaging studies. Functional data has been restricted to treatment studies using selective serotonin reuptake inhibitors (SSRIs) for depression and dopaminergic compounds for movement disorder. PD is predominantly a disease of dopaminergic pathology, however the failure of dopaminergic treatment to completely normalise cognition in early-in-the-course PD (Kulisevsky et al., 2000) – or attenuate depressive symptoms (Okun & Watts, 2002) – suggests these problems might have a non-dopaminergic basis; alterations in cholinergic, noradrenergic and serotonergic systems are thought to be involved (Levin & Katzen, 1995).

Serotonin is a neurotransmitter that “… has been implicated in almost every conceivable physiologic or behavioral function – affect, aggression, appetite, cognition, emesis, endocrine function,
gastrointestinal function, motor function, neurotropism, perception, sensory function, sex, sleep, and vascular function” (Aghajanian & Sanders-Bush, 2002, p. 15), nutrient intake, nociception, as well as cardiovascular and respiratory activity (Jacobs, 1995). To influence such a diverse range of activities, serotonergic pathways from a small number of cell bodies clustered in the brain stem project to all regions of the brain and spinal cord (Palmer et al., 1987). A number of different receptors act presynaptically and post-synaptically to mediate the activity of 5-HT. This functioning system is so complex that a comprehensive picture of its workings, even in healthy persons, is yet to be determined (Jones & Blackburn, 2002).

It is vital the body of knowledge on the serotonergic system is expanded since serotonergic medications are being used in a variety of the somatic and psychiatric conditions of older persons, without robust evidence (Ghazi-Noori et al., 2006; Leroi et al., 2006) and, sometimes, with undesirable side effects (Jankovic, 2002). Moreover, a degenerating or dysfunctional serotonergic system, either on its own or in concert with other neurotransmitter systems, may create problems that could be ameliorated with serotonergic-acting compounds. Ideally a large study comprising participant representatives from all ages of life is best suited to investigate this functional change. Smaller comparative studies, however, contribute valuable insights within an integrated and interdisciplinary paradigm.

A number of studies have investigated different aspects of the serotonergic system using the technique of acute tryptophan depletion (ATD). This has proven to be a safe and effective method for inducing a drastic but temporary reduction in central serotonin levels. ATD is an experimental technique that involves participants ingesting a treatment of amino acids in liquid or capsule form, waiting five hours and then undertaking a series of assessments – biological, behavioural, or electrophysiological. Generally the experiment uses a double-blind, placebo-controlled, counterbalanced, randomised, and crossover design.

ATD studies with young adult participants have produced a consistent pattern of cognitive and affective results. Namely, significant consolidation impairments in memory and significant effects in mood in vulnerable groups only. Neurotransmitter systems change during normal ageing and, in light of the main effects in two studies in the elderly that were anomalous to the pattern in younger persons, it is possible ATD has different effects in the healthy elderly. Cholinergic activity in the hippocampus and entorhinal areas declines in healthy adults after middle age (Perry et al., 1993c) and is particularly reduced in PD (Bohnen et al., 2003). ATD has been shown to have a particular and divergent profile in older patients having severe cholinergic dysregulation and it is also possible the ATD-induced worsening in global cognitive status seen in these groups is part of a bigger picture in older patient groups in general.

The primary focus for a series of studies, including those of Porter and colleagues (Porter et al., 2003b; Porter et al., 2000; Porter et al., 2005), was to investigate the role of serotonin in cognition and mood
when neurotransmitter systems – in particular, the serotonergic, cholinergic and dopaminergic systems – are dysregulated as a result of ageing or neurodegeneration. For the purposes of the present research, four studies were instigated to examine the functional role of 5-HT in healthy older persons and patients with PD and DLB. The first study was explorative and, in comparing two different ATD doses, informed the choice of dose for the other studies.

The patient groups had two co-morbid and severely compromised neurotransmitter systems: the dopaminergic and the cholinergic. The overarching hypothesis was that ATD would worsen global cognitive status in patients with PD and DLB compared to healthy older persons. However, there is a particular profile of cholinergic changes in PD, PDD and DLB, thus a comprehensive battery of psychometric neuropsychological tasks was devised to assess, not only the cognitive functions previously shown to be affected by ATD, but those that have been shown affected in PD and DLB. The important role of 5-HT in affective states meant mood scales were incorporated into the testing schedule. A standardised PD motor scale was used to assess the potential effect of ATD on movement and tremor in particular.

For the purposes of this thesis three studies are presented in three separate chapters and discussed with reference to what is known about serotonin and what is understood about behaviour in these groups. Background chapters on the serotonergic system, ATD, and PD precede the study chapters to inform the reader of the biological and behavioural elements associated with each of these factors; and to highlight the issues yet to be resolved. Together they demonstrate that serotonergic functionality is not the same in older and younger persons, and that the nature of this difference extends also to neurodegenerative disorders having marked cholinergic and dopaminergic deficits. The fourth study investigating ATD in patients with PD and dementia (PDD) and DLB is ongoing, but preliminary data is presented in Appendix J.

With this in mind, the following overview of the thesis is presented herewith.

Chapter 2 reviews the serotonin system, including interactions between the serotonergic and other neurotransmitter systems. There is a particular focus on the behaviours of movement, mood, and cognition; and the variations that occur in older adults and between genders.

Chapter 3 describes the technique of acute tryptophan depletion and the efficacy of this method for investigating the serotonin system and behaviour.

Chapter 4 comprises a literature review of serotonergic function in movement, mood, and cognition, with specific sections on age and gender; also of the studies using ATD in mood and cognition.

Chapter 5 reviews Parkinson’s disease; in particular, the classical symptoms and biological basis for these symptoms in pathological movement, mood, cognition, and in dementia. Evidence for serotonin’s role in the symptoms of this disease is also discussed.
Chapter 6 outlines the methodology used in the subsequent three chapters. These chapters examined the effects of ATD on a wide range of movement, mood, and cognitive tasks. Details of these tasks and the experimental procedure – including treatments – are included in this methodology chapter, although the specific tasks and procedures used are delineated separately at the beginning of each study’s chapter.

Chapter 7 describes a study that investigated the effects of using two different doses of ATD. It involved pooling the data from healthy older persons who acted as the controls in two separate studies with patient groups. This study informed the optimal treatment dosage and selection of tasks for the subsequent two studies.

Chapter 8 describes a study using ATD to investigate the role of serotonin in PD. The four areas investigated were biological, movement, mood, and cognition.

Chapter 9 describes a further ATD study, initiated to examine the same wide range of behaviours but in a large group of healthy older persons.

Chapter 10 concludes the thesis with a summary of the main findings, a critique of the weaknesses and strengths of the research and a guide for future directions.
2 SEROTONIN

2.1 Introduction
The following chapter forms part of the background for the three ATD studies in this present research in that it outlines some of what is known about serotonin in the central nervous system (CNS). It begins with a review of the central serotonergic system, continuing to review other neurotransmitter systems that interact with the serotonergic system to affect mood, movement and cognition. This section is followed by a discussion of gender differences in the serotonergic system, concluding with the effects of normal and pathological ageing.

2.2 The Central Serotonergic System
Serotonin is a biologically active compound which, along with dopamine (DA), noradrenaline (NA) and acetylcholine (ACh), is classed a biogenic amine. In the CNS, it is synthesised in axon terminals from tryptophan (TRP) with the assistance of a number of cofactors. Synthesis is limited by the dietary availability of these substrates and is regulated by brain activity (Cooper et al., 2003), stress, elevated cortisol levels, vitamin B6 deficiency, and kynurenine levels (Birdsall, 1998). Also, to the expression of tryptophan hydroxylase via the TPH2 gene which is preferentially expressed in the brain stem raphé nuclei and involved in 5-HT synthesis, TPH1 being expressed largely, but not solely in the gut, pineal gland, spleen, and thymus (Patel et al., 2004; Walther & Bader, 2003; Zhang et al., 2004). There are a number of pharmacogenetic studies linking the THP2 gene with psychiatric disorders and aggressive behaviour (e.g., De Luca et al., 2004; Harvey et al., 2004; Lopez de Lara et al., 2007; Popova, 2006; Reuter et al., 2007; Zill et al., 2004).

Once synthesised, 5-HT is packaged in vesicles in the pre-synaptic terminal ready for release into the synapse. Synaptic activity is completed primarily by the uptake of 5-HT into the pre-synaptic neuron by the 5-HT reuptake transporter. 5-HT is metabolised by monoamine oxidase (MAO) into 5-hydroxyindoleacetic acid (5-HIAA) which is excreted in urine (Kaplan & Sadock, 1994).

2.2.1 Serotonergic Receptors
There are 16 known types of serotonin receptors (Naughton et al., 2000), which are categorised into seven families according to their molecular structure, pharmacology, and signal transduction pathways (Barnes & Sharp, 1999; Meneses, 1998). All members of the 5-HT1 type tend to have pre- and post-synaptic inhibitory actions, while all those of the 5-HT2 type have excitatory actions (Aghajanian & Sanders-Bush, 2002). These two subtypes (5-HT1 and 5-HT2) have dense concentrations in the dorsal raphé nuclei, hippocampal pyramidal cell layer, substantia nigra pars reticular (SNpr), cranial motor neurons, and cerebral cortex (Aghajanian & Sanders-Bush, 2002).

Pre-synaptically, the 5-HT1A receptor acts as an autoreceptor which, in the dorsal raphé nuclei, inhibits the release of 5-HT. This regulatory action on 5-HT release in the dorsal raphé nuclei is complemented by the activity of 5-HT2A/C-mediated local gamma-aminobutyric acid (GABA) and glutamate neurons,
and by post-synaptic 5-HT$_{1A}$ receptors in the medial prefrontal cortex as part of a long-loop feedback (circuit) system (Aghajanian & Sanders-Bush, 2002). Subtype 5-HT$_{2}$ receptors are present in high numbers in certain areas of the frontal cortex; neurons in the substantia nigra par reticular (SNpr) are activated via these receptors (Aghajanian & Sanders-Bush, 2002).

5-HT$_{3}$ provides rapid excitatory effects and in the CNS is widely distributed in the cerebral cortex and parts of the hippocampus. The 5-HT$_{4}$ receptors appear in several discrete regions of the CNS, including the frontal cortex, striatum, substantia nigra, and hippocampus (Aghajanian & Sanders-Bush, 2002; Buhot et al., 2000).

### 2.2.2 Serotonergic Function

The organisation of serotonergic neurons and axon terminals is a primitive one found in virtually all vertebrate brains (Jacobs, 1995). In humans there are eight serotonergic nuclei in the brain and they have an omnipresent influence in the CNS. Nuclei located in the medial and dorsal raphé of the brainstem project extensively to the subthalamic nucleus, globus pallidus (GP), striatum, limbic system, and cerebral cortex (Gerhardt & van Heerikhuizen, 1997).

Serotonin has a modulatory role in these areas (Spoont, 1992) and this is believed to be largely inhibitory. It is in this manner that 5-HT acts to constrain the activity of other neurotransmitters in a wide variety of behaviours (Lucki, 1998). The mechanism for inhibition seems to be via a G-protein initiated hyperpolarisation, which reduces Ca$^{2+}$ influx. The effect is to manipulate the release of neurotransmitters such as NA, ACh, GABA, and glutamate (for reviews see, Barnes & Sharp, 1999; Schechter et al., 2002). Many widely prescribed psychiatric medications exert their effects through serotonin 5-HT receptors, 5-HT transporters, or both (Kroeze & Roth, 1998).

The overarching role of the serotonergic neurons in the dorsal raphé nuclei appears to be as a highly regulated pacemaker (Kaplan & Sadock, 1994). By maintaining a slow and tonic clock-like rate of firing, 5-HT neurons are able to maintain an important homeostatic function, with feedback coming through 5-HT autoreceptors and the activity of other neurotransmitters, notably NA (Aghajanian & Sanders-Bush, 2002). The brain’s axonal architecture supports this hypothetical role for 5-HT, in that axons from the dorsal raphé nuclei and median raphé nucleus neurons have storage vesicles beading along the axon, implying multiple communication ports along the length of the axon (Palmer et al., 1987), in a manner akin to NA axons. This suggests these neurons do not communicate in the point-to-point tradition, evidenced by the post-synaptic thickenings of other neurotransmitters.

### 2.3 Other Neurotransmitter and Neuroendocrine Systems

As a neurotransmitter, serotonin plays a major role in many types of behaviour (Buhot, 1997; Sirvio et al., 1994; Steckler & Sahgal, 1995). The precise nature of this functional role is as yet unclear (Meneses, 1998), but it seems likely to occur through interactions with its own and other neurotransmitter systems, in particular, the cholinergic, dopaminergic, noradrenergic, glutaminergic, and GABAergic systems (Buhot et al., 2000; Cassel & Jeltsch, 1995; Hsiao et al., 1993; Lanctot et al.,
2001; Little et al., 1995; Matsuda et al., 2002). These other systems are important in cognition, mood and movement, and disruptions to their function caused by pathophysiological deviations are inherent in neurodegenerative diseases such as SDAT, PD, and DLB. The functioning systems of the CNS may also be challenged by age-related changes in any aspect and in any of the components.

2.3.1 Acetylcholine
Acetylcholine neurons have widespread axonal projections throughout the brain and, therefore, like 5-HT, has a widespread influence on brain activity (Kaplan & Sadock, 1994). Cholinergic tracts project from the pedunculopontine nucleus (PPN) in the reticular system to the cerebral cortex, limbic system, hypothalamus, and thalamus. There are also major projections to the cerebral cortex and limbic system from the cholinergic basal forebrain, including the nucleus basalis of Meynert, which degenerate in SDAT and in Lewy body disorders, including PD. Depending on the nature of the post-synaptic receptors, ACh action may be excitatory or inhibitory (Tortora & Grabowski, 1996).

There are two ACh receptor types in the brain – muscarinic and nicotinic – and these are located in a variety of cortical areas. They are both found in the substantia nigra pars compacta (SNpc) and the substantia nigra pars reticular (SNpr) (Bonnet, 2000). The muscarinic receptors are prominent in the neuronal projections in the septohippocampal-GABA pathway crucial for learning and memory, while nicotinic receptors may be important during attentional and spatial working memory tasks (Picciotto et al., 2002). Tobacco smoking and hence, nicotinic agonism, has been linked to a reduced risk for developing PD and SDAT (Cooper et al., 2003; Kaplan & Sadock, 1994).

2.3.1.1 Acetylcholine and Ageing
Several lines of research have provided evidence for age-related declines in cholinergic function, not only in senile dementias but in normal ageing (McEntee & Crook, 1990). For example, studies have shown diminished levels of choline acetyltransferase (ChAT, the enzyme used in ACh synthesis) and acetylcholinesterase (AChE, the enzyme involved in ACh synthesis) in the hippocampus of healthy older persons (DeKosky et al., 1985; Perry et al., 1993c).

2.3.1.2 Acetylcholine and Cognition
Acetylcholine has a well documented role in behaviour; in particular, arousal and sleep, motivation and reward, motor, and cognitive function. By way of example, Picciotto et al. (2002) have differentiated the influence of cholinergic neurons in (a) the basal forebrain in arousal and regulating the sleep state and (b) the pedunculopontine tegmental nucleus with reward reinforcement behaviour and motor perseveration. There is strong evidence from a variety of sources that ACh also participates in cognition (for reviews see, Cummings & Back, 1998; Everitt & Robbins, 1997). For example, lesioning of cholinergic neurons produces cognitive deficits in animals (Dubois et al., 1985), specifically, attention, arousal, short-term spatial (or working) memory (Everitt & Robbins, 1997; Voytko, 1996), and ACh drugs alter cognitive performance in humans (Drachman & Leavitt, 1974; Molchan et al., 1992; Parrott & Dreary, 1992). Experimental manipulation of ACh activity via acute
nicotinic blockade has been shown to provoke significant cognitive impairments in older but not younger adults – comparable to those seen in dementia – indicating the vulnerability of the cholinergic system in older persons (Newhouse et al., 1992, 1994).

Importantly, cholinergic dysfunction has been well documented as being intrinsically involved in the cognitive impairments of SDAT (Iversen, 1997), PD (Bohnen et al., 2006), and DLB (Samuel et al., 1997), with losses of cholinergic forebrain neurons in PD, and especially Parkinson’s disease with dementia (PDD), being greater than occurs in SDAT (Bohnen et al., 2003). Post-mortem studies, like the latter, are usually performed on victims in late-in-the-course which may lead to overestimation of apparent damage. However, functional studies in early- and late-in-the-course PD also show the significant losses of cholinergic function. Three imaging studies have shown decreased binding of a marker for AChE in temporal regions in late-onset SDAT patients but extensive cortical decrements in PDD, and limited cortical decreases in PD patients without dementia (Bohnen et al., 2006; Bohnen et al., 2003; Kuhl et al., 1996). There are few double-blind randomised placebo-control studies investigating the efficacy of cholinesterase inhibitors in PDD and DLB (Liepelt et al., 2007), however, other types of studies have demonstrated marked improvements in cognitive function – with no, or improved effect, on parkinsonism – to the extent that sudden withdrawal from this medication produces acute cognitive and behavioural decline (Aarsland et al., 2003a; Bullock & Cameron, 2002; Leroi et al., 2004; Liepelt et al., 2007; Minett et al., 2003; Aarsland et al., 2004b; Werber & Rabey, 2001).

It has been hypothesised that the role of ACh in PD is via activity in the frontal cortex, especially since it was demonstrated that acute anticholinergic manipulation induced a cluster of cognitive deficits similar, but not as severe, as those occurring patients with frontal lobe lesions; and longer term anticholinergic treatment induced deficits in executive function (Bedard et al., 1999). Also, because positron emission tomography (PET) has shown performance on tasks assessing attention and executive function (Digit Span, Trails, Stroop) correlates with AChE activity (Bohnen et al., 2006).

It is probable cognitive impairments are attributable to compromises in the cholinergic, as well as the dopaminergic system. The involvement of both systems may be autonomous or synergistic (Werber & Rabey, 2001). Such a multifactorial hypothesis takes into account (a) the severe impairments demonstrated in tasks of executive function – in the presence of preserved functioning in other cognitive domains – in patients given anti-cholinergic medication (Bedard et al., 1999; Dubois et al., 1990) and (b) the differential effects on cognition of dopaminergic and anticholinergic medications in early-in-the-course PD patients (Cooper et al., 1992).

### 2.3.1.3 Acetylcholine and Movement

The CNS role of ACh in motor function arises from this neurotransmitter’s role in activating the interneurons of the corpus striatum (i.e., a structure within the basal ganglia comprising the caudate nucleus, putamen and nucleus accumbens, and involved in motor and cognitive processes) (Govoni et
ACh and dopamine have a reciprocal balancing relationship in the striatum. When the ACh/DA ratio is affected by dopamine depletion, there is a relative excess of cholinergic activity, and vice versa. For example, long-term pharmacotherapy targeting enhancement of the cholinergic system to improve cognition is associated with unwanted extrapyramidal side effects (Forstl, 1999); and in PD, dopaminergic drugs used to treat motoric symptoms may be accompanied by anticholinergic drugs designed to restore the balance in this neurotransmitter ratio. Also, the side effects of potent D2 receptor antagonists are overcome when co-administered with anticholinergic medication.

2.3.1.4 Acetylcholine and Serotonin

There is mounting anatomical, neurochemical, pharmacological, and electrophysiological evidence to suggest that interactions between the cholinergic and serotonergic systems may underpin cognitive function and other behaviours in animals and humans (Buhot et al., 2000; Cassel & Jeltsch, 1995; McEntee & Crook, 1991; Meneses, 1998; Richter-Levin & Segal, 1993; Ruotsalainen et al., 1998; Steckler & Sahgal, 1995). By way of example, (a) cholinergic neurons project to the raphé nuclei (Woolf & Butcher, 1989), medial raphé neurons terminate on ACh neurons in the hippocampus, and dorsal raphé neurons project to cholinergic neurons in cortical and amygdaloidal areas (Steckler & Sahgal, 1995); it appears that release of ACh may be modulated by post-synaptic 5-HT1A receptors (Barnes & Sharp, 1999), (b) neurochemical lesioning of cholinergic neurons in the nucleus basalis magnocellularis increases concentration and upregulation of 5-HT2A receptors (Lown et al., 1992), and (c) TRP infusions combined with fluoxetine (5-HT agonist) resulted in lower ACh efflux (Rada et al., 1993).

In functional terms, this relationship between the cholinergic and serotonergic systems is synergistic. As demonstrated, for example, in an experiment in which a pronounced memory impairment in rats was coupled with simultaneous loss of ACh and 5-HT to a greater extent than the effect of individual losses (Markowska & Wenk, 1991; Nilsson et al., 1988). Also, in an experiment in humans when the combination of ACh and 5-HT antagonists slowed P3 latency (i.e., subjects take longer to evoke a brain P300 wave after the presentation of a meaningful stimuli, indicative of reduced attention, interest, or arousal) to a greater extent than each drug was able to do its own (Meador et al., 1995).

At other times, however, the relationship is reciprocal with 5-HT being involved in the regulation of ACh release. For example, (a) depleting 5-HT by inhibiting synthesis or lesioning serotonergic neurons potentiates ACh release in the hippocampus and cerebral cortex (Barnes et al., 1989), (b) 5-HT3 antagonism can facilitate ACh release (Barnes et al., 1989), and (c) enhanced learning following neurochemically-induced 5-HT depletion has been blocked by lesions to cholinergic neurons originating from the nucleus basalis magnocellularis (Normile et al., 1990).

The synergism and reciprocity intimates that the interaction is possibly more complex than the action of two systems working exclusively. Most of the 5-HT receptor activity on the ACh system occurs in brain regions that underpin learning and memory (Buhot et al., 2000) and – although experiments have
shown that direct administration of 5-HT or treatment with different 5-HT agonists and antagonists has stimulated the release of ACh in rat brains – the effects on cognition in living animals and humans is orchestrated by many different actions at multiple receptor types, positioned at both pre- and post-synaptic sites located in different brain areas.

2.3.2 Dopamine

Dopamine is another neurotransmitter with a pivotal role in coordinating behaviour. Along with 5-HT and NA, DA neurons comprise less than 1% of the total neuronal population, but the extensive distribution of axons and terminals from these neurons (Palmer & DeKosky, 1993) mean they are critically positioned to influence cognition, mood, and movement. There are three distinct DA projections from neurons in the substantia nigra-ventral tegmentum area complex that have widespread projections to frontal brain areas; namely, the nigrostriatal (or the more appropriately labeled, mesostriatal), mesolimbic, and mesocortical pathways (Bjorklund & Dunnett, 2007).

There are five dopamine receptor types and numerous subtypes (Sokoloff & Schwartz, 1995). Among other locations in the brain, the D_3 to D_5 receptors are found in the nucleus accumbens, ventral putamen, nucleus basalis of Meynert, cortex, pituitary gland, hypothalamus, amygdala, hippocampus, and the cerebellum (Bonnet, 2000). The most relevant to PD and other neurodegenerative disorders are the D_1 and D_2 receptors, each having a differential and selective effect: D_1 in the nigrostriatal pathway and D_2 in the pallidostriatral pathway. They either oppose – for example, in modulating attentional function in the prefrontal cortex (PFC) (Granon et al., 2000) – or enhance each other’s activity (Seeman et al., 1989) – for example, in spatial working memory (Ellis et al., 2005).

Like 5-HT, DA operates under a strong self-regulatory mechanism which ensures equilibrium between internal and external environments (Grace, 2002). To achieve this, DA neurons maintain spontaneous spike firing via autoreceptors. However, unlike its monoaminergic counterpart 5-HT – which is a major initiator of inhibition and downstream excitation – DA acts more like a scrutineer of cellular and behavioural states in order to optimise behavioural strategy (Grace, 2002). Examples of this occur in the basal ganglia when DA mediates the response of striatal neurons to other neurotransmitters like glutamate or to the striatal initiated release of GABA and glutamate (Grace, 2002). These latter neurotransmitters are controlled by excitation at the D_1 receptors in the direct pathway and at inhibitory D_2 receptors in the indirect pathway of the corpus striatum (Govoni et al., 2001) (refer Box 1, p. 75). When this process is interrupted, as occurs in PD due to drastically reduced DA levels, the direct pathway to the thalamus is less active and the indirect becomes overactive, giving rise to classic parkinsonian symptoms.

2.3.2.1 Dopamine and Ageing

There is a significant loss of dopaminergic function with ageing (for a comprehensive review see, Barili et al., 1998; Hornykiewicz, 1987). Striatal dopaminergic function, particularly D_2, reduces from early to late adulthood (Scherman et al., 1989; Volkow et al., 1998) and this differs substantially from
that typically associated with PD (Kish et al., 1992; Zigmond & Burke, 1995). There are reports that by 60 years of age, a healthy adult has an approximate 30-50% loss of neurons in the substantia nigra; in the striatum this loss would be accompanied by a 50% reduction in DA (see Palmer & DeKosky, 1993 for review of the content of DA and other dopaminergic system elements in older adults). In comparison a person with PD will have 50% cell loss in the substantia nigra pars compacta and an accompanying 91% loss in striatal DA (Hornykiewicz, 1998; Jellinger, 2002). Nyberg (1984) reported post-mortem data from 76 neurologically healthy brains showing DA levels decreasing significantly with age in the corpus striatum, especially in the brains of people aged over 75 years of age and that the rate of this decrease increases in those over 85 years. These decrements mirror the age-related reductions in substantia nigra nucleoli volume (Mann & Yates, 1979) and DA concentrations in the midbrain, hippocampus, and corpus striatum (Adolfsson et al., 1979).

Significantly, gender – and probably gender-age effects – has a significant effect on DA neurons in the substantia nigra. This has been demonstrated by (a) the higher number of DA cells in female ovariectomised compared to male monkeys, (b) the significant reduction in cell numbers after the withdrawal of oestrogen administration, and (c) the increase in density of tyrosine hydroxylase-immunoreactive cells when oestrogen replacement was briefly resumed (Leranth et al., 2000). Similar effects of oestrogen on the dopaminergic system (Dluzen & McDermott, 2002; Dluzen et al., 2001; Dluzen et al., 1996a, b) are not necessarily mirrored by the male gonadal hormone, testosterone (Dluzen, 1996), which may be important in neurodegenerative disorders such as PD, where the ratio of affected males to females is higher (REF). However, testosterone has been linked with non-motor symptoms, such as apathy (Okun et al., 2006; Okun et al., 2002a; Okun et al., 2002b; Ready et al., 2004); apathy is linked to frontal system dysfunction and testosterone administration has been shown to improve working memory in older males (Cherrier et al., 2005; Janowsky et al., 2000), thus it is possible low testosterone levels operate in concert with low DA levels to affect frontally-directed functions in male PD patients.

2.3.2.2 Dopamine and Cognition

Experimentation with the dopaminergic system in animals has produced profound effects on cognition (Brozoski et al., 1979; Castner & Goldman-Rakic, 2004; Collins et al., 2000). Functional studies in humans, however, have produced mixed results depending on the nature of the task assessed (i.e., stimulus type, response style, level of difficulty), nature of the pharmacological challenge used (i.e., dose, potency, selectivity, administration duration), and individual differences of the subjects (i.e., age, baseline performance, genetic polymorphisms) (for reviews see, Barch, 2004; Chamberlain et al., 2006b; Mehta & Riedel, 2006; Robbins, 2003). Amidst the complexity, however, there does seem to be a pattern of dopaminergic involvement in working memory (e.g. digit ordering) (Cooper et al., 1992) and spatial working memory (Harmer et al., 2001; Luciana & Collins, 1997; Luciana et al., 1992; Mehta et al., 1999). Spatial working memory describes the processes that allows an object’s location in extrapersonal space to be held ‘on-line’ – or maintained, either with or without
manipulation – for brief intervals, after which the information can be used to guide behaviour (Luciana et al., 1998). Spatial working memory is possibly mediated by dorsal striatum neurons projecting to the lateral prefrontal, premotor, and motor cortices (Mehta et al., 2005). Optimal levels of DA are required for efficient working memory because, at least in experimental animals, too much or too little seems to impair performance (Grace, 2002).

DA is associated with tonic arousal and the readiness to respond, both behaviours that would describe the mental activity variable of attention (Pribram & McGuinness, 1975; Robbins, 1997). Stimulation of the dopaminergic system by both amphetamine and methylphenidate has enhanced performance on tasks of sustained attention (or vigilance) (Koelega, 1993). Research has suggested that the role of DA in attention is via the projections of ventrostriatal to ventromedial and orbitofrontal regions (Robbins, 2003; Wilkinson et al., 1998). This would seem likely given the influence of DA in orientating the subject to novel, as opposed to pre-presented (or predicted), stimuli (Grace, 2002). It is interesting to note that people with hypofunctioning dopaminergic systems have difficulties in performing tasks with a novelty element. For example, Ivory et al. (1999) found patients with PD were impaired when learning occurred incidentally but when they knew that what they were learning was to be assessed as part of a memory task, they performed as well as controls.

However, if DA were responsible for cognitive deficits in PD, then DA therapy would attenuate or correct these deficits. Overall the literature on patients both on and off DA medication has reported variable effects of exogenous DA on cognition (Cools et al., 2006). Some research shows no variation in cognitive performance between the on and off phases of DA enhancement (Girotti et al., 1986). Other studies, however, show differential effects for these twin phases and differential effects depending on what cognitive function is being assessed (Bowen et al., 1975; Delis et al., 1982; Fournet et al., 2000; Gotham et al., 1988; Malapani et al., 1994; Mohr et al., 1987). The effects have been hypothesised to indicate frontal lobe impairment as a result of dysfunction in the mesocortical DA system (Malapani et al., 1994; Mohr et al., 1987). This hypothesis has been supported, at least during a planning task, by blood flow changes in the PFC during PET scanning (Cools et al., 2006).

### 2.3.2.3 Dopamine and Movement

Perhaps the most observable behavioural effect of the dopaminergic system is in motor function with research focused on ameliorating, or avoiding, the motor impairments seen in native movement disorders, or in the iatrogenic dyskinesia of Parkinson’s disease and tardive dyskinesia (Rascol & Fabre, 2001). Eighty percent of the DA in the human brain is found in the basal ganglia. Tonic inhibition provided at DA terminals in the substantia nigra is essential for effective motor function and lesioning of DA neurons in this location has been shown to impair motor performance (Andersson et al., 2006). D2 receptors intrinsically suppress the activity of the caudate nucleus and, because this structure has a gate-like effect on motor activity, changes in DA availability will affect the extrapyramidal system. Blockade of D2 receptors at the end of the nigrostriatal tract by classic
antipsychotic drugs induces parkinsonian side effects, while endogenous reductions in activity at these same receptors allow the neurons in the caudate nucleus to excessively dampen motor activity, resulting in bradykinesia (Kaplan & Sadock, 1994). Second generation antipsychotics with mixed properties of DA_2/5-HT_2A antagonism, e. g., olanzapine, are associated with few extrapyramidal side effects (King & Waddington, 2004).

2.3.2.4 **Dopamine and Mood**

Because of its role in the emotion-laden behaviours of reward seeking and motivation, DA has also been linked to depression (Agren & Reibring, 1994; Ordway et al., 2002). A review (Brown & Gershon, 1993) of dopamine’s role in depression across the lifespan concluded decreased activity of this neurotransmitter is likely to underlie psychomotor retardation, diminished motivation, depressed mood, or all of these. Alterations in DA concentrations contribute to an hypothesis of DA involvement in depression, or at least, to its role in the differential of depressive subtypes. For example, (a) reduced DA activity may cause motor retardation in melancholic depression (Malhi & Berk, 2007) whereas an excess may contribute to bipolar and psychotic depression, (b) increased MAO-B (an enzyme that breaks down biogenic amines) activity is possibly associated with the higher incidence of depression in older adults, and (c) a lowered brain DA/ACh ratio, combined with a deficit of 5-HT, may be the underlying cause of the depression in PD (Brown & Gershon, 1993).

In support of the DA hypothesis, some studies have found low levels of DA metabolites in depressed patients and levodopa has been shown to cause mania. Also, amphetamines may be effective antidepressants. They produce their effects by inducing the release of catecholamines (DA and NA) and are especially effective on the release of DA from the dopaminergic-producing neurons in the ventral tegmentum area which project to the cerebral cortex and limbic areas. They also effect significant changes in the serotonergic system by causing a rapid release of 5-HT (Kaplan & Sadock, 1994).

Monoamine depletion studies, however, have not supported the hypothesis that DA is a key aetiological factor in the mood of healthy adults (for reviews see, Booij et al., 2003; Ruhe et al., 2007) but they have provided evidence for a vulnerability in patients treated with serotonin-noradrenergic reuptake inhibitors (SNRIs) to a mood lowering response (selective serotonin noradrenaline reuptake inhibitors, SNRIs, Booij et al., 2005a). Monoamine depletion is a gross rather than a target manipulation, so the outcome of this review does not negate the implication of DA in depression.

2.3.2.5 **Dopamine and Serotonin**

The ratio of homovanillic acid (HVA) to 5-HIAA is used as an index of the interaction between the dopaminergic and serotonergic neurotransmitter systems (Hsiao et al., 1993). The intimacy of these monoamine neurotransmitter systems is such that one system will act as proxy for the other. For example, cross neuronal type uptake exists, serving as a compensatory backup when a specific
transporter is dysfunctional, as was demonstrated in a rodent study when DA neurons were shown to store 5-HT for possible use as a ‘false neurotransmitter’ (Zhou et al., 2002).

There is some evidence to show 5-HT content in the dorsal raphé nuclei is moderated by DA (Lee & Geyer, 1982), however, more studies have provided anatomical evidence for an interaction weighted in favour of 5-HT mediating DA activity (Agren et al., 1986; Herve et al., 1987; Miller et al., 1975; Smith et al., 1997a; van der Kooy & Hattori, 1980). The behavioral outcomes of this in the corpus striatum are the modulation of tonic motor activity (Jacobs, 1995; Rosengarten et al., 2006), the control of motor output at the expense of sensory input (Jacobs & Azmitia, 1992), and the optimal running of motor programmes (Brooks, 2001). The behavioural outcome of 5-HT acting on the dopaminergic system in the forebrain may be tonic control on behavioural inhibition (Passetti et al., 2003) because 5-HT depletion and amphetamine administration have similar effects on impulsivity (Harrison et al., 1997; Lucki, 1998).

To understand and highlight the reciprocity of the serotonergic and dopaminergic systems in the forebrain, Luciana et al. (1998) compared the cognitive effects of acute challenges from a DA (bromocriptine) and a 5-HT agonist (fenfluramine) and found that whereas spatial working memory performance was enhanced by DA, it was impaired with enhanced 5-HT. This was further examined in a study comparing the effects of 5-HT and DA precursor depletion, e.g. ATD versus APTD (acute phenylalanine-tyrosine depletion) (Nathan et al., 2002). In this study, memory consolidation was selectively impaired by ATD while working memory performance was selectively impaired by acute phenylalanine-tyrosine depletion (APTD).

Behavioural comparisons have been made between the dopaminergic and serotonergic systems using depletion techniques (ATD and catecholamine with alpha-methylparatyrosine) to investigate mood. Results from these studies showed TRP and catecholamine depletion both lowered mood but TRP, not catecholamine, depletion demonstrated this effect to a greater extent in females than in males (Moreno et al., 2006). When females alone were studied, both ATD and APTD demonstrated similar lowering effects on mood but this effect was not statistically significant until participants had been exposed to a psychological stressor (Leyton et al., 2000b). Another precursor comparison study of ATD and APTD, again in females only, however, found no effects on mood but greater fatigue during ATD (Harrison et al., 2002b).

2.3.3 Noradrenaline

Most NA neurons are located in the locus coeruleus (Kaplan & Sadock, 1994). These neurons have projections to the cerebellum and cerebral cortex and, along with other brain stem NA neurons, have axons that collateralise extensively to provide innervation over wide areas of the brain and spinal cord (Palmer & DeKosky, 1993).

There are two broad subgroups of NA receptors – the α-adrenergic and β-adrenergic receptors – with the former inhibiting and the latter stimulating the formation of cAMP (cyclic adenosine
monophosphate) (Kaplan & Sadock, 1994). The $\alpha_1$ receptors are located post-synaptically and the $\alpha_2$ receptors are located both pre- and post-synaptically; $\beta_1$ receptors are localised on neurons and $\beta_2$ on glial cells (Bonnet, 2000). Central noradrenergic and adrenergic neurons are located in the pons and medulla oblongata of the brainstem. One of these cell groups – the locus coeruleus – projects extensively to the cortex, hippocampus, amygdala, thalamus, and the SNpc, among other regions. Thus NA neurotransmission is ideally positioned to orchestrate brain function both directly and via its synaptic modulation of other monoamines (Ordway et al., 2002).

NA is believed to have a role in affective, autonomic, cognitive, arousal, and sleep-wake processes (Ashton-Jones, 2002). A variety of neurochemicals exert strong excitatory and inhibitory actions on the locus coeruleus neurons, including glutamate, adrenaline, GABA, enkephalin, histamine, and hypocretin (Ashton-Jones, 2002).

2.3.3.1 Noradrenaline and Ageing

There is a marked age-associated loss of locus coeruleus neurons culminating in a 30% loss after, but not before, 65 years of age (Brody, 1976; DeKosky & Palmer, 1994). This is accompanied by pronounced reductions in noradrenergic activity, particularly in hypothalamic regions (Palmer et al., 1987), where turnover is also reduced (Joseph et al., 1978). Significant age-related reductions in NA concentrations in limbic structures (i.e., cingulate gyrus, hippocampus, and hypothalamus) and in cell volume in the locus coeruleus were found by Nyberg et al. (1982). Nyberg et al. also suggest age-related NA deficiencies occur in limbic but not striatum structures (Nyberg, 1984).

2.3.3.2 Noradrenaline and Cognition

Post-synaptic activity by NA has been implicated in working memory, arousal, the formation of new memories, and the encoding of reactivated memories, in particular, through activity at its $\beta$ receptor sites (Chamberlain et al., 2006b; Clayton & Williams, 2000a, b, c; Li et al., 1999; Mao et al., 1999; Przybyslawski et al., 1999; Williams et al., 2000). It has also been suggested that a long-loop feedback system between the peripheral nervous system and the CNS – involving adrenaline in the periphery and NA in the CNS – may be especially important in the processing of emotionally salient events into memories (Ashton-Jones, 2002).

The noradrenergic system appears to be involved also with arousal, vigilance, and focused attention (Chamberlain et al., 2006a; Coull, 1994), a mental activity which seems particularly vulnerable to the effects of ageing (Riedel & Jolles, 1996). NA is not thought to be involved in sustained attention (or vigilance) (Delagrange et al., 1993; McGaughy et al., 1997; O'Hanlon et al., 1998).

2.3.3.3 Noradrenaline and Mood

Significantly, NA has a role in the psychopathology of affective disorders. For example, depressive symptoms were increased when depressed patients medicated with noradrenaline reuptake inhibitors were administered alpha-methylparatyrosine (i.e., a compound that inhibits the synthesis of NA by
blocking tyrosine hydroxylase) (Miller et al., 1996). Moreover, drugs that target the biogenic amines, such as tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs), have been effective in alleviating symptoms of depression, while β-receptor antagonists like propranolol have attenuated anxiogenic symptoms (Kaplan & Sadock, 1994).

There has been a concern in the past that beta-blockers may increase the risk for developing depression in cardiac patients. However, a quantitative review of randomised trials employing data from nearly 11,000 patients, found no differences between beta-blockers and placebo for depression risk (Ko et al., 2002).

### 2.3.3.4 Noradrenaline and Serotonin

The affective effects of the noradrenergic system are indicative of a strong link between this neurotransmitter system and the serotonergic system (Asnis et al., 1992a; Asnis et al., 1992b; van Praag et al., 1990). Moreover, this functional reciprocal relationship may also be operating during learning and memory (McEntee & Crook, 1991) as evidenced by strong anatomical connections and physiological ‘cross-talk’, corroborated from immunocytochemical, histochemical, and autoradiographic studies (Asnis et al., 1992b). By way of example, NA acting through α1- and α2-adrenergic receptors has been shown to accelerate the intrinsic pacemaker activity of 5-HT (for review see, Mongeau et al., 1997) and 5-HT is purported to act on locus coeruleus afferents via post-synaptic 5-HT1A receptors (Barnes & Sharp, 1999).

It has been suggested the reason for 5-HT’s inhibition of noradrenergic activity is to restrain response to arousing stimuli in the environment; the opposite occurs when 5-HT activity is eased (Lucki, 1998).

### 2.3.4 GABA and Glutamate

GABA is the primary inhibitory neurotransmitter found almost exclusively in the CNS, particularly in the superior and inferior colliculi, thalamus, hypothalamus, and occipital lobes (Tortora & Grabowski, 1996). GABA is the primary neurotransmitter in intrinsic neurons, mediating the local activity of feedback loops (Kaplan & Sadock, 1994). GABA neurons also provide external inhibitory input to the locus coeruleus and raphé nuclei (for review see, Ordway et al., 2002). Thus GABA is able to influence behaviour either on its own or via its interaction with other monoaminergic neurotransmitters. For example, 5-HT modulates ACh release indirectly via its interaction with the GABAergic neurons that synapse onto the medial septum ACh neurons (Farr et al., 1999).

The GABAergic neurons represent a major neuronal population in the basal ganglia (Di Cara et al., 2003) and act as an inhibitory neurotransmitter in the direct output pathway (Govoni et al., 2001) (see Box 2 p.74), thus contributing to the excitatory input of the basal ganglia to the cerebral cortex. In the indirect pathway, the subthalamic nucleus uses the excitatory neurotransmitter glutamate to inhibit this usually excitatory pathway and thus lessen excitation to the cerebral cortex. The subthalamic nucleus receives input from the corpus striatum via the external segment of the globus pallidus (GPe); the initial message from the corpus striatum to the GPe is activated by GABA (Govoni et al., 2001).
Raphé nuclei afferents synapse onto GABAergic neurons (Herve et al., 1987) and may exert a phasic inhibitory control on striatal GABA transmission, thus a decrease in 5-HT neurotransmission may contribute to changes in striatal GABA neuronal activity by increasing their reactivity (Di Cara et al., 2003). A systematic inhibition of excitation to the cortex would be especially important in diseases like PD because the direct pathway to the SNpr and globus pallidus interna (GPi) is less active and the indirect pathway, through disinhibition of subthalamic nucleus, is overactive (Govoni et al., 2001).

Glutamate is a primary excitatory neurotransmitter. Of particular relevance, it also mediates many of the cortical inputs to the extrapyramidal system (Govoni et al., 2001). There are three glutamate receptors – NMDA, AMPA, and kainite – and they are all found in the striatum, but also variously in the hippocampus, hypothalamus, SNpr, GPe, and olfactory bulb (Bonnet, 2000). Glutamate is involved with response inhibition through its activation in SNpr (Hauber, 1998).

2.3.4.1 Glutamate and Ageing

Glutamate activity does not tend to decline in healthy ageing (Perry et al., 1993c) but reduced binding in the hippocampus and entorhinal cortex has been observed (Court et al., 1993).

2.3.4.2 Glutamate and Serotonin

The role of 5-HT and particularly 5-HT$_{1A}$ receptor regulation of glutamate transmission in a number of brain regions has been investigated, largely because glutamate has been implicated in a number of neuropsychiatric and neurodegenerative disorders (Schechter et al., 2002; Tsapakis & Travis, 2002). It is possible antagonism of activity at this 5-HT site may remove the inhibitory effects of endogenous 5-HT on pyramidal neurons and enhance glutaminergic activation, which would be especially potent in SDAT where significant hypoactivity of glutamate has been associated with cognitive impairment (Schechter et al., 2002).

2.3.5 Summary

Behaviour occurs in response to multitudinous activity in the CNS. Nuclei in the dorsal raphé nuclei send and receive innervation to and from broad areas of the brain, as well as intrinsically. Release of 5-HT by these neurons and the pre- or post-synaptic action they generate as a consequence is highly regulated. The nuclei are physiologically integrated with other transmitter systems via afferent and efferent pathways, but are also functionally selective in that activation by other neurotransmitters – or 5-HT itself – elicits a selective topographical pattern of release (Lucki, 1998). Abnormality or deviations in any of the components may disrupt signaling not only in the ‘home’ system but also in the neighbouring systems. Awareness of gender differences and normal age-related changes in the 5-HT system means normal variation may be used as a standard to judge deviations from this norm.

2.4 Normal Variation in the Serotonergic System

Up to this point in the chapter the basics of the serotonergic and other neurotransmitter systems and their relevance to mood, movement, and cognition have been discussed. The effects of gender and age
have been mentioned in relation to these other transmitter systems. The following section outlines how these two variables manifest in the serotonergic system to delineate normal variations in adult behaviour.

2.4.1 The Serotonergic System and Gender

There is a small but growing body of literature pointing to gender differences within the serotonin system. Research with functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) has found that, depending on the brain areas examined, men and women synthesise and utilise 5-HT at different rates, but this difference does not appear to be restricted to any specific region (Biver et al., 1996; Nishizawa et al., 1997; Okazawa et al., 2000; Sakai et al., 2006). It is possible that these will differentially influence behaviour. It is also possible behaviour may be influenced differentially by the oestrogenergic hormones.

2.4.1.1 5-HT Synthesis, Levels, Turnover and Gender

Early studies investigating TRP, 5-HT, and 5-HIAA across several areas of the rat brain found significantly higher levels of each in females suggesting higher levels, and higher rates of synthesis and turnover in female mammals (Carlsson & Carlsson, 1988a, b; Carlsson et al., 1985; Haleem et al., 1990; Rosecrans, 1970). It has been suggested that the gender difference in the rat is restricted to the hippocampus, where regional 5-HT and 5-HIAA concentrations in females have been shown to be, respectively, 34% and 36% higher than in males (Haleem et al., 1990).

A recent study using functional brain imaging of a brain trapping constant used as a proxy for 5-HT – and which in the rat brain correlates with conversion of tryptophan into 5-HT – found female rats had lower rates of synthesis to male throughout the cortex, but found no gender difference in the subcortex (Sakai et al., 2006).

In humans, a group of studies investigating cerebrospinal fluid (CSF) levels of TRP and 5-HIAA in pathological conditions, found greater concentrations in females, suggesting 5-HT turnover is possibly faster in this gender (Agren et al., 1986; Asberg et al., 1973; Young et al., 1980). Similar weighting in females was observed in the post-mortem brains of victims with neurologic, metabolic and psychiatric disease (Bucht et al., 1981).

Studies that experiment with reducing central 5-HT levels with acute tryptophan depletion (ATD) in humans have reported firstly, a decline in 5-HT turnover of nine and a half times in males and forty times in females during acute tryptophan depletion; the rate of synthesis was approximately 50% higher in males (Nishizawa et al., 1997). And secondly, a differential rate of 5-HT synthesis for men and women in different brain regions (Okazawa et al., 2000). This lower synthesis but higher turnover in females may underlie the susceptibility of women to 5-HT related psychiatric and neurological disorders.
There is evidence that oestrogen increases TPH1 mRNA expression in animals (Gundlah et al., 2005). Dietary studies have shown that a calorie restricted diet can reduce peripheral TRP levels by approximately 10% in women but not in men, suggesting a greater reserve in men (Anderson et al., 1990; Smith et al., 2000). This change in plasma TRP has been shown to result in upregulation of certain neuroendocrine measures of 5-HT function. Interestingly this upregulation does not appear to occur in women with a history of depression (Anderson et al., 1990; Smith et al., 2000).

The evidence is tentative, with inconsistencies arising possibly from methodological artefacts. For example, (a) measurement of CSF levels of 5-HIAA may be confounded by other processes during the metabolite’s transportation out of the spine (Nishizawa et al., 1997) and, (b) 5-HT levels may be misread because 5-HT deteriorates during the first few hours of death (Nyberg, 1984). These aside, animal and human studies do point to a gender difference in the serotonergic system such that males have greater synthesis and females have faster turnover, however, this is still uncertain.

### 2.4.1.2 5-HT Receptor Numbers and Gender

PET data investigating the 5-HT$2_r$ receptor in post-synaptic locations indicates greater binding capacity in males than in females in most brain regions, and a “…consequent apparent ‘downregulation’ of these receptors in women compared to men” (Biver et al., 1996, p. 27). Other PET studies have found gender differences in 5-HT$2_A$ (Baeken et al., 1998) and 5-HT$1_A$ binding but conversely a greater binding in females (Arango et al., 1995; Parsey et al., 2002).

### 2.4.1.3 5-HT Function and Gender

Pharmacoendocrine studies have generated evidence for gender differences in serotonergic activity. McBride et al. (1990) found females had a greater prolactin response to fenfluramine, which is a response used to assess the integrity of the serotonergic system. Challenges with TRP depletion, and the 5-HT agonists m-chlorophenylpiperazine or buspirone, in different subject groups, induce significantly stronger effects on affective state in females than in males (Delgado et al., 1989; Kahn et al., 1991; Meltzer & Maes, 1994).

Oestrogen is a term used for a group of hormones present in both males and females but to a much higher level in women of reproductive age. Between the ages of 40 and 50, the ovaries become less responsive to stimulation from gonadotropic hormones, with a consequential reduction in oestrogen and progesterone production (Tortora & Grabowski, 1996). There are number of symptoms experienced by women around the time of menopause – which include changes in mood and cognition (Miller et al., 2002) – which may mean a role for oestrogen in these behaviours. Women have increased vulnerability to major depression, especially postpartum, and are more sensitive than men to mood disturbance secondary to ATD (Booij et al., 2002; Ellenbogen et al., 1996; Smith et al., 1997b). Women may experience memory impairment and mood change during the premenstrual phase of the oestrus cycle (Symonds et al., 2004), which can be restored after tryptophan loading (Schmitt et al., 2005), suggesting a relationship between this hormone and 5-HT.
Two recent studies (Amin et al., 2006; Newhouse et al., 2007) have investigated the interaction of the serotonergic system and oestrogen on mood using ATD. Neither study demonstrated a change in mood on the test day but a positive effect on mood was observed in the former study the day following TRP and oestridial manipulation (Amin et al., 2006).

Another recent study investigated the memory effects of acute tryptophan depletion in women (Sambeth et al., 2007) and found the disruption in memory induced by this technique was especially pronounced during the follicular phase of the cycle, a phase that is controlled by oestradiol, the most potent of all oestrogens. The effect of age was also examined in this study to see if the menopause would attenuate the ATD induced memory deficits. Interestingly, in finding no effect of age, the authors also suggest oestrogen levels may not affect memory during ATD. However, a study comparing the effects of oestrogen therapy and ATD found oestrogen attenuated a prior ATD-induced impairment in verbal memory (Amin et al., 2006). This was the same study, above, that reported enhanced mood the day following ATD. There is a possibility, therefore, that a disruption to either oestrogen or 5-HT may affect memory and mood in post-menopausal women.

2.4.1.4 Commentary

There appears to be gender differences in the serotonergic neural system which may manifest in a gender-biased vulnerability to changes in 5-HT availability. That is, whole brain synthesis of 5-HT is lower, but metabolism is higher, in females compared to males. There is also a suggestion that oestrogen may interact with the serotonergic system to influence mood and cognition. Given that neuroanatomical changes throughout adulthood unfold along different time scales for males and females (Cowell et al., 2007), it is essential gender differences in serotonergic activity are examined as part of any study investigating the role of 5-HT in behaviour.

2.4.2 The Serotonergic System and Ageing

Serotonin neurons are one of the first brainstem neurons to emerge during prenatal development and the serotonergic system is the first to innervate the earliest cells in the cerebral cortex (Azmitia, 2001). From this nascent appearance through normal development to old-old age, 5-HT participates in maintaining peripheral and central processes (i.e., in blood vessel, platelet, skeletal muscle, cytoskeletal, and neuronal functioning, as well as cell maturation/proliferation/apoptosis) (Azmitia, 2001). Thus it is not surprising the serotonergic system is investigated for its aetiological – and therapeutic – role in the morphological changes and psychopathological plasticity that can appear in later life.

Although there are reports that concentrations of 5-HT and 5-HIAA are not correlated to age, for example, Wester et al. (1984), there is other research, including post-mortem data (Gottfries, 1990; Marcusson et al., 1984), functional imaging (Meltzer et al., 1998b; Rosier et al., 1996; Wong et al., 1984), and functional challenge (Lerer et al., 1996; McBride et al., 1990), that is painting a picture, albeit complex, for age-associated changes in the 5-HT system (Meltzer & Reynolds, 1999).
2.4.2.1 5-HT Synthesis, Level, Turnover and Ageing

Rehman and Masson (2001) reported total levels of 5-HT decrease with age, to the extent levels in the putamen are reduced 50% in the 60-90 year age span (Gottfries, 1990). Previously, McEntee and Crook (1991) and Nyberg (1984) asserted that post-mortem studies had failed to provide evidence for a decline in 5-HT concentrations or those of 5-HIAA.

Age-related changes in serotonergic function have been reported by Agren et al. (1986), Asberg et al. (1973), Bowers and Gerbode (1968), and Gottfries et al. (1971) who all found turnover of 5-HT, as assessed by cerebrospinal fluid (CSF) levels of 5-HIAA, was positively correlated with age. The effect appears unrelated to serum oestrogen level or contraception medication.

It is possible the number of 5-HT metabolite excretions may be abnormally increased with ageing (e.g., kynurenine, kynurenic acid, and N-alpha-acetylkynurenine), perhaps indicating an increase in 5-HT anabolism and catabolism in older persons (Crepaldi et al., 1975). Alternatively, synthesis may be altered because a high proportion of older adults have suboptimal levels of pyridoxine – a necessary cofactor in hepatic kynurenine metabolism and in the conversion of 5-HTP into 5-HT (Madigan et al., 1998).

2.4.2.2 5-HT Receptor Number and Ageing

A large number of studies (for review see, Meltzer, 1999), have demonstrated widespread cortical reductions in 5-HT$_{2A}$ binding with age, suggestive of a 50% loss in this receptor in older (61-76 years) compared to younger adults (18-29 years). A recent study placed these losses in middle rather than late life (Sheline et al., 2002). 5-HT$_{2A}$ receptors have been implicated in the pathophysiology of mood disorders (Baeken et al., 1998) suggesting a potential link between these receptors, age, and mood.

5-HT$_{1A}$ receptor density and binding sites of the serotonin transporter (SERT) do not seem to decline with age (Parsey et al., 2002; Rabiner et al., 2002; van Dyck et al., 2000).

In the post-mortem study by Palego et al. (1997), female brains showed age-related decrements in maximal binding (i.e., demonstrating receptor density) in the parietal cortex and hippocampus, and increases in occipito-cortical membrane. Conversely, in a PET study using healthy living participants, Meltzer et al. (2001) observed that binding in males was inversely related to age. Both studies included a broad age range (21 - 83 years).

Also, post-menopausal decreases in oestrogen levels may be associated with alterations in central 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors densities as well as circulating 5-HT levels (Meltzer et al., 1998a).

2.4.2.3 5-HT Function and Ageing

Despite evidence for 5-HT involvement in cognition in aged rats (Richter-Levin & Segal, 1993), research investigating serotonin and cognition in aged humans is sparse (Palmer et al., 1987). Bucht et al. (1981), Banki and Molnar (1981), Nyberg (1984), and Wester et al. (1984) have found no
consistent pattern of age-related changes in the main indices of serotonergic function (e.g., 5-HT receptor binding, pharmacological effects, and 5-HIAA), but experimental reduction of 5-HT has been reported to worsen global cognitive status in a group of older adults (Porter et al., 2000). The pattern of cognitive impairment secondary to this challenge in older adults differed from that in younger subjects and thus deserves further examination (Porter et al., 2003b).

Cognitive dysfunction is common in older persons suffering depression (Nebes et al., 2003) – possibly attributable to comorbid vascular pathology (Alexopoulos et al., 2002) – and depression coexists with cognitive impairment in dementia (Gray et al., 1999). SSRIs are well tolerated in dementia patients (Gray et al., 1999) and have demonstrated cognitive enhancing properties for both depressed and dementia patients (Gottfries, 1996; Kerr et al., 1993). They also appear to have no detrimental effect in healthy older persons (Knegtering et al., 1994), so their serotonergic-enhancing properties may well be an indicator for the usefulness of serotonergic strategies in improving depressive and cognitive abnormalities in older persons. Age-related declines in 5-HT_{2A} receptor density and age-related vulnerability are hypothesised to underlie depression in older age (Lerer et al., 1996; Meltzer et al., 1998b), but definitive evidence for this association has not been forthcoming from binding and pharmacological challenge studies.

2.4.2.4 Commentary

There is no complete picture of decline in the serotonergic system in healthy ageing. Turnover rate is increased, some receptor types are reduced, and a number of processes involved with syntheses and metabolisation are altered as part of natural ageing. However, the net effect of these changes is difficult to assess in humans. Autopsy studies provide valuable data but are limited by a number of caveats, for example, the instability of neurochemicals post-mortem and the incapability of demonstrating functional activity. Other methods, including neuroimaging and biochemical challenge, are available to assess function in terms of behavioural outcomes in a wide variety of healthy and patient populations. It is vital age is considered in studies investigating mood and cognition because significant effects of age have been observed in depressed older persons on a number of neuropsychological tasks, especially those assessing psychomotor speed and executive function (Lockwood et al., 2002; Tarbuck & Paykel, 1995).

2.5 Abnormal Variation in the Serotonergic System

The final section of the chapter touches on abnormal variations in the serotonergic system, the variations that arise through the interactions this system has with other, now, dysfunctional systems in the neurodegenerative disorders of SDAT, PD, and DLB, and how these changes affect behaviour. For example, degeneration in the nuclei that produce ACh, 5-HT and DA – the basal forebrain, dorsal raphé, and substantia nigra – may underlie the cognitive impairment, depression and vigilance impairment that are concomitant in PD, irrespective of dementia (de Vos et al., 1996). Treatment research for the cognitive and mood problems in all three neurodegenerative disorders has focused on
finding a replacement for the neurotransmitters whose activities are lost, in much the same manner that levodopa and DA agonists have proven to be an effective therapy for motor symptoms in PD.

Other clinical symptoms in these disorders include memory loss and other cognitive impairment – e.g., visuospatial problems – as well as psychosis (e.g., hallucinations), mood (e.g., depression and anxiety), aggression, and sleep disturbance (Court & Perry, 1991; Lanctot et al., 2002). The serotonergic system has a role in these behaviours, thus it seems reasonable to consider that in SDAT, PD, and DLB, this system is either directly dysregulated or dysregulated as a result of deficits in neurotransmitter systems generally reactive to 5-HT regulation. There may also be alterations in the balance that underlies the oppositional or synergistic effects of 5-HT and these transmitter systems.

2.5.1 Serotonin, Neurodegenerative Disorder and Cognition

The role of 5-HT in cognition is most likely to occur through its modulation of the cholinergic system, in particular, through activity at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{3} receptor sites (Meltzer et al., 1998a). This has been illustrated in two rodent studies when (a) lesioning of medial and dorsal raphé neurons resulted in decreased 5-HT concentration but increased ACh turnover in the rat hippocampus, cerebral cortex, or both, but not the striatum (Robinson, 1983) and, (b) combined administration of the 5-HT_{2A} antagonists ketanserin and the acetylcholinesterase inhibitor physostigmine enhanced memory in the rat (Normile & Altman, 1992). In healthy humans, combined administration of metachlorophenylpiperazine (m-CPP: mixed 5-HT agonist/antagonist) and scopolamine exaggerated the cognitive impairment of scopolamine administered alone (Little et al., 1995). It is not surprising these effects occur since most of the 5-HT receptors interact with the cholinergic system in the hippocampus and the frontal cortex (Buhot et al., 2000) – two areas of the brain intimately associated with cognition and cognitive impairment of SDAT.

Neurodegenerative dementia is associated with marked abnormalities in the cholinergic system, even in the earliest stages of disease progression (refer to tables in, Court & Perry, 1991 on p. 425 and p. 426). In SDAT this is linked to memory impairment and in PD and DLB also with hallucinations (for reviews see, Court & Perry, 1991; Emre, 2003; Simard et al., 2000). As noted earlier, 5-HT performs an inhibitory role on the cholinergic system and could be hypothesised to dampen an already compromised system. This is particularly so when the inhibition of ACh release occurs in two brain areas associated with learning and memory (Lanctot et al., 2001). Animal evidence suggests age-related dysfunction in the cholinergic and serotonergic systems is associated with age-related memory impairment (Buhot et al., 2003). In PD and DLB, cholinergic loss is extensive and more severe than that seen in SDAT (Bohnen et al., 2006; Candy et al., 1983; Langlais et al., 1993; Perry et al., 1985; Perry et al., 1993b; Perry et al., 1995; Tiraboschi et al., 2000a; Tiraboschi et al., 2000b), and correlates with the severity of cognitive impairment and hallucinogenesis (Perry et al., 1990b). Moreover, in hallucinating DLB patients, reductions in a marker for ACh function which demonstrates this cholinergic loss is accompanied by increases in a marker for 5-HT function in the temporal and parietal cortex (Court & Perry, 1991). Reducing the inhibition of 5-HT may be an initiative for...
therapeutic strategies aimed at improving the cognitive deficits and hallucinations associated with SDAT, PD, and DLB.

The role of DA in cognition in SDAT is less clear, but is important in PD because treatment with typical antipsychotics can cause motor symptoms and atypical antipsychotics can cause memory impairment and confusion (Hanagasi & Emre, 2005). The concern is even greater in DLB because antipsychotic treatment with DA antagonists is related to an adverse neuroleptic response which leads to unremitting rigidity and death (McKeith et al., 1992a). It is important, therefore, that individual neurotransmitter functions and their interactions as they operate in the different disorders are clearly understood. Pharmacotherapy for one symptom may create or exacerbate other symptoms, such that anticholinergic and chronic levodopa therapy for motor symptoms in PD can precipitate memory loss and hallucinogenesis (Dubois et al., 1990; Katzenschlager et al., 2003; Zoldan et al., 1995).

These examples suggest polypharmacotherapy may help or hinder treatment and that knowledge of the different neurotransmitter systems in these conditions may inform strategies using cholinergic, dopaminergic, and serotonergic compounds having enhancing, blocking, or combination activity, based, perhaps, on their preferential effects across different brain regions.

2.5.2 Serotonin, Neurodegenerative Disorder and Mood

The same may be hypothesised for the interactions between 5-HT and NA, 5-HT and DA, or the pathological alterations in serotonergic function with respect to depression in these disorders (Ballard et al., 2002a; Mendlewicz et al., 1981; Perry et al., 1990b; Zubenko et al., 1990). PET data has updated indirect evidence (e.g., animal models, post-mortem human studies, measurements of 5-HT binding, and CSF metabolite levels) but a direct match between pathophysiology and clinical symptoms is not yet available (Meltzer et al., 1998a). Depression is common in the senile dementias (Burn, 2002; Meltzer et al., 1998a; Swanberg & Cummings, 2002) leading to questions over which neural substrate or substrates underlie affective states.

Despite several open trials of 5-HT precursors (Mayeux et al., 1988) and SSRIs (Hauser & Zesiewicz, 1997) there has been no satisfactory placebo controlled trial of antidepressant therapy for depression in PD or DLB (Aarsland & Cummings, 2002). With evidence that side effects of treatment include hallucinations and confusion, the authors of a Cochrane Review (Chung et al., 2003) suggest there is insufficient data with which to make recommendations for their use in PD. One study has looked at the effect of reducing 5-HT in SDAT and found cognition but not mood was affected (Porter et al., 2003b). However, no study has looked at a 5-HT challenge in demented PD patients or those with DLB and when the present research was commissioned none had investigated this in non-demented PD patients. After the start of the current study, one group published work on the effects of ATD in PD (Leentjens et al., 2006; Scholtissen et al., 2006b).

The parkinsonism of PD and DLB is attributed to DA and possibly, given the correlation between depression and cognitive impairment (Stefanova et al., 2006) and the hallucinatory nature of
anticholinergics for treating motor symptoms in some PD and DLB patients (Sanchez-Ramos et al., 1996), to ACh losses (Aarsland et al., 2001b). Pharmacological treatment currently involves levodopa in patients with prominent motor symptoms, however, this and other DA agonists can exacerbate hallucinations, thereby outweighing the potential benefits of increased mobility (Swanberg & Cummings, 2002).

2.5.2.1 Commentary

Understanding of the serotonergic system in the neurodegenerative disorders of SDAT, PD, and DLB is limited and still evolving. A number of neurotransmitter systems are implicated in a variety of clinical symptoms, in particular psychiatric, cognitive, affective, and motor signs. Unfortunately the dearth of functional studies investigating these symptoms means insights from pathophysiological studies lack a certain perspective. The cost of these symptoms to patient and care-giver and, sometimes, the adverse side effects of symptom treatment (Gray et al., 1999), mean it is timely for functional studies to be undertaken.

2.6 Summary

The serotonergic system has been investigated in a number of ways to broaden our understanding of how humans think, act, feel, and move; how people behave when development is normal and how they behave when it is not. Gender and age are pertinent factors which, at the most basic level, point to the morphological and physiological differences that may arise in this neurotransmitter system. Very little is known about the way age and gender manifest in the serotonergic system of the healthy brain and very little is known about their affect on 5-HT function in the behaviour of healthy living humans. Both age and gender have tentatively been shown as risk factors for serotonergic hypofunction, but larger and more comprehensive studies are required with which to make robust interpretations and recommendations for serotonergic agents in treatment strategies.

Many somatic and psychiatric disorders include loss of normal brain morphology and patients may experience cognitive and emotional breakdown as a result of these changes. A preliminary 5-HT challenge study has been conducted in SDAT patients (Porter et al., 2003b), but there are other disorders in older persons that may have links to the serotonergic system. Patients with these disorders have symptoms that are being treated with serotonergic strategies whose safety and efficacy have not been solidly evidenced-based (Deakin et al., 2004). Some older persons have cognitive impairments and these may be ameliorated when clinical depression is treated. Moreover, neurodegenerative disorders having prima facie cases for aberrations in systems other than the serotonergic, may have a symptom profile that includes dysregulation in the relationship these neurotransmitter systems have with the serotonergic system. A functional investigation into the role of 5-HT in one of these disorders, PD, would provide useful knowledge for treatment options and pharmaceutical restorative or compensatory strategies, as well as providing possible markers for diagnosis and prognosis.
3 SEROTONIN, TRYPTOPHAN, AND ACUTE TRYPTOPHAN DEPLETION

Serotonin is manufactured in the body from the amino acid tryptophan. Some protein foods are naturally higher in TRP than others (Markus et al., 2002), but the content is small compared to that of the other amino acids which compete with TRP for cellular uptake. Increasing the consumption of good quality protein, however, will not necessarily increase the amount of 5-HT in the brain because, paradoxically, its greater molecular weight and numerical disadvantage reduces brain TRP levels (Maurizzi, 1990).

This information has been utilised experimentally in animals and humans to investigate the serotonergic system. A large number of studies have manipulated TRP levels to examine the integrity of the serotonergic system under normal conditions, as well as the degree to which behaviours can be altered in abnormal conditions. This chapter provides a description of TRP and of its conversion into central 5-HT. The chapter explains the technique of acute tryptophan depletion for lowering central levels of 5-HT and its effectiveness. This is followed by an overview of the procedure, the peculiarities of age and gender, and a concluding section on the limitations and advantages of the technique.

3.1 Tryptophan

Tryptophan is an amino acid which is the precursor of a number of biologically active compounds, including serotonin, melatonin, tryptamine, nicotinamide, kynurenic acid, quinolinic acid, and xanthurenic acid. TRP has been associated with weight control, pain, aggression, affective disorder, and sleep disorder (for reviews see, Knapp et al., 2003; Sainio et al., 1996). Physiologically, TRP plays an active role in protein synthesis and the creation of niacin (Birdsall, 1998; Sainio et al., 1996).

Of the two major pathways of tryptophan metabolism in humans, the most quantitatively important, accounting for 90% of TRP catabolism, is the kynurenine pathway. Several neuroactive metabolites arise in this pathway as a result of degradation by tryptophan pyrrolase (tryptophan-2,3-dioxygenase) in the liver (Pakes, 1979) and indoleamine-2,3-dioxygenase in various tissues (Sainio et al., 1996). Tryptophan pyrrolase is induced by TRP and cortisol (Sainio & Sainio, 1990) and its products include intermediates like kynurenic acid, and by-products like xanthurenic acid, picolinic acid, quinolinic acid, and niacin (nicotinic acid, Knapp et al., 2003), NAD (nicotinamide adenine dinucleotide) and its phosphate NADP. The other major pathway – the indoleamine pathway – is induced by interferon gamma (Taylor & Feng, 1991) and produces neuroactive metabolites including serotonin, melatonin, and tryptamine (Sainio et al., 1996). It is this pathway that provides the focus for the investigations in this thesis.

TRP is an aromatic indolypropionic acid and is one of eight essential amino acids necessary in human nutrition (Sainio et al., 1996), which means it must be derived from the diet (see point a in Figure 3-1). It is the only amino acid bound to plasma albumin (McMenamy & Oncley, 1958) and its concentration in the body is the most modest of all amino acids (Sainio et al., 1996). Both these factors are important
in determining CNS levels of 5-HT because any 5-HT in the brain is synthesised solely from the TRP that crosses the blood brain barrier, there being no equilibration between 5-HT in the body and 5-HT in the brain.

TRP competes with five other large neutral amino acids (LNAAs, i.e., phenylalanine, methionine, tyrosine, valine, leucine, and isoleucine) for a specific membrane carrier to transport across the blood brain barrier into the brain (at point c in Figure 3-1, Fernstrom, 1994; Pakes, 1979). A homeostatic ratio means the brain level of each individual amino acid will depend not only on the plasma level of each, but the plasma levels of all the others competing for the same transport system.

Once in the brain, TRP is converted into 5-HTP (see point f in Figure 3-1) by the enzyme tryptophan hydroxylase (TH, see point e in Figure 3-1) and the co-factors oxygen, iron, tetrahydrobiopterin, and ascorbic acid (vitamin C) (Birdsall, 1998; Cooper & Melcer, 1961). 5-HTP is then decarboxylated by aromatic acid decarboxylase, along with the cofactor pyridoxine (vitamin B6) into 5-HT (see point g in Figure 3-1, Knapp et al., 2003; Rose & McGinty, 1968). After a half-life in healthy adults of 2.7 hrs (Pakes, 1979) or 2.65 hrs in depressed patients (Knapp et al., 2003), the final degradation of central TRP occurs when monoamine oxidase catabolises TRP into 5-HIAA (Pakes, 1979).

3.1.1 Summary
TRP in the diet is involved in several physiological processes before it enters the CNS and is converted into 5-HT. There are a series of steps and products involved in the metabolism of TRP into 5-HT and its excreted end-product, 5-HIAA.

3.2 Determinants of CNS Serotonin Levels
Serotonin levels in the brain are dependent on several factors in addition to the dietary intake of its precursor TRP, other LNNA substrates, and the cofactors. This includes the amount of TRP saturating the key regulatory enzyme tryptophan hydroxylase (Fernstrom, 1994). Because this enzyme is approximately 50% saturated with TRP (Young & Gauthier, 1981), the hydroxylation of TRP by tryptophan hydroxylase is the rate limiting step in the conversion of TRP into 5-HT. TRP concentration in the brain is thus the major determinant of 5-HT synthesis (Fadda, 2000); a fact underpinning methods that use TRP intake to manipulate brain levels of this neurotransmitter. Also included is the amount of TRP available for uptake from plasma. Critically, plasma TRP concentration and central 5-HT levels can be affected by:

a. The requirement of the body to use TRP in the manufacture of proteins. An ingested load of amino acids will initiate this process in the liver (see point b in Figure 3-1).

b. The catabolic pathway initiated by tryptophan pyrrolase (i.e., L-tryptophan-2,3-dioxygenase) (Young, 1986).

d. The conversion of TRP to kynurenine because elevated kynurenine levels induce tryptophan pyrrolase (Young, 1986).

e. The competition between TRP and kynurenine, and between TRP and the other LNAAs for the same transport molecule to cross into the brain at point e in Figure 3-1 (Fukui et al., 2007; Pakes, 1979; Russo et al., 2003a; Sidransky, 1997).

f. The regulation of central 5-HT levels by pre-synaptic autoreceptors.

These critical determinants (i.e., TRP, LNAAs, cofactors, tryptophan hydroxylase, cortisol, tryptophan pyrrolase, kynurenine, and pre-synaptic receptors) help regulate the level of 5-HT in the brain. Alterations in any one of them will affect 5-HT levels, which is an opportunity used by researchers to investigate the function and integrity of serotonergic system.

Figure 3-1 Mechanism for conversion of dietary tryptophan to 5-HT in the CNS.

Amino acids from the diet (a) are circulated to the liver for protein synthesis (b) or to the CNS where they must compete with other LNAAs for transport to cross the blood brain barrier (c). Once in the brain, tryptophan (d) is synthesised into 5-HTP (f) with the help of tryptophan hydroxylase (e), and thence into 5-HT (g). 5-HT = serotonin; CNS = central nervous system; LNAAs = large neutral amino acids (Drawn from a blank brain template sourced, HowStuffWorks, 1998-2007).

Techniques used by some researchers incorporate manipulation of the precursor TRP, through either TRP loading, dieting, or TRP depletion. For example, intravenous TRP administration has been shown to increase plasma TRP levels and the TRP/LNAA ratio (Price et al., 1991), but to significantly lower mood (Sobczak et al., 2002b). This counterintuitive outcome highlights the complexity of this system, with the authors hypothesising TRP activity was probably lowered via a negative feedback loop.
Extreme dieting has been shown to lower plasma TRP levels by restricting intake of all amino acids at point a in Figure 3-1. For example, a 1000 calorie daily diet for 3 weeks reduced plasma tryptophan levels only by about 10% and plasma TRP/LNAA ratio only by 15% in healthy females (Anderson et al., 1990; Smith et al., 2000), and even less in men (Anderson et al., 1990).

The preferred way to severely and robustly reduce serotonin levels, however, is not through diet but through rapid TRP depletion (Young & Leyton, 2002). Based on processes that metabolise TRP into 5-HT, there are several vectors for effecting this rapid reduction. These are:

a. Inhibiting metabolism at the rate limiting step (i.e., at point d in Figure 3-1) by inhibiting the activity of tryptophan hydroxylase with p-chlorophenylalanine (PCPA). However, this method is limited in humans by toxicity (Reilly et al., 1997).

b. Preventing access to the brain by increasing the load of another amino acid (i.e., at point c in Figure 3-1). This has been done successfully with valine loading (Williamson et al., 1995).

c. Limiting TRP (i.e., at points a, b, and c in Figure 3-1) by loading the body with amino acids other than tryptophan in order to increase firstly, protein synthesis by the liver with a subsequent decrease in circulating TRP (Biggio et al., 1974) and secondly, competition for the membrane transport molecule. This challenge test is called acute tryptophan depletion (ATD).

3.2.1 Summary

The amount of 5-HT in the CNS is regulated by the concentrations of amino acids in circulation, the uptake of these amino acids by the liver for protein synthesis, the competition between TRP and the other LNAAs, the dynamic activity of TRP pyrrolase, kynurenine and cortisol, and the pre-synaptic 5-HT receptors. ATD utilises a number of these factors to significantly lower CNS levels of 5-HT.

3.3 Acute Tryptophan Depletion

ATD is a technique that robustly effects a temporary and tolerable reduction in central 5-HT activity (for reviews see, Moore et al., 2000; Reilly et al., 1997). The technique has been shown to reduce central 5-HT synthesis in animals (Gessa et al., 1974) and humans (Nishizawa et al., 1997); and in animal brains, to a change in 5-HT release (Fadda et al., 2000; Stancampiano et al., 1997). There have been 220 studies investigating ATD in over 3,900 people and not one has reported serious adverse side effects (Booij & Van Der Does, 2007).

To produce a reduction in 5-HT, ATD comprises a TRP deficient amino acid or protein treatment containing large amounts of LNAAs but devoid of TRP (Hood et al., 2005). The composition of amino acids in the ATD drink is in the same proportion as food meant for human consumption (i.e., human milk), except it is missing aspartic acid and glutamic acid – because of toxicity concerns – and, of course, TRP (Young et al., 1989; Young et al., 1985).
After the protein drink is ingested, the digestive system cleaves the peptides into amino acids (Feigin et al., 1971) and circulates them in the blood to different locations around the body. Most of the tryptophan (65-78%) in this blood is loosely bound to plasma albumin (Lipsett et al., 1973) to be used in peripheral processes, including conversion to 5-HT in the kidneys, liver, stomach, and small intestine (Knapp et al., 2003). Although some of this circulating TRP is bound— which may in a very limited way cross the blood brain barrier (Etienne et al., 1976; Yuwiler et al., 1977) – it is the unbound (or free) TRP that is the most likely to be associated with changes in brain 5-HT and subsequent behavioural outcomes in humans (Delgado et al., 1990).

The ingested load of amino acids, plus any amino acids already in circulation, are also drawn into the liver for protein synthesis (Biggio et al., 1974). During ATD this early manufacturing process results in a severe reduction of TRP levels in both blood and tissue (Young & Leyton, 2002).

ATD takes advantage of this protein building process (Moja et al., 1991) as it does from another physiological process, the quantitative rivalry of other amino acids for the same transport molecule to cross into the brain.

Only one of three amino acid transport systems is accessible for the transport of TRP into the brain (Oldendorf & Szabo, 1976; Pakes, 1979; Pardridge, 1986) and, as previously mentioned, TRP must compete for this carrier with the other LNAAs (Pakes, 1979). TRP concentrations in the brain, therefore, are determined not only by how much TRP is available in the diet and how much is unbound – or free – in the blood, but on the ratio of TRP to other LNAAs, i. e., the TRP/LNAA ratio (Moja et al., 1989).

The type of food ingested is fundamental to the integrity of this ratio. A meal high in protein has no effect on the TRP/LNAA ratio, whereas it raises the tyrosine-phenylalanine/LNAA ratio; a non-protein, or carbohydrate meal, will not influence the tyrosine-phenylalanine/LNAA ratio but will increase the TRP/LNAA ratio. Serotonin synthesis rapidly reflects these meal effects (Fernstrom, 1990). In everyday terms, this counter-intuitively means a high protein meal does not raise brain TRP levels since it also increases the levels of other amino acids; a carbohydrate-rich meal on the other hand will raise TRP levels and the TRP/LNAA ratio (Madras et al., 1973; Markus, 2007; Wurtman et al., 1980). The reason for this is that carbohydrates stimulate the release of insulin, insulin then draws the branched chain amino acids (leucine, isoleucine, and valine) out of circulation and into muscle tissue; TRP levels in the brain increase because there is less competition for the transport molecule from these now curtailed rivals (De Montis et al., 1978; Sainio et al., 1995).

Availability of TRP, binding to albumin, protein synthesis, competition with other LNAAs for the transport molecule to cross into the brain are thus crucial factors in determining how much TRP crosses the blood brain barrier to be synthesised into 5-HT. Clearly, the extent to which the technique effects a reduction in 5-HT level is important if behavioural outcomes are to be attributable to reduced 5-HT levels.
3.3.1 Summary
ATD involves the ingestion of a load of amino acids minus TRP. It has been used reliably to produce a significant reduction in 5-HT synthesis, release, and turnover.

3.4 Efficacy of ATD
Several biological mechanisms have been investigated to assess changes in central serotonergic metabolism in animals and humans after ATD; this includes plasma TRP levels, LNAA levels, 5-HT synthesis, 5-HT release (in animals only), and 5-HT turnover (with 5-HIAA appraisal).

There is good evidence that ATD reduces plasma TRP, central 5-HT (synthesis, levels, or release), CSF 5-HT, and 5-HIAA levels in rats (Bel & Artigas, 1996; Biggio et al., 1974; Gessa et al., 1974; Moja et al., 1989; Stancampiano et al., 1997), primates (Young et al., 1989), and humans (Carpenter et al., 1998; Fadda, 2000; Nishizawa et al., 1997; Sakai et al., 2006; Williams et al., 1999) without affecting other monoaminergic neurotransmitters or their metabolites (Young et al., 1989).

Both free and total (i.e., unbound + bound) plasma TRP levels have decreased to a maximum of 60-90% within 5 – 7 hrs after ATD (Delgado et al., 1990; Moore et al., 2000; Reilly et al., 1997; Van der Does, 2001a; Young et al., 1989; Young et al., 1985); following a placebo treatment, they have increased by variable increments (for table of effects see, Fusar-Poli et al., 2006). TRP depletion coincides with significant reductions in 5-HT synthesis (Nishizawa et al., 1997).

Attempts have been made with microdialysis to determine the degree of 5-HT release in animals from serotonergic neurons (Fadda et al., 2000). Depletion induced a 50% reduction in 5-HT release in the frontal cortex, plus declines of 38.5% in hippocampal and 35% in striatal regions (Brown et al., 1998). The same percentage reduction was induced by depletion in rats pre-treated with an SSRI (Bel & Artigas, 1996).

PET brain scans substantiate the effect of ATD by demonstrating the involvement of most brain regions in reduced synthesis, and that this effect is not just a result of general brain metabolism (Cahir et al., 2007; Harrison et al., 2004; Leyton et al., 2000b).

The effect of ATD has also been observed in melatonin, with secretion of this 5-HT product attenuated across all (1 hr or 2 hr) nocturnal observation points in every participant under study (Zimmermann et al., 1993).

Concentrations of monoamine metabolites in the CSF reflect central catabolism, thus the level of 5-HIAA in CSF is a potential pointer to central serotonin turnover (Seifert et al., 1980). Evidence for an ATD-induced decrease in 5-HT turnover in humans came from a study by Carpenter et al. (1998), which showed plasma total TRP and CSF TRP reached, respectively, a nadir at approximately 6 hours with a mean reduction from baseline of 85% and at 7 - 12 hours with a mean decrease of 92%. Moreover, this study showed that with a mean 31% drop from baseline in the CNS level of serotonin’s metabolite, 5-HIAA in the 8 - 12 hours after baseline level – and no change in homovanillic or other
metabolites – ATD results in an unmistakable decline of serotonin turnover. A study by Williams et al. (1999) found 5-HIAA level was maximally reduced at 12 - 14 hrs by 24-40%.

Finally, the effects of ATD are most likely to be specific to reductions in TRP. That is, they are not due to protein synthesis inhibition because an amino acid was left out of the diet. This has been demonstrated by studies comparing ATD with lysine reduction (Klaassen et al., 1999a) and tyrosine combined with phenylalanine reduction (Harrison et al., 2004). Both studies found mood and memory outcomes specific to the challenge compound. Neither are the behavioural effects of ATD due to an artifact of the challenge design because comparisons made between depletion-placebo, depletion-baseline, and placebo-baseline scores, have shown the significant differential effect is between the two treatment scores, and thus curtailed 5-HT synthesis (Schmitt et al., 2000).

3.4.1 Summary
Several investigative techniques have proven the efficacy of ATD in lowering central 5-HT levels and shown that the reduction is specific to the depletion of TRP and not some other cause.

3.5 ATD and Procedure

3.5.1 ATD and Dose
The first human ATD study (Young et al., 1985) – to which many ATD studies refer – assessed male participants only, but deployed both 50 g and 100 g sized drinks. Both sizes were effective in reducing free and total TRP from baseline levels. Several studies have employed ATD doses ranging from 25 g to 104.8 g of amino acids and, where data have been published, all doses have decreased both plasma TRP levels and the TRP/LNAA ratio during depletion, but to varying degrees. For example, the 100 g dose – which incidentally contains an equivalent amount of amino acids to a 500 g steak – lowers plasma TRP concentrations peripherally by up to 80% within 5 - 7 hours (Young et al., 1989); the smaller 25 g drink lowers plasma TRP by approximately 40% (Booij et al., 2005b). On the placebo arm, plasma TRP levels have increased but the TRP/LNAA ratios have not changed significantly from baseline for the smaller doses (See Section 3.5.3 and also, Evers et al., 2005; Sobczak et al., 2002d). A 20% increase in this ratio has been noted for the higher placebo dose at 5 hrs post treatment (Schmitt et al., 2000). One ATD study (Booij et al., 2005b) specifically examined the effect of dose in remitted depressed patients without comparing with placebo or control group. A high dose (100 g) produced a transient return of depressive symptoms in 35% of participants, which did not occur with the low dose (25 g) despite the 46.8% depletion of plasma tryptophan reached with this dose. This finding may be critical, considering a hypothesised threshold for plasma TRP depletion of around 60% to achieve mood effects in this group of patients (Van der Does, 2001b). The high dose in the Booij et al. study induced cognitive changes irrespective of mood effects, notably improved focused attention in the neutral Stroop task but worsened performance in the emotional Stroop task (i.e., a variant of the traditional Stroop task that assesses attentional bias for emotional material as well as response inhibition, attention); the high dose, relative to the low dose, improved executive function for females
but tended to impair it for males. The differential effects on mood and cognition, plus the gender effect varied with strength in a dose-dependent manner.

The cognitive effects of ATD have been investigated with different treatment doses in groups of healthy young male and female adults, with varying effects. For example, in males, neither a 52 g nor a 78 g dose has been shown to affect cognition (Hughes et al., 2003; Van der Veen et al., 2006), whereas a 100 g has been shown to affect memory (Riedel et al., 1999). In healthy females, a 75 g dose has been shown to improve executive function (Evers et al., 2006a) but an 87 g dose has been reported to both impair executive function (Murphy et al., 2002) and have no significant effect (Matrenza et al., 2004). An improvement in visual memory was noted in older female SDAT patients having an 83 g dose (Porter et al., 2003b).

### 3.5.1.1 Summary

The size of the dose is important in effecting behavioural change. There are likely to be variable effects based on gender, as well as dose.

### 3.5.2 ATD and Type of Administration

There are three main methods for effecting ATD:

a. An amino acid powder made up into a drink with water and flavouring. This traditional amino acid mixture is made up of a number of amino acids, as described in section 6.1.1. Sometimes this mixture is supplemented with sunflower oil and maltodextrin (a carbohydrate) so that participants have sustenance to last the day (e.g., Schmitt et al., 2000). The matched placebo mixture is generally the same as the active except maltodextrin is replaced by TRP. The drink has an unpleasant taste.

b. An amino acid powder and capsule combination. Because the taste of the amino acid drink is unpleasant and is associated with nausea and vomiting, some studies have removed the sulphur-containing compounds (i.e., arginine, cystine, and methionine) from the mixture and encapsulated them (Schmeck et al., 2002). The drink-capsule combination induce sufficient depletion (Wolfe et al., 1995) and may reduce these side effects but at the cost of ingesting 50 capsules along with the drink of remaining amino acids (Booij et al., 2003). The remaining amino acid mixture (total of 31.5 g) comprises: isoleucine (4.2 g), leucine (6.6 g), lysine (4.8 g), methionine (1.5 g), phenylalanine (6.6 g), threonine (3.0 g), and valine (4.8 g). The matched placebo capsules contain 31.5 g of lactose.

c. A gelatin-based protein. A TRP-free protein-carbohydrate mixture, e.g., gelatin-based protein, has been devised which comprises all amino acids (except TRP) in the form of peptides, rather than as individual amino acids. The composition of the nutritional and amino acid content of the gelatin-based protein for the placebo is outlined in
Lieben et al. (2004a); the placebo protein-carbohydrate mixture contains an extra 0.28% TRP of total protein. In rats, this method has been shown to increase tolerability and further reduce plasma and central TRP levels and 5-HT levels compared to the traditional amino acid drink (Lieben et al., 2004a; Lieben et al., 2004b). It has also effectively lowered plasma TRP levels in humans (Blokland et al., 2004; Sambeth et al., 2007).

3.5.2.1 Summary

There are three vectors for getting the amino acids into the digestive system. The most widely used involves a drink of amino acids to the same composition as human milk. The drawback to this mixture is its unpleasant taste which could prevent participants returning for a second visit.

3.5.3 ATD and Neutrality of Placebo

As noted above, there are a number of alternate placebos and some concern in the literature that the placebo may not be a neutral control. The traditional placebo contains an identical composition of amino acids plus TRP; others contain a 25% strength preparation (Krahn et al., 1996) or lactose only. The problem arises when these placebo variants are used to compare the effect of ATD on behavioural outcomes since they also have an effect on plasma TRP; the 25% preparation has caused small to moderate reductions (Van der Does, 2001a) but the balanced mixture has caused a varied increase in plasma total TRP levels up to 247% from baseline levels for the traditional amino acid mixture (Golightly et al., 2001) and free TRP levels up to 221% for the drink-capsule combination (Yatham et al., 2001). A modest increment of around 45% in plasma TRP, as noted by Booij et al. (Booij et al., 2005b) and others (Markus et al., 2002), will affect mood and cognition, so it is possible the placebo is not a neutral treatment. Moreover, TRP loading has been shown to induce performance decrements in verbal working memory and motor speed but improvement in vigilance (Luciana et al., 2001). Although the Luciana et al study did not include other LNAAs in the challenge and, thus, the TRP load did not undergo the same competitive process as occurs with a balanced placebo load, it may be important to take the level of plasma TRP after the placebo, and the potential of its non-neutrality, into account when interpreting the significant ATD effects.

To counteract a potentially non-neutrality placebo, some studies have investigated the effect on plasma TRP and the TRP/LNAA ratio using (a) the balanced amino acid drink and either double the amount of TRP, or half the amount of other amino acids, or (b) a more neutral gelatin-based protein (Blokland et al., 2004; Evers et al., 2005; Weltzin et al., 1994). ATD has thus been shown to produce either no change to the ratio (Golightly et al., 2001) or and increase (Scholtissen et al., 2006b). The latter induced an effect on only one cognitive variable with no effects on mood.

The placebo treatment comprising a balanced amino acid mixture (traditional and drink-capsule combination) may also, but not always, significantly lower the TRP/LNNA ratio (Weltzin et al., 1994; Wolfe et al., 1995). A decrease in the TRP/LNAA is unlikely to increase TRP entry into the brain and
thus lead to an overestimation of ATD effects because in this case TRP entry is competitively
disadvantaged. It could, on the other hand, lead to an underestimation of effects if TRP entry into the
brain is reduced. Van der Does (2001b) proposed there is a threshold that requires a reduction in
plasma TRP of at least 60% before effects are found. Studies report a much smaller change in this
ratio than they do of plasma TRP level, so if decreases in the TRP/LNAA ratio were as much as the
40-50% reduction that occurred in studies reviewed by Hood et al. (2005), then it is possible ATD
results are underestimated.

3.5.3.1 Summary

There is a serious issue that the results of ATD compared with a non-neutral placebo might lead to an
over- or under-estimation of effects. The TRP/LNAA ratio can be used to monitor the ‘neutrality’ of
the placebo.

3.5.4 ATD and Diet

There are differences among the ATD studies in food ingestion prior to, and during, the test days. That
is, participants are asked (a) to partake in a low TRP diet on the days preceding ATD, (b) to fast
overnight and eliminate breakfast, (c) to fast overnight but have a low TRP breakfast, (d) to partake in
a low TRP diet for 24 hrs (supplemented with TRP containing capsules before the placebo drink days
(Delgado et al., 1990), or (e) to freely ingest certain foods – including carbohydrates – during the test
day (Evers et al., 2005; Riedel et al., 1999). Delgado’s group thought the 24 hr diet would enhance the
effect of the treatment and at the same time, reduce dietary-induced variations in baseline levels for the
active and placebo conditions (Delgado et al., 1990). Riedel’s group argued the addition of
carbohydrate and fat would not alter the TRP/LNAA ratio because mixed meals – unlike

A potential confound to the diet conundrum is the possibility that receptors up- or down-regulate in
response to a change in 5-HT availability attributable to dietary factors other than the ATD challenge.

3.5.4.1 Summary

There are several schools of thought regarding diet both on and prior to the test days. Most ATD
studies include an overnight fast. An absence of any difference in plasma TRP at baseline on the ATD
and the placebo days suggests a long-term low TRP diet is unnecessary and could lead to a possible up- or down-regulation in receptors in response to chronically reduced 5-HT.

### 3.5.5 ATD and Receptor Adaptation

There is some discussion as to whether the effects of ATD may be mediated by compensatory adaptations in receptor density or affinity, and that this could differentiate the responses of different study groups. That is, would putative differences in the 5-HT$_2$ receptor of depressed patients or the 5-HT$_{1A}$ receptor of SDAT patients incline these patients to be more responsive to the effects of ATD? Certainly, the effect of long-term TRP depletion in animals has shown this to occur with the upregulation of the post-synaptic 5-HT$_{2C}$, but not the 5-HT$_{1A}$ receptor (Franklin et al., 1999). When an investigation using PET found ATD did not significantly affect mood in healthy women but a reduction in the number of 5-HT$_2$ receptors in various cortical regions, the authors hypothesised on the basis of animal evidence that receptors downregulated as part of a protective adaptation against depressive symptoms (Yatham et al., 2001). There is known to be an association between decreased 5-HT$_{1A}$ and 5-HT$_2$ receptor density and some antidepressant drugs, and that this decrease can occur within 24 hrs of treatment onset (Klimek et al., 1994; Yatham et al., 1999). Combined with the understanding that 5-HT$_2$ receptors do not fit the traditional upregulation-in-response-to-challenge model – since both agonist and antagonists at this site induce downregulation (Leysen & Pauwels, 1990) – there is a possibility that the serotonergic system may adapt within the 6 - 7 hr period within which most ATD behavioural assessments are administered.

Receptor adaptation has been investigated in two ATD studies with unmedicated depressed participants. At the time of maximal plasma TRP depletion, one study challenged with infusion of metachlorophenylpiperazine (mCPP) (Price et al., 1997), the other with infusion of TRP (Price et al., 1998). Both studies used the correlation between plasma TRP and cortisol level to estimate serotonergic system integrity, and both concluded the negative correlation attributable to a compensatory upregulation in 5-HT$_2$ receptors but no corresponding compensatory upregulation of 5-HT$_{1A}$ receptors. This may explain the enhanced mood observed in some untreated depressed patients the day after ATD (see section 4.2.3.10).

#### 3.5.5.1 Summary

There is a possibility the effects of ATD could be due to a down-regulation in some receptors within the time frame of the testing schedule.

### 3.5.6 ATD and Time of Testing

Different testing schedules have been used to assess behaviour during ATD (e.g., Evers et al., 2005). However, it is generally accepted that testing should begin between four and six hours post treatment with ATD preparation and conclude a maximum of nine hours later (Riedel et al., 2002b). This window of maximal depletion was demonstrated during 24 hr sampling of plasma TRP levels and the TRP/LNAA ratio after treatment at 9.00 am (Klaassen et al., 1999b; Schmitt et al., 2000). This is
based also on research that showed plasma TRP levels, after an initial rise in the first hour, decline sharply to reach maximal reduction at four to five hours post treatment (Benkelfat et al., 1994; Weltzin et al., 1994); CSF levels reach nadir seven to ten hours post treatment (Carpenter et al., 1998). It is at this point that levels will reach 9-55% of baseline levels (Van der Does, 2001a).

The usefulness of performing behavioural assessments approximately five hours post treatment was highlighted in two studies (Riedel et al., 1999; Schmitt et al., 2000). Firstly, by memory impairment when the word presentation list was presented at 6 hrs, and secondly, when the effects of depletion were shown to be more marked at 5 hrs than at 9 hrs. In both studies the participants were given until 11 am to consume the amino acids, but time to assessment was measured from 9.00 am.

3.5.6.1 Summary

It is important if behavioural effects are to be investigated that testing be achieved in a five hour time frame at least four hours after the ATD mixture is ingested.

3.6 ATD and Gender

There are few studies investigating gender differences during ATD. However, one key paper (Nishizawa et al., 1997) using PET to investigate 5-HT synthesis during ATD found that, although synthesis decreased in a uniform manner in all brain regions examined for both genders, the reduction was more than four times greater in females than in males.

Most ATD and cognition studies do not differentiate between gender; however, of the few that have, two (Riedel et al., 1999; Schmitt et al., 2000) found no difference between placebo and depletion in any of the biochemical parameters or verbal learning task scores. One study investigating women only (Harrison et al., 2004), reported an impairment in the consolidation of verbal items. A further gender study (Gallagher et al., 2003) with men only this time, reported ATD improved attention. A recent pooled analysis found ATD impaired performance to a greater extent in females than in males (Sambeth et al., 2007).

Other studies have found ATD either significantly lowered mood in women but not men recovered from depression (medicated and nonmedicated, Booij et al., 2002) – and this seemed to be an ATD-specific not a monoamine depletion-specific response (Moreno et al., 2006) – or tended to lower mood in healthy women with a family history of affective disorder but not men with the same family history (Benkelfat et al., 1994; Ellenbogen et al., 1996). No gender differences were demonstrated in a nonmedicated group of recovered depressed (Moreno et al., 1999) or a healthy group with a family history of bipolar disorder (Quintin et al., 2001).

3.6.1 Summary

The turnover of 5-HT is greater in females than in males. This may mean females are more vulnerable to mood effects after ATD.
3.7 ATD and Age

Prior to commencement of the current research, most ATD studies had been undertaken with healthy young adults or older patients. The latter groups include a group of SDAT patients (Newhouse et al., 2002; Porter et al., 2003b; Porter et al., 2000), a group of people recovered from depression (Porter et al., 2005), and a case study of one PD patient (McCance-Katz et al., 1992). Before experiments for the current research were completed, two papers were published reporting ATD effects from the study of the same PD patients (Leentjens et al., 2006; Scholtissen et al., 2006b). However, not one of these six papers reported any independent effects of ATD and age; perhaps due to the limited age range in each study.

ATD has not induced changes in mood in older persons and has not been used to assess movement prior to the commissioning of the present research. A recent study used ATD to examine mood and cognition in a group of menopausal women recovered from major depression (Epperson et al., 2007). At the time of study, these women were taking either oestrogen replacement, fluoxetine, or a combination, to improve their mood. In comparison to studies in younger people recovered from depression – but in common with the older people in the above study by Porter et al. – these women experienced no relapse of depression or significant worsening of mood, despite the vulnerability to which their SSRI medication predisposes them (Booij et al., 2002).

3.7.1 Summary

There are no reports of ATD-induced effects being influenced by age. ATD did not induce significant mood effects in older persons, despite some of the groups tested having a vulnerability to these mood effects.

3.8 Limitations of ATD

As yet there is no direct evidence that ATD alters 5-HT release in human brains (Young & Leyton, 2002). Observed effects therefore, and the attribution of these effects to reduced brain 5-HT brain activity, may not necessarily be a result of the depletion. Other factors may be at play. For example:

a. A change in the amount of circulating TRP as a result of altered protein synthesis, or changes in gastrointestinal absorption which may, as a result of age or disease, differentiate experimental groups more than the differential between treatments (Kilkens et al., 2004; van Nieuwenhoven et al., 2004). For example, the gastrointestinal system in the older persons is often characterized by decreased hepatic function, gastric acidity and the absorption of certain foods and substances could be affected by this.

b. A potential for the load of amino acids to cause nausea, vomiting, and diarrhea. These gastrointestinal effects and the unpleasant taste of the drink could mean some participants do not complete the two day testing protocol.
c. An unwitting increase in DA and NA synthesis. The ratio of one LNAA to the others is critical in determining corresponding neurotransmitter synthesis. This was investigated by Badawy (2005) who calculated that during tyrosine-phenylalanine depletion, the tyrosine-phenylalanine/LNAA ratio underwent an approximate forty-five-fold decrease but the TRP/LNAA ratio underwent a three-fold increase.

d. A depleting effect of the placebo, since the increased load of amino acids may lower the TRP/LNAA ratio and thus preclude TRP entry to the brain (Weltzin et al., 1994). The potential non-neutrality of the placebo has been assessed with the Profile of Mood States (POMS) rating scale (McNair et al., 1992), however, and scores were shown to be worse during ATD but not the placebo (Lam et al., 2000; Rosse et al., 1992).

e. Alterations in compounds other than 5-HT which might confound the effects of ATD. There are metabolites of TRP including tryptamine, quinolinic acid, kynurenic acid, and nicotinamide. These metabolites are psychoactive and responsive to changes in TRP levels. Along with dietary availability and protein synthesis, kynurenine is the main valve to TRP abundance in the blood and, hence, levels of 5-HT in the CNS. That is, when there is too much TRP in peripheral circulation TRP is degraded to kynurenine and when kynurenine levels are high, TRP transport into the CNS is curtailed (Birdsall, 1998). Kynurenine and the enzyme (indoleamine-2,3-dioxygenase) that catalyses the cleaving of TRP into kynurenine, are distributed across all anatomic regions of brain (Gal & Sherman, 1980). Of the products manufactured in this metabolic pathway, kynurenic acid acts as an antagonist at glutamate receptors, and quinolinic acid acts as an agonist at glutamate receptors (Freese et al., 1990; Sainio et al., 1996; Stone, 2001). Awareness of these kynurenines is important in TRP studies not only because they modulate the excitatory transmitter, glutamate, and thus may influence behaviour (Blokland et al., 2004), but because quinolinic acid carries an inherent neurotoxic danger (Freese et al., 1990). Another metabolite of TRP, tryptamine, is directly decarboxylated from TRP in the indolamine pathway. Although tryptamine is a trace amine it is able to modulate the action of 5-HT on neurons (Sainio et al., 1996).

3.9 Caveats of ATD

There are several medical issues that researchers using ATD need to be aware of to make robust interpretations. They also need to isolate, and control for, as many confounding factors in the process of 5-HT metabolism as possible. These include (a) testing with placebo or depletion within two weeks of each other to avoid circannual variations – except when testing younger females when it is critical re-testing occurs at the same stage of menstrual cycle, (b) asking participants to fast before and during testing to avoid insulin uptake of amino acids, (c) timing biological and behavioural observation points
to take account of the staggered time lags in 5-HT metabolism, and (d) accounting for or controlling gastrointestinal effects (nausea and diarrhoea). Researchers also need to be aware that rapid depletion may produce quite different effects to chronically low levels of TRP.

3.10 Advantages of ATD

There are a number of advantages in using ATD to investigate the role of serotonin in behaviour. These advantages include:

a. Ease of administration, reliability, safety, tolerability, and reversibility.
b. Speed of effect – lowers central 5-HT levels within hours.
c. The specificity of the technique to TRP and no other amino acid depletion.
d. The relative certainty that pre-synaptic 5-HT function is reduced.
e. Evidence that post-synaptic 5-HT function is related to effect behavioural change.
f. Its adaptability to animal or human subjects.
g. The investigation of dynamic processes in the same subject at different time intervals.
h. The flexibility of use in a variety of healthy and patient populations.
i. The ability to study a wide range of behaviours including affect, impulsiveness, psychosis, eating disorder, cognition, sleep, brain-gut responses, motion sickness, drug interactions, cortisol release, and various brain mechanisms or regional activations.
4 THE ROLE OF SEROTONIN IN BEHAVIOUR

Serotonin has a multitude of different physiological actions, which are exerted at a variety of receptor sites. Although the neurons are located in a relatively circumscribed area, they reticulate to most parts of the brain and spinal cord (Azmitia & Whitaker-Azmitia, 1991), interacting with other neurotransmitter systems to influence numerous normal and abnormal behaviours. Clearly, the pharmacology of the serotonergic system is complex (Jones & Blackburn, 2002), however, there is an aggrandisement of data revealing the physiological stasis and functional activity of this system in healthy humans; and the aberrations that occur during ill health. To the extent there are methods to assay physiological stasis (e.g., concentrations of 5-HT and metabolites, neuronal numbers, and axonal projections) in deceased humans, there are a number of ways 5-HT function can be investigated in the CNS in vivo. These include:

a. Challenges to the serotonergic system with compounds, such as TRP, fenfluramine, buspirone, m-CPP, and several SSRIs. Plus, the vicarious assessment of the system’s integrity through an endocrine response, for example, cortisol (Porter et al., 2007b), growth hormone, and prolactin (Flory et al., 1998; Porter et al., 2003a; Ricaurte et al., 2000).

b. Manipulation of 5-HT levels with ATD and the assessment of, first, central 5-HT levels with peripheral plasma TRP level and the TRP/LNAA ratio, and second, behaviour with a number of behavioural measures.

Three behaviours which are assessable in this manner are movement, mood, and cognition. This chapter reviews the literature on 5-HT challenge and manipulation in these three domains. The first section describes two motor behaviours where 5-HT has an observed function and goes on to discuss the idea that 5-HT could have a putative, and possibly therapeutic, role in a number of native and drug-induced movement disorders. The second section discusses the role of 5-HT in depression and the evidence that demonstrates some groups are at risk for a mood lowering response to ATD but some are resistant. The final section provides a synopsis of cognition and the role of 5-HT in cognitive function; most specifically, the effects of ATD on performance in each of the cognitive domains tested by technique.

4.1 Serotonin and Movement

Movement difficulties – akinesia (i.e., slowness, lack of movement, or both, and difficulty initiating movement), rigidity (i.e., increased resistance to passive movement), chorea (i.e., jerky involuntary movement), and tremor – are symptoms of movement disorders. Through its modulation of other neurotransmitters 5-HT may be influencing these aberrant motor activities. For example, an imbalance in the DA/5-HT ratio is implicated in chorea; and a combination of reduced DA and 5-HT afferents to the striatum results in resting tremor (Fahn et al., 1971).
Serotonin neurons project to many areas of the brain but there is a particularly intense innervation to motor areas in the cortex, brainstem, and spinal cord. Serotonin appears to have a role in motor activity possibly as a modulator of other neurotransmitters, since evidence from the rat brain has demonstrated it produces a strong effect on motorneurons when combined with the direct application of other neurotransmitters but has little or no effect on its own (Jacobs & Fornal, 1993).

Raphé neurons discharge in a stereotypical, clock-like manner (see section 2.2.2) with an intrinsic frequency of 1-5 spikes per second during alert wakefulness (Jacobs & Fornal, 1993). During quiet awakening the spike rate is reduced to 3 per second and in REM sleep, when body movement is paralysed (i.e., when motorneurons regulating muscle tone are inhibited), 5-HT neuronal activity remains silent. Research in animals using interventions to control motor output during various states of arousal suggests a strong relationship between tonic motor activity and 5-HT neuronal discharge (for list of studies see, Jacobs & Fornal, 1993).

As well as involvement in the sleep-arousal-wake cycle there appears to be a role for 5-HT in gross movement. This is evidenced by the dense innervation to motorneurons in areas of (a) the spinal cord involved with gross repetitive movements, as opposed to fine or discreet movement and (b) the brainstem involved with jaw and facial muscles, as opposed to fine eye movements. Jacobs and Fornal (1993) have suggested the function of this innervation may be to smooth motor outputs. Further, that in increasing their firing rate before movement, these neurons may serve priming and timing functions for these motor outputs.

Thus, with a primary role in facilitating motor output in tonic and repetitive modalities, 5-HT may hold an overarching but integrating role with other systems in effecting normal and abnormal motoric behaviour. This idea opens the way for investigations of 5-HT in movement as a possible therapeutic strategy for movement disorders and to this end, animal studies already suggest that serotonergic inhibition of dopaminergic function could have a beneficial effect on the extrapyramidal side-effects of neuroleptic treatment (Hertel et al., 1997; Korsgaard et al., 1985; Miyawaki et al., 1997). There is also the possibility that 5-HT agents may ameliorate motor symptoms in PD.

In humans, post-mortem examinations have indicated lower 5-HT concentrations in the basal ganglia of PD patients while antemortem studies have demonstrated reduced CSF levels of 5-HIAA, indicating reduced 5-HT synthesis and turnover (Chase, 1972, 1974). There is intimate communication between the serotonergic and dopaminergic neurotransmitter systems in the basal ganglia with 5-HT tonically inhibiting cells in the SNpc and ventral tegmentum area (Ugedo et al., 1989).

Anatomical studies have shown that the afferent and efferent nerve fibres of the corpus striatum are organised in a somatotopic fashion (Fahn et al., 1971). Thus the presence and quantity of neurotransmitters in different parts of the corpus striatum influences a corresponding set of tissues elsewhere in the brain. A synopsis of the communication circuitry in the basal ganglia of the healthy human brain is that (a) DA is evenly distributed throughout the corpus striatum except for the caudal...
tail (Fahn et al., 1971), (b) ACh in the corpus striatum is inhibited by DA and when DA is decreased  
ACh release is increased and vice versa. In PD this means less inhibition in DA and over activity of  
ACh; while in chorea the deficiency in ACh may be compensated by antagonising DA inhibition  
(McGeer et al., 1976), (c) GABA is the main neurotransmitter of interneurons throughout the larger  
basal ganglia, and (d) 5-HT is distributed evenly throughout the striatum, perhaps reflecting its  
modulatory role in neurotransmission. Raphé-nigra pathways appear tonically to inhibit the activity  
of dopaminergic neurons (Sandyk & Fisher, 1988). As both DA and 5-HT activation inhibits ACh  
turnover, 5-HT in the striatum may also serve to inhibit ACh neurons (Samanin et al., 1978).  
The overall outcome of these different neurotransmitters and their interactions on the thalamus is  
inhibitory; to apply the brakes on one function in order to effect activity in another. For example,  
when a person wants to move, the brakes are placed on postural reflexes but released on those  
constraining voluntary movement; the opposite occurs when a person wants to stand still. When  
lesions or dysfunctional neurotransmitter activity alters this finely tuned process, the outcomes are  
likely to be an absence – or limitation – in initiating movement and performing voluntary movements,  
as occurs in PD. Or it may result in extraneous movements, as occurs in Huntington’s disease.  
With 5-HT cells firing in anticipation of movement and having an influence in the smoothness of  
movement, plus 5-HT having such an ubiquitous – and one assumes purposeful – presence in the  
striatum, could it be hypothesised that adjustments in 5-HT levels be used to treat bradykinesia and  
tremor in PD or neuroleptic parkinsonism?  
Enhancement of the serotonergic system with TRP and 5-HTP has elicited distinctive and replicable  
motor effects in animals and humans including, among other behaviours, tremor and rigidity (Jacobs  
& Fornal, 1993). Moreover, side effects from SSRIs include the development of parkinsonism within  
weeks or days of treatment onset (for list of studies see Miyawaki et al., 1997). These medications  
have been linked to extrapyramidal symptoms in older persons, as well as patients taking concurrent  
antipsychotic medication and PD patients (Caley, 1997; Gormley et al., 1997; Lambert et al., 1998;  
Lane, 1998; Schillevoort et al., 2002). A recent review article highlighted the increased risk of falls  
associated with SSRI use, such that the risk may be higher than that associated with TCA use  
(Hartikainen et al., 2007).  
Given these reports, a recent prospective trial looked at the motor effects of four SSRIs (citalopram,  
fluoxetine, fluvoxamine, and sertraline) in 62 consecutive non-demented, non-fluctuating, depressed  
patients with PD. After one, three, and six months, there were no significant differences in motor  
severity scores (Dell’Agnello et al., 2001). In the context of intact 5-HT innervation, however, there is  
still the possibility that 5-HT may exert influence over motoric behaviour and that strategies target this  
nerve system to benefit aberrant movement. It is with this in mind that Miyawaki et al. (1997) suggest  
agonism at the 5-HT3 receptor site may result in downstream dopaminergic neurotransmission, thus  
leading the way for reducing 5-HT activity in order to improve movement symptoms.
Serotonergic systems are very much the focus of antipsychotic psychopharmacology because 5-HT has a reciprocal working relationship with DA. The beneficial effect of atypical antipsychotics in avoiding extrapyramidal symptoms such as parkinsonism, akathisia, and dystonia arises because of their combined 5-HT$_{2A}$ and DA$_2$ receptor antagonism. Ritanserin, for example, has been shown to be an effective treatment for parkinsonism and akathisia (Cunningham Owens, 1999). The effect of its combined serotonergic-dopaminergic action was demonstrated when pre-treatment by the 5-HT depleting drug, para-chlorophenylalanine (PCPA) reversed the increase in firing ritanserin induced in the SNpc and ventral tegmentum area (Ugedo et al., 1989). Clinical observations and animal research has increasingly suggested that drugs blocking the 5-HT$_{2A/C}$ receptors can benefit patients with certain extrapyramidal movement disorders (Oh et al., 2002). One study using quetiapine (i.e., an antipsychotic with mixed DA, NA and 5-HT antagonism) in PD patients showed this compound successfully treated psychosis without incremental parkinsonian side effects, as assessed by the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., 1987) total and motor subscale score (Juncos et al., 2004; Starkstein & Merello, 2002).

4.1.1 Commentary
The definitive role of indoleamine involvement in movement is not yet clear but it seems to have an effect on dopaminergic neurotransmission and, on current evidence, it may partly be active in chorea and tremor. There is good evidence that reducing 5-HT activity at the 5-HT$_{2A}$ receptor site may have beneficial effects in parkinsonian syndromes.

4.2 Serotonin and Mood
Clinical and pre-clinical research has provided enough evidence for a therapeutic effect of 5-HT in affective disorders (for reviews see, Cowen, 1993; Jones & Blackburn, 2002; Maes & Meltzer, 1995; Ordway et al., 2002; Sobczak et al., 2002c; Young & Leyton, 2002) to support an important role of 5-HT dysregulation in the pathophysiology of mood. It has been suggested that certain individuals have a serotonergic vulnerability, or increased sensitivity, to alterations of the serotonergic system and that this functional abnormality may predispose these individuals to depression (Law et al., 2007). For example, acute reductions of TRP with ATD have induced a mild and transient lowering of mood in some, but not all healthy adults, and a relapse of depressive symptoms in adults with a past history of depression (or reviews see, Bell et al., 2001; Booij et al., 2003). ATD has also reversed the antidepressant effects of compounds that primarily enhance 5-HT neurotransmission (SSRIs and MAOIs) in current inpatients (Delgado et al., 1990; Delgado et al., 1994; Delgado et al., 1991) and recovered depressives (for tables of studies in healthy or depressed people see, Moore et al., 2000; Moreno et al., 1999). Moreover, the effect of this acute change in 5-HT has been observed as reduced brain metabolism in the dorsolateral prefrontal cortex (DPFC), orbitofrontal cortex (OFC), and thalamus of SSRI-treated patients with major depression experiencing a depletion-induced relapse correlating with their depressive symptoms (Bremner et al., 1997).
Law et al. (2007) have proposed a model for predicting risk for depression based on the threshold hypothesis of combined vulnerability factors. That is, family history of depression, female gender, personality, sex hormones, stress, drug use, genetic and immune system factors. However, despite this and numerous studies investigating other aspects of the serotonergic system – e.g., plasma TRP levels (Moller et al., 1986), serotonin transporter availability (Little et al., 1997), receptor densities (Agren et al., 1991; Drevets et al., 1999; Stockmeier et al., 1998), neuronal numbers (Baumann et al., 2002; Baumann & Bogerts, 2001; Underwood et al., 1999), and neuroendocrine effects (Cowen & Charig, 1987; Deakin et al., 1990; Heninger et al., 1984; Porter et al., 2003a) – the specificity of a relationship between the serotonergic system and depression pathology is still to be determined (Cowen, 1996).

The most consistent evidence seems to suggest depression is related to reduced activity at the 5-HT$_{1A}$ receptor site and increased activity at the 5-HT$_{2}$ receptor site. PET scanning studies have confirmed that the number of 5-HT$_{1A}$ receptors is reduced in depression, and that this continues following treatment with SSRIs (Drevets et al., 1999; Sargent et al., 2000). Human neuroendocrine studies investigating 5-HT$_{1A}$ receptor function have used intravenous TRP or direct 5-HT$_{1A}$ receptor agonists to examine the relationship of this receptor to mood. The latter, with the exception of studies using the agonist buspirone, have generally shown attenuated responses. These responses are thought to reflect reduced 5-HT$_{1A}$ receptor activity at both pre- and post-synaptic sites (Lesch et al., 1990; Meltzer & Maes, 1995; Price et al., 1991; Riedel et al., 2002a; Shapira et al., 2000). However, results from a more recent study have not supported this and suggest that, since in this study patients with major depression were not taking psychotropic medication, and the basal cortisol, DHEA and the cortisol-DHEA ratio did not differ between patients and controls, the effects in earlier studies of TRP infusion may be due, instead, to hypercortisolaemia or psychotropic medication (Porter et al., 2003a).

Studies using putative 5-HT$_{2}$ agonist challenges (5-HTP and mCPP) have generally suggested an upregulation of these receptors in depressed compared with healthy control participants (Maes et al., 1987; Price et al., 1997; Riedel et al., 2002a).

4.2.1 Older Age and Mood
Depression is common in older persons, with estimates reporting somewhere between 10% – 35% of hospitalised and non-hospitalised persons having depression (Gareri et al., 2002; Meltzer et al., 1998a). This differs in character from the depression seen in younger adults (Taylor & Doraiswamy, 2004) in that there is an increased risk of suicide, higher non-suicide mortality, more anxiety and psychosis, greater polyopathy, and dementia, as well as a diminished tolerance to treatment and failure of treatment (Casey, 1994; Gareri et al., 2002; Gottfries, 1998; McGuire & Rabins, 1994; Meltzer et al., 1998a). Genetic factors seem to be less important for developing late-onset depression (> 40 yrs) than they are for early-onset (< 40 yrs) (Baron et al., 1981) and depression is more likely to develop as a result of structural and biochemical changes in the brain (Gareri et al., 2002; Gottfries, 1998; Krishnan et al., 1997) – possibly associated with the HPA axis or frontostriatal dysfunction (Gareri et al., 2002) – and associated vascular and neuroendocrine disease (Baldwin & O'Brien, 2002;
Psychosociogenic factors, however, are important, for example, 30% spouses of demented patients develop depression (Gottfries, 1998) and older persons are likely to get depressed as a side effect of polypharmacotherapy.

Cognitive dysfunction is significant in depression, particularly in the elderly (Porter et al., 2007a). The cognitive impairment persists after depression remits (Bhalla et al., 2006; Lee et al., 2007; O’Brien et al., 2004) and may well be a risk factor for later irreversible dementia (Alexopoulos et al., 1993).

Biochemical research looking at the aetiology of major depression in older persons has focused on DA, NA, and 5-HT neurotransmitter systems, and clinical research on the efficaciousness of increasing 5-HT neurotransmission (Gareri et al., 2002). For example, (a) after being administered a 5-HT agonist, healthy older adults had a decreased behavioural response (Lawlor et al., 1989) and (b) change in serotonergic functioning – observed as a blunted prolactin response to an SSRI challenge – could make older individuals more vulnerable to depression or more likely to increase the number of episodes (Lerer et al., 1996).

The cortisol response of older recovered depressed patients and healthy matched controls was investigated during ATD – both during the acute challenge and during the day following the challenge – with no significant effects on its secretion (Porter et al., 2007b). ATD had been shown previously to have no effect on mood in this older group (Porter et al., 2005) and contrary to the evidence presented in the preceding paragraph – and to evidence in younger adults – may be suggesting there is no specific vulnerability of the serotonergic system in this older group.

There are few published randomised, placebo-controlled trials of antidepressant medication in this cohort. One study found a comparable efficacy and tolerability between the noradrenaline reuptake inhibitor, nortriptyline, and the SSRI, paroxetine, in the acute treatment of older depressed patients (Mulsant et al., 1999). A review of studies concluded that antidepressants are effective in older persons, but more research is required in people without significant comorbidity (Taylor & Doraiswamy, 2004).

4.2.1.1 Antidepressant Medication and Older Age

It seems appropriate given the prevalence of depression in older persons to include a discussion of the possible concomitant effects of SSRIs in this cohort. Older age is the main aetiological factor in PD and, in fact, a risk factor for the development of other neurodegenerative diseases (Govoni et al., 2001). Several conditions often co-exist and treatment of one condition may result in iatrogenesis. For example, in older persons, SSRIs are linked to the increased risk of (fragility) fractures, falls, and syncope, as discussed previously in section 4.2.1. Moreover, although not necessarily associated with normal healthy ageing (Greenblatt et al., 1982a), there are changes in pharmacokinetic and pharmacodynamic processes like drug distribution and biotransformation, as well as problems with drug clearance delay, accumulation, or both, in this group (Gareri et al., 2002).
Because of the significant loss in aminergic neurotransmitter level, receptor number, and function in older persons (Hornykiewicz, 1987), pharmacological treatments may result in undesirable side effects. For example, TCAs have anticholinergic and antihistaminergic properties (Nathan et al., 2000) alongside their serotonergic and noradrenergic enhancing ones which may inflict unwanted cognitive impairments.

SSRIs rapidly increase central 5-HT in monkeys and the consequential raised level of 5-HT is maintained at a constant rate until the medication is discontinued (Anderson et al., 2005). In older humans pharmacokinetic differences mean these drugs and their active metabolites can stay in the body longer. This not only increases their clinical activity but may produce unpleasant side effects (Fairweather et al., 1993).

One problem with SSRI treatment is the adverse side effect profile, for example, gastrointestinal bleeding (Paton & Ferrier, 2005). For older persons, the risk of taking this treatment include (a) an increased risk of falls (Hartikainen et al., 2007; Pacher & Ungvari, 2001); a two-fold increased risk for clinical fragility fractures with daily use by persons aged over 50 years, even after falls and bone density are controlled for (Richards et al., 2007) – it was noted by these authors that the magnitude of risk was similar to that of corticosteroid use, (b) an increased risk of syncope, even after cardiovascular disease is controlled for (Cherin et al., 1997), and (c) although they do not have the adverse cardiovascular profile of TCAs, SSRIs may have cardiovascular depressant effects (Pacher & Ungvari, 2001).

It is important to deepen scientific understanding of drug dispositions in older persons because therapeutics have been prescribed based on research with younger people, anecdotal data, clinical impression, and trial and error (Greenblatt et al., 1982b). Studies in humans investigating a range of SSRIs in younger adults have reported conflicting effects on cognition (Harmer et al., 2002; Levkovitz et al., 2002; Riedel et al., 2005; Schmitt et al., 2001). In depressed older persons, 12 week treatment with either paroxetine or nortriptyline medication did not improve cognition to the level of healthy controls (Nebes et al., 2003). What then is the hypothesis for cognition in older persons using these drugs? The answer to this question is all the more critical when side effects from chronic treatment limit optimal function for daily activities.

4.2.2 Gender and Mood

Depression is more common in females, with women being twice as likely to experience depression as men. For major depression, females have a lifetime prevalence rate of 21.3% compared to a prevalence rate in men of 12.7% (Kornstein, 2001). Women have more severe symptoms, more psychomotor retardation, and more functional impairment than men (Kornstein et al., 1995). They are also more responsive to rapid experimentally-induced reductions in 5-HT (Booij et al., 2005c). Men, on the other hand, are more likely to have a history of co-morbid chemical abuse (Kornstein et al., 2001). Epidemiological studies have provided mixed reports of the prevalence rate in older compared
to younger women (Bijl et al., 1998; Brown et al., 1992; Cyranowski et al., 2000; Zunzunegui et al., 1998).

There is recent neuroimaging data to suggest gender differences in regional brain activation, volume, and lateralisation; males demonstrate more brain lateralisation and females more brainstem activation during emotion-eliciting tasks (Wager et al., 2003).

Despite the lack of evidence indicating menopause as a risk factor for depression, oestrogen has a role in the treatment of mood in perimenopausal and post-menopausal women (Kornstein, 2001). Oestrogen alters the expression of genes in the serotonergic system (see Section 2.4.1.2, Gundlah et al., 2005), which raises the possibility that oestrogen may influence the vulnerability of females to depression via its effect on serotonergic function (Law et al., 2007). The results of a study investigating the efficaciousness of fluoxetine combined with oestrogen replacement therapy, suggests oestrogen therapy may enhance the antidepressant effects of SSRIs in post-menopausal women (Schneider et al., 1997).

4.2.3 ATD and Mood

Mood was first altered in healthy adults during ATD by Young et al. (1985). Subsequent depletion studies have either reproduced an acute or subclinical lowering of mood, or had no effect in a number of psychiatric and healthy adult populations. The following section summarises the research findings from these ATD studies on symptoms of mood only. Risk refers to statistically significant vulnerability; Resistance to a non-significant response.

4.2.3.1 Healthy people

Risk The significant lowering of mood demonstrated in the above study (Young et al., 1985) was replicated by the same authors, again in a male only study (Smith et al., 1987) and in another study at 6 hrs in a mixed gender group, irrespective of affective family history (Klaassen et al., 1999b). It was observed also in healthy females but not males in a small sampled study and a meta-analysis (Ruhe et al., 2007; Smith et al., 1997b). A modest increase in the change scores of the depression item score of the POMS was reported in one study investigating scalp recordings of electroencephalogram (EEG) activity during ATD (males only studied, Knott et al., 1999) and again, through a visual analogue scale, during another EEG study (Ahveninen et al., 2002).

Resistance However, five reviews and a meta-analysis of the literature are consistent in stating that, although healthy adults may be susceptible to transient mild dysphoria, they are not vulnerable to clinical depressive symptoms during ATD (Bell et al., 2001; Booij et al., 2003; Fusar-Poli et al., 2006; Jans et al., 2007; Ruhe et al., 2007; Van der Does, 2001a). Further, SSRI treated healthy adults are not susceptible to mood effects during ATD like depressed persons (Barr et al., 1997). Interestingly, the absence of mood effects occurs in many ATD studies where cognitive changes are manifest (Talbot & Cooper, 2006).
4.2.3.2 Healthy People with a Family History of Major Affective Disorder

Risk Two experimental studies and one meta-analysis found people with a family history of major affective disorder, generally a first degree relative, have a vulnerability to the mood lowering effects of ATD (small effect and only males were studied, Benkelfat et al., 1994; Klaassen et al., 1999b; Ruhe et al., 2007). People with a family history of bipolar I disorder experienced lower mood during ATD; conversely those with a family history of bipolar II disease had an elevation in mood (Sobczak et al., 2002a).

Resistance A group of women having a family history of affective disorder did not demonstrate mood effects of ATD but the study’s high exclusion criteria – that included a multi-, but not uni-generational family history of three documented cases of bipolar or recurrent major depressive disorder, including affected first degree relative – may have biased this result (Ellenbogen et al., 1999).

4.2.3.3 Current Major Depression

Resistance Two studies (Price et al., 1997; Price et al., 1998) did not demonstrate mood effects in a group of patients – with heterogeneous diagnoses of major depression – currently untreated, but having a history of treatment from a range of medications. This non-significant result was also observed in an earlier study with a group of drug naïve depressed patients; although some patients became more depressed the day after depletion, some improved during the day of depletion (Delgado et al., 1994). It may be that ATD produces a floor effect in the already compromised serotonergic system of depressed patients. However, because these ATD results have also been observed in studies depleting other monoamines, it may also be that mood in major depressive disorder does not correlate with 5-HT or NA level (Ruhe et al., 2007).

4.2.3.4 Currently Treated and Clinically Remitted Major Depression

Risk There is a strong indication that 50-60% of people recovered from depression, but still being treated for it, are vulnerable to these mood effects (Van der Does, 2001a). This is particularly so for the subgroups having (a) past or current treatment with SSRIs (pooled analysis, Booij et al., 2002; Bremner et al., 1997; Delgado et al., 1990; confirmation of the pooled analysis, Delgado et al., 1999; pooled analysis, Delgado et al., 1991; meta-analysis, Ruhe et al., 2007; Smith et al., 1999b; Spillmann et al., 2001) or MAOIs (Delgado et al., 1990; Smith et al., 1999b), (b) female gender, (c) suicidal ideation (Booij et al., 2002) and, (d) recurrent and particularly chronic episodes (Booij et al., 2002).

Resistance Patients who have not responded to ATD with significant mood effects are older remitted patients (Porter et al., 2005), menopausal women treated with SSRIs (Epperson et al., 2007), or those treated with (a) non-serotonergic antidepressants (Delgado et al., 1990; Delgado et al., 1999; Delgado et al., 1991), (b) cognitive behavioural therapy (O’Reardon et al., 2004), (c) electroconvulsive therapy (Cassidy et al., 1997), and (d) the NA antidepressant bupropion (Evans et al., 2002).
4.2.3.5  Currently Untreated and Clinically Remitted Major Depression

**Risk** ATD induced clinically significant depressive symptoms in treatment-free patients currently in clinical remission (more so in patients with a family history of affective disorder, Leyton et al., 2000a; drug-free for at least 3 months, Moreno et al., 1999; only females studied, Smith et al., 1997c). A meta-analysis of studies confirmed a moderate decrease in mood in these participants (Ruhe et al., 2007).

**Resistance** Not all studies have found an effect of ATD on mood in untreated remitted patients (Leyton et al., 1997).

4.2.3.6  Other affective disorders: Seasonal Affective Disorder and Bipolar Disorder

**Risk** The effects of ATD in patients with seasonal affective disorder (SAD) parallel those in major depression (Van der Does, 2001a) in that 50% of phototherapy- and naturally-remitted unmedicated patients have responded with worsened mood (Lam et al., 2000; Neumeister et al., 1997a; Neumeister et al., 1998a; Neumeister et al., 1998b), however, currently depressed patients were unaffected (Neumeister et al., 1997b).

**Resistance** Lithium treatment is associated with protection from the mood effects of ATD (history mania, Cassidy et al., 1998; current bipolar, Hughes et al., 2000; current bipolar and unipolar depressed, Johnson et al., 2001).

4.2.3.7  Other 5-HT Vulnerable Groups

**Risk** A worsening of mood was observed in one group of patients with obsessive compulsive disorder currently treated with SSRI and other drugs (Barr et al., 1994) but not in another group with a past history of SSRI treatment (Smeraldi et al., 1996). ATD worsened, or slightly worsened, mood in females with, respectively, unmedicated remission or current bulimia nervosa (Smith et al., 1999a; Weltzin et al., 1995).

**Resistance** No effect of ATD was observed in a group of unmedicated patients with panic disorder (Goddard et al., 1994). People with irritable bowel syndrome (IBS) are successfully treated with serotonergic acting medication and thus may be susceptible to a mood response to ATD, however no differential treatment effects during the traditional ATD-placebo experiment have been found (Kilkens et al., 2004; Shufflebotham et al., 2006).

4.2.3.8  Neurodegenerative Disorder

**Risk** There is a case report of a person with PD experiencing lowered mood during ATD (McCance-Katz et al., 1992).

**Resistance** Patients with SDAT experienced no mood effects during ATD (Porter et al., 2000).
4.2.3.9 Other Groups

Risk There are several groups demonstrating a mood response to ATD based on genotype. In particular the polymorphism (5-HTTLPR) in the gene that encodes the 5-HT transporter protein (5-HTT). Different alleles have been associated with a mood response to ATD (Moreno et al., 2002; Neumeister et al., 2002; Pierucci-Lagha et al., 2004). Heavy smokers also seem susceptible to depressive symptoms during ATD, irrespective of past history for major depression (Pergadia et al., 2004). However, although medication-free for six months, the history of SSRI use in this group was not reported.

Resistance The prevalence of depression in healthy older persons and older people with psychiatric complaints (Forsell & Winblad, 1997) combined with age-related declines in the 5-HT system, has led Meltzer (1999) to include older adults within the cohort of people having a biological vulnerability to depressive illness. Two studies specifically investigating mood response to ATD in healthy older women (Epperson et al., 2007) and older persons recovered from depression (Porter et al., 2005) found no effect. Other studies in patients with schizophrenia (Golightly et al., 2001) or men with a family history of alcoholism found no treatment effect on mood (Crean et al., 2002).

4.2.3.10 Post test day effects

A post test day change in mood was reported in a study with a group of currently depressed and medicated patients when 23% patients experienced worsened mood and 37% improved mood (Delgado et al., 1994); also in another study when baseline rate was resumed after an initial lowering of mood at 6 hrs (Klaassen et al., 1999b).

Risk Mood worsened for 42% of a similar patient group during the evening of the depletion day which, because it occurred after the experimental observation time, could not be statistically evaluated (Aberg-Wistedt et al., 1998).

Resistance A study of pooled data showed an improvement in mood symptoms the day after, but not during, the ATD test day, in 30% of currently depressed but unmedicated patients (Delgado et al., 1991). An improvement in mood occurred the following day for a group of depressed patients currently treated with the SNRI venlafaxine – but not SSRIs – receiving high dose depletion (100 g, Booij et al., 2005a) and for a group of menopausal women treated with oestradiol (Amin et al., 2006). There was no interaction of ATD by time for older remitted depressed patients on the MADRS assessed 27 hours post ingestion (Porter et al., 2005).

4.2.3.11 Predictive Effects

The ATD technique has also demonstrated its effectiveness in predicting individuals at risk for a relapse in depression within the following year (Moreno et al., 2000; Neumeister et al., 1999).
Table 4-1 Factors Affecting a Mood Response to ATD

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>non Risk Factors</th>
</tr>
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<tbody>
<tr>
<td>Remitted depression</td>
<td>Remitted depression</td>
</tr>
<tr>
<td>Recurrent episodes or chronicity</td>
<td>SSNRI treatment</td>
</tr>
<tr>
<td>Female gender</td>
<td>ECT treatment</td>
</tr>
<tr>
<td>Partially remitted</td>
<td>CBT treatment</td>
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<tr>
<td>Suicidal ideation</td>
<td></td>
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<tr>
<td>Cognitive reactivity</td>
<td></td>
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<tr>
<td>Family history psychiatric disorder</td>
<td></td>
</tr>
<tr>
<td>SSRI or MAOI treatment</td>
<td></td>
</tr>
<tr>
<td>Responsive to SSRIs</td>
<td></td>
</tr>
<tr>
<td>Remitted SAD</td>
<td>Remitted or current BPD (lithium treatment)</td>
</tr>
<tr>
<td>Bulimia Nervosa</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>Panic Disorder</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>IBS</td>
</tr>
<tr>
<td>OCD (SSRI treatment)</td>
<td></td>
</tr>
<tr>
<td>Genetic polymorphisms</td>
<td></td>
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<tr>
<td>Family history of affective disorder</td>
<td></td>
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<tr>
<td>Smoking</td>
<td></td>
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<tr>
<td>Neurotocism</td>
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<tr>
<td>Older age</td>
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<td>Current depression</td>
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<tr>
<td>Menopause</td>
<td></td>
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<tr>
<td>OCD (SSRI treatment history)</td>
<td></td>
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</tbody>
</table>

Note: ECT = electroconvulsive therapy; BPD = bipolar disorder; CBT = cognitive behavioural therapy; IBS = irritable bowel syndrome; OCD = obsessive compulsive disorder; MAOI = monoamine oxidase inhibitor; SAD = seasonal affective disorder; SNRI = selective serotonin noradrenaline reuptake inhibitor; SSRI = selective serotonin reuptake inhibitor.

4.2.4 Conclusion

There are a number of factors which predispose people to a mood lowering response during ATD, as presented in Table 4-1. In a pooled analysis of studies, Booij et al. (2002) concluded that, in people recovered from depression, chronicity is the strongest predictor of a depressive response to ATD, along with female gender, suicidal ideation, and prior – but not necessarily current – treatment with SSRIs. Chronicity was not found to be a risk factor in a more recent meta-analysis (Ruhe et al., 2007), but these authors defer to the findings of the previous study because it included a larger number of studies. The most consistent finding from the meta-analysis of Ruhe et al. (2007), was that ATD induced a decrease in mood and relapse into depressed state in currently medicated remitted depressed patients.

Although there is considerable evidence that dysregularities in the serotonergic system are responsible for aberrations in mood, it is still unclear where or what these are. Several neurotransmitters have been implicated, either on their own or in unison with other transmitter systems. There are some patient groups who have a biological vulnerability to the mood lowering effects of ATD.

Mood in healthy young people is not affected by ATD, but at present there is no robust experimental evidence that this resistance is paralleled in healthy older persons. Older people are vulnerable to mood disorders, not necessarily because they have a genetic risk, but because depression in this group is particularly related to vascular changes. Older persons are also more likely to have one or a combination of social factors (e.g., bereavement and isolation) and lifestyle habits, somatic,
psychological, and treatment co-morbidities. ATD studies have not demonstrated mood lowering vulnerability in this older cohort.

Females are more prone to depression than males, and have more severe symptoms and functional impairment. There may be a role for oestrogen in treating depression in females.

4.3 Serotonin and Cognition

Many reviews of animal and human studies have highlighted the role 5-HT plays in cognitive function (Booij et al., 2003; Buccafusco & Terry, 2000; Buhot, 1997; Buhot et al., 2000; Chamberlain et al., 2006b; Chudasama & Robbins, 2006; Ellis & Nathan, 2001; McEntee & Crook, 1991; Meneses, 1999; Palmer & DeKosky, 1993; Richter-Levin & Segal, 1996; Riedel & Jolles, 1996; Robbins, 1997, 2000; Roth et al., 2004; Sirvio et al., 1994). The precise nature of this role is unclear but it seems likely to occur through interactions with other neurotransmitter systems, in particular the cholinergic, dopaminergic, noradrenergic, GABAergic, and glutaminergic systems (Buhot et al., 2000; Cassel & Jeltsch, 1995; Lancot et al., 2001; Leckman et al., 1980; Little et al., 1995; Matsuda et al., 2002; Richter-Levin & Segal, 1993; Steckler & Sahgal, 1995). Under normal conditions, these interactions occur at a number of receptor sites in different brain areas. Investigation of individual receptor activity, however, has been difficult in living subjects, thus, the pattern of 5-HT action has generally been inferred from performance on cognitive tasks.

The following section outlines the findings on some of these tasks, but I would like to first make clear (Box 1) the range of behavioural domains these cognitive tasks assess. Lezak (1995) defines cognition as one of three behavioural functions, the others being personality and executive functioning. This thesis will be exploring executive functioning and the cognitive functions of memory, including working memory. It will also examine three mental activity variables (i.e., attention, activity rate and consciousness) which she says energise the activity of the cognitive functions.

**Box 1**

*Memory* comprises a number of systems (Squire, 2004) that allow the brain to store traces of information and later create memories. Memory requires an interaction between newly learned information and currently stored memories, with different brain areas and neurotransmitter mechanisms (Izquierdo & McGaugh, 2000) being recruited to acquire, or learn, (i.e., encode and consolidate) information and different ones required to retrieve a memory. Some brain regions are preferentially active during short-term and working memory (e.g., PFC), storing factual information for later retrieval from long-term memory (e.g., hippocampus and related cortical areas), or modulation of the storage related to emotional events (e.g., amygdala) (Baddeley et al., 2000).

The main structures critical for memory acquisition of factual information are those comprising the hippocampal complex in the mesial temporal lobe; those involved with the retrieval of this type of memory are located in nonmedial temporal regions (Tranel & Damasio, 2002). The PFC is also
involved in memory when executive guidance or control is required (Stuss & Benseon, 1987). The left prefrontal cortex is more involved than the right during the encoding of episodic memories and the right prefrontal cortex during the retrieval of these memories. This hemispheric encoding/retrieval asymmetry (HERA) model operates for both verbal and non-verbal matter (Habib et al., 2003; Tulving et al., 1994).

This region specialty, however, is not a complete explanation of the way the brain functions as an integrated unit. Its processes are multifarious. In the PFC for example, activity is sometimes anatomically and functionally fractionated, limiting activity to a localised region or network. This occurs in the superior medial region when energisation, or concentration, for a task is required; at other times, regions along prescribed and ‘geographically’ diverse networks are activated when tasks are more demanding (Stuss, 2006).

The issue for neuropsychological assessment is discerning which function, or functions, among the myriad of potentially confounding processes operating within a dynamic system is being assessed by a particular test. A further issue in neuropsychological assessment is that brain function can be fractionated and characterised but only at a conceptual level. In reality several brain functions operate to initiate and co-ordinate behaviour. For example, posterior and basal regions are involved in mood, motor, memory, cognition, attention, alertness, for example, but anterior regions subserve these regions in a superordinate manner providing executive control and overarching consciousness (Stuss & Benseon, 1987).

For the sake of simplicity, however, this thesis makes several conceptual divisions in cognition. To begin with, a distinction is made between long-term and working memory. Whereas working memory is about memory for the present, long-term memory comprises two systems: one which is past-oriented in that a person has to travel back in time to a given episode to access the required information (declarative); and one which is prosoplic in that information is acquired for future use with no requirement to travel back in time (procedural) (Nilsson, 2003). The distinction is based on whether information acquired is brought to conscious awareness at the time of retrieval, or not.

Declarative memory is dichotomised into semantic memory (i.e., memory for facts) and episodic memory (i.e., memory for particular episodes) (Squire, 2004). The remainder of this thesis will be referring to episodic memory when discussing memory.

Episodic memory involves the organisation of past information within a spatiotemporal context. This information is organised in such a way that retrieval of the memory enables a flexible behavioural response (J. McGaugh in, Baddeley et al., 2000). Episodic memory is assessed with recall (i.e., the ability to remember something in the context of something else) and recognition (i.e., the ability to remember previously presented information when subsequently re-offered) tasks.

Executive Functions generally refer to those mechanisms that optimise performance by allowing the simultaneous operation of a number of different cognitive processes (Owen et al., 1998b) and in doing
so, cover such functions as anticipation, planning, goal selection, monitoring (Stuss, 2006); also selection and perception of relevant information, decision making, reasoning, initiation, behavioural control and adaptation to environmental changes, cognitive flexibility, and task management. They are the mental operations which are critically involved in adaptation to novel situations and where, because prior experience or external aids are lacking, a heuristic plan of action and auto-monitoring are required (Taylor & Saint-Cyr, 1995). They differ from memory in that a person can sustain large losses in memory, but remain independent; however, when executive functions are impaired, they may not. Executive functions are assessed by a variety of tasks; they are the ‘how’ and ‘why’ type tasks.

A novel concept has been suggested linking executive functioning and working memory to the creation of memories. L. Nadel in Baddeley et al. (2000) hypothesised the hippocampus as establishing a static template that links together various cortical and limbic representations of all information in an event with the frontal cortex providing the mechanisms for sequencing these disparate traces. It is within this frontal process that working memory functions since working memory holds a sequence record of recently experienced things.

**Working Memory** allows a limited amount of new information to be temporarily held and inspected against previously stored information, while at the same time holding information on-line. The classic model encompasses three main components: verbal, visuospatial, and central executive (Baddeley, 1992), although additional details have been added e.g., the episodic buffer (Baddeley, 2000). Working memory is assessed with verbal or visual spans, and tasks involving maintenance, monitoring or manipulation of information.

A review (D'Esposito, 2001a) of original neuroimaging data from a number of studies provides support for the role of the PFC in working memory, the lateral areas in particular. The left and right hemisphere PFC areas are generally associated with ‘type of information’ (i.e., spatial and non-spatial) (Baker et al., 1996; Smith et al., 1996; Smith et al., 1995), while the ventral and dorsolateral PFC areas are associated with the ‘type of operation’ (i.e., maintenance or manipulation) (D'Esposito, 2001b). For example, studies (Cabeza & Nyberg, 2000a, b) showing stimulation in the lateral PFC during working memory tasks suggest tasks related to holding or simple maintenance of information over a short period of time may be related to ventrolateral PFC (D'Esposito et al., 1999; Klingberg et al., 1997; Tsukiura et al., 2001); other tasks that require participants to hold and actively manipulate or monitor information have been shown to activate both ventrolateral PFC and DLPFC areas (D'Esposito et al., 1999; Klingberg et al., 1997; Manoach et al., 1997; Owen et al., 1996b; Salmon et al., 1996; Tsukiura et al., 2001).

The DLPFC is generally considered one of the main brain regions underpinning the central executive function within the tripartite system of working memory because of its top-down control. Patients with lesions in this area have problems with sustained attention, working memory, and maintaining or shifting-set in response to task demands (Malloy et al., 1993). Executive function, working memory,
and attention are three processes that activate regions in the PFC – like the DLPFC – that are
intimately connected to basal ganglia structures. In his comprehensive description and review of the
frontal lobes, Petrides (2000a) explains that cognition studies are concerned with the anterior (or
prefrontal) part of what is otherwise a very large part of the human cerebral cortex. Excluding the
motor or premotor cortices, the PFC has distinct regions with specific connections to subcortical and
other cortical areas with similar functional specialisation. The anterior cingulate cortex (ACC), OFC,
and the DLPFC are three frontal areas forming frontostriatial circuits and are critical in executive
functions (Zgaljardic et al., 2003).

The OFC is associated with response inhibition, especially in affective processing (Dias et al., 1996),
that is, inhibiting extraneous associations which do not comply with the task requirements; rule breaks
in the COWA, for example (Malloy et al., 1993). This region is connected to with limbic structures in
the cingulate and anterior temporal lobes and hence the role for the OFC in integrating motivational
and emotional processes (Malloy et al., 1993). In humans, disruptions in the OFC can lead to
emotional instability (e.g., disinhibition, euphoria, social irresponsibility, diminished affect, and poor
reasoning and decision-making abilities) in patients with conditions like depression and obsessive
compulsive disorder (Zgaljardic et al., 2003). Psychometric neuropsychological tasks to assess OFC
are lacking because it is difficult to separate OFC from DLPFC functions, however, patients with OFC
lesions are unable to inhibit their responses in Go-No Go tasks (Malloy et al., 1993).

The ACC is more often associated with affect and behaviour, however, there are a number of theories
suggesting it is also involved in cognition – particularly in evaluative processes when strong control is
required – for example, awareness, response intention, sustained attention, spontaneous response
production, response monitoring, and in particular, maintaining the attentional demands of monitoring
errors in a task requiring conflicting responses (MacDonald et al., 2000; Zgaljardic et al., 2003). This
function therefore allows for sudden adaptive changes in behaviour in response to a given context
(Luu & Pederson, 2004).

A conflict-monitoring hypothesis would be represented by increased activation in the ACC
accompanied by improved performance on behavioural tasks representative of focused attention
(Botvinick et al., 2004). This was demonstrated when high ACC activity and low interference scores
were correlated with incongruent trials during the Stroop task (Kerns et al., 2004). The authors in this
study noted the relatively strong activation in DLPFC following trials with greater ACC engagement,
consistent with the role of this area in top down control. A further example of this phenomenon
occurred during ATD interference when scores for incongruent colour words decreased during the
experiment (Evers et al., 2006b). It is possible ATD triggers greater cognitive control, greater focused
attention, and thus improved performance on this task.

There are extensive and reciprocal connections between the DLPFC and areas in the parietal, occipital,
and temporal lobes for integrating sensory information from multiple modalities (Malloy et al., 1993).
The DLPFC role is also to maintain and monitor events in working memory (Petrides, 2000a, p. 76). It follows that the DLPFC works with other brain regions that are critical for the manipulation of working memory since manipulation also requires the monitoring of current information.

The frontal regions of the brain act in concert with each other and with other non-frontal regions to ensure the execution of smooth, integrated behaviour (Malloy et al., 1993). Neuroimaging studies also suggest that different frontal regions demonstrate dissociable functions in tasks that are classified as either executive function, working memory, or attentional. For example,

Firstly, the understandings favoured by the HERA model, above.

Secondly, during a modified Stroop task, activation observed in neuroimaging scans was strong in the ACC during conflict monitoring and in the DLPFC (Brodmann’s area 9) during successful task implementation (e.g., naming the colour, not the word). The investigators interpreted this as consistent with a role in the implementation of control, whereas activation in the ACC (Brodmann's areas 24 and 32) when responding to incongruent stimuli, was interpreted as consistent with a role in performance monitoring (MacDonald et al., 2000). Similarly, during Go-No Go tasks which require shifting cognitive set (e.g., the Intra-Extra Dimensional Set-Shift, IED, task from CANTAB) (Robbins et al., 1994b). Within these tasks a person trained to respond to a particular stimulus dimension (e.g., a shape or colour) is asked to (a) transfer the learned rule to a new set of exemplars within the same stimulus dimension, that is, make an intra-dimensional (ID) shift, (b) shift to a set in an alternative, previously irrelevant dimension, that is, make an extra-dimensional (ED) shift (Owen et al., 1991), or (c) perform both these set shifts when the stimulus reinforcement pairing (e.g., green √ / red x) is reversed without warning, that is, make a stimulus-reward set reversal (also labeled reversal shift or reversal learning).

And thirdly, commentators on the Wisconsin Card Sorting Task (WSCT) (Grant & Berg, 1948) – which involves similar set shift components to the IED – have associated the task with the working memory process of maintaining task relevant information after comparing it with new information. This is because the brain regions activated (Berman et al., 1995) have been shown to be similar to those in a sequential letter n-back task (Cohen et al., 1997). A more recent neuroimaging study, however, has parsimoniously attributed ED set shift to attentional control (Rogers et al., 2000). Specifically, to the requirement of overriding an acquired attentional bias, if a response to a new stimulus dimension is to be made and the attentional pull back to the previously relevant stimulus is to be ignored.

It is probable that different brain regions are active during different aspects of tasks (e.g., reversal set shift activates cells in the OFC and ED set shift activates cells in the DLPFC) (Rogers et al., 2000). It is equally probable that different neurotransmitters are involved and thus, classifying the WSCT as solely on executive function, working memory or attention task is too simplistic. For example, animal studies have shown lesions to the ascending cholinergic neurons produce specific deficits in reversal shift (Roberts et al., 1992), whereas, lesions to DA neurons projecting to the PFC produce specific
deficits in ED shift (Roberts et al., 1994); human studies have found that the performance of PD patients taking dopaminergic medication was improved during an ED set shift task, but was impaired during a reversal shift task (Cools et al., 2001). The authors of the latter study hypothesised that the integrity of the DLPFC is dependent on circuitry from the caudate nucleus – an area profoundly affected by DA depletion in PD – thus augmentation would improve performance in a task reflecting function in this region; the OFC, on the other hand, is dependent on ventral striatal circuitry – which is relatively spared in PD – demonstrating supraoptimal doses of DA to this region may prove detrimental (Cools et al., 2001).

Attention relates to the receptivity of an animal or human to internal and external stimuli and how processing of these stimuli is initiated. Attention requires arousal. Two types of attention are relevant to this thesis. Sustained attention (or the medically oriented term, vigilance) involves actively attending to a cognitive operation over a period of time. This ability to sustain alertness is thought to be mediated by the reticular activating system and NA (Ponsford, 2000). Focused attention and the related term selective attention refer to the ability of attending to a relevant stimulus while inhibiting irrelevant stimuli. Anterior brain regions and the basal ganglia are important in focusing attention and inhibiting irrelevant information (Ponsford, 2000). Focused attention is linked to awareness and the conscious control of information processing. Information processing has a limited capacity and rate so that heavy demands on attention will limit task performance, e.g., when attention is divided.

Attention is essential in the formation of episodic memories; when not given full attentional focus, an episode is likely to be forgotten, despite attempts to remember it (Craik et al., 1996). For example, when a task is given full attention, or is divided by the demands of an easy secondary task, successful encoding activates the left PFC, hippocampus, and parahippocampal gyrus; when attention is divided by the demands of a hard task these areas are less activated (Fletcher et al., 1995). When the encoded information is later recalled it is not as rich or detailed with a hard task as a consequence of the distraction from dividing attention, possibly indicating a reduction in explicit memory traces (Kensinger et al., 2003).

There is no single test of attention (Ponsford, 2000); focused and sustained attention are often assessed with reaction time and accuracy (i.e., hits minus false alarms) in timed tasks. The problem with timed tasks is that they are often multifactorial and do not necessarily correspond to the constructs of attention. For example, the Trail Making Test-Part B can be loaded on to a factor measuring mental processing, psychomotor speed, and focused attention (Ponsford, 2000), but it also requires dexterity, movement speed, and mental flexibility and the ability to shift attention.

Activity Rate includes both psychomotor speed (i.e., the speed of mental activity; information processing speed) and motor response speed. Psychomotor speed reflects the cognitive load of a task, that is, the degree of concerted effort required to perform a task. Thus, healthy persons will complete passive tasks (e.g., recognition tasks) more quickly than they will tasks with greater cognitive load.
Psychomotor speed is the response time (or alternatively labeled, reaction time or latency) recorded in a wide variety of tasks (e.g., simple and choice reaction time tasks) and refers to the time between stimulus onset and initiation of a response; motor response speed (or alternatively labeled, movement time) is the time from initiation of response to termination. Accuracy of response also needs to be considered when assessing processing speed because an inordinate number of false hits may imply heightened impulsivity.

Consciousness refers to awareness, rather than merely being awake. It is the degree to which someone is aroused or receptive to stimulation. It is assessed either clinically or with tasks of attention, reaction time, or both. Disturbances of consciousness like hallucinations are assessed quantitatively and qualitatively. Fluctuations in consciousness are assessed quantitatively.

As noted earlier, 5-HT has a role in cognition, but the precise nature of this role is, as yet, unclear. A number of studies have investigated 5-HT and cognition. Animal research is helpful in providing behavioural and anatomical comparisons but results can only suggest what may be happening in live humans; likewise, post-mortem human studies. Challenges to the serotonergic system in living healthy and patient human groups allow insights into the functional role of this neurotransmitter.

Cognition has been thus investigated secondary to mood in pharmacological trials of SSRIs and TCAs. Unfortunately, two problems with these studies are that (a) they use an open-label design so that there is no control and no blinding of treatment group with which to compare cognitive effects and (b) the non-5-HT specificity of the TCAs (e.g., additional anticholinergic, antihistaminergic, antidopaminergic, and antinoradrenergic properties) make them unreliable vectors for examining cognitive effects of 5-HT enhancement (Amado-Boccara et al., 1995). 5-HT agonists and antagonists – with varying specificities – have also been used but not extensively (Schmitt et al., 2006). Other methods include TRP loading and depletion, and both have been used in healthy volunteers as well as groups having hypothesised 5-HT hypofunction (e.g., people with various affective and psychiatric disorders, sleep disorder, pain, fatigue, neurodegenerative disorders, eating disorder, irritable bowel syndrome, or older age).

The following section summarises the research findings from these challenge studies on cognitive function only. 5-HT+ refers to the effects of serotonergic enhancement; 5-HT- refers to the effects of serotonergic reduction.

### 4.3.1 5-HT and Global Cognitive Status

#### 4.3.1.1 5-HT+ and Global Cognitive Status

In two recent PD studies, the SSRI citalopram and its S-enantiomer escitalopram had no effect on global cognitive status (Menza et al., 2004; Weintraub et al., 2006). Studies investigating SSRIs in
patients with SDAT and vascular dementia have reported no clear improvement in cognition (Nyth & Gottfries, 1990).

### 4.3.1.2 5-HT- and Global Cognitive Status

Serotonin level has an influence on global cognitive status in older patient groups in that acute tryptophan depletion induced a significant impairment on the Modified Mental State examination (3MS) (Teng & Chui, 1987) in concurrently medicated older people recovered from depression (Porter et al., 2005) and in SDAT patients (Porter et al., 2000).

**Commentary**

Enhancement of 5-HT does not seem to alter global cognitive status in neurodegenerative disorders, compared to young patient and healthy adults in all age groups. However, there is evidence to suggest it may be impaired in these diseases when 5-HT is reduced, suggesting older patient groups may be at risk for cognitive effects after ATD.

### 4.3.2 5-HT and Memory

There is much animal support for the role of 5-HT in memory. Studies in rats have demonstrated that peripheral TRP depletion lowers 5-HT activity in the hippocampus, while TRP loading has the opposite effect (Gartside et al., 1992; Sharp et al., 1992; Stancampiano et al., 1997). Examples like this are outlined in several of the comprehensive reviews available on animal literature regarding 5-HT and cognition (Buccafusco & Terry, 2000; Buhot, 1997; Buhot et al., 2000; Chudasama & Robbins, 2006; McEntee & Crook, 1991; Meneses, 1999; Richter-Levin & Segal, 1996; Sirvio et al., 1994; Steckler & Sahgal, 1995).

The success of these animal models has prompted the search for serotonergic drugs which may have prospective cognitive enhancing properties (for review see, Buhot et al., 2000). Drug affinities to various receptor subtypes produce selective effects on behaviour, but can also influence seemingly similar 5-HT-mediated behaviours in quite different ways (Lucki, 1998). For example, 5-HT$_3$ antagonists (Arnsten et al., 1997; Terry et al., 1996) and 5-HT$_4$ agonists (Terry et al., 1998) have both been shown to enhance the performance of aged macaque monkeys in a delayed-matching-to-sample task.

McEntee and Crook (1991) stimulated interest in the possibility that reduced 5-HT levels will improve learning and memory. Their optimism was based on results from animal studies using passive avoidance tasks. Other studies have been using the ATD paradigm to establish this hypothesis in humans, using verbal and visual recall and recognition tasks. Whereas the animal data used in the nine studies reviewed by McEntee and Crook confounded pre-training (pre-retention) with post-training performance – the former impairing, the latter enhancing performance – the data from human studies have been more consistent.
Cognition is more than learning and memory, however, and as such the following review of studies investigating the effects of 5-HT challenge studies in healthy young adults and some patient groups includes executive function and the mental activity variables.

### 4.3.2.1 5-HT+ and Memory

There have been mixed reports on the effects of 5-HT enhancement in memory in healthy young adults. Studies have used SSRIs, 5-HT agonists, and TRP supplementation to investigate short-term memory and long-term memory. Depending on the compound being tested, SSRIs can either improve (Furlan et al., 2001; Harmer et al., 2002), impair (Riedel et al., 2005; Riedel et al., 2002a; Schmitt et al., 2001), or have no effect (Schmitt et al., 2001; Siepmann et al., 2003). TRP supplementation has impaired long-term memory (Sobczak et al., 2003). Taken together the evidence for 5-HT enhancing memory is not viable in young adults, possibly because they may be already operating at ceiling levels (Schmitt et al., 2006).

No effects of two SSRIs were observed in a study investigating memory in healthy older persons (Furlan et al., 2001).

People with depression, irrespective of age, generally have some cognitive impairment which could include memory, attention, executive function, or cognitive flexibility (Schmitt et al., 2006). When the serotonergic system has been enhanced there have been significant improvements in memory (Levkovitz et al., 2002), improvements that preceded improvement in mood (Riedel et al., 2002b).

There have been no SSRI studies investigating cognition per se in SDAT (Schmitt et al., 2006). In PD, escitalopram had no effect on memory (Weintraub et al., 2006).

### 4.3.2.2 5-HT- and Memory

ATD studies have produced a definitive pattern of episodic memory impairment during recall tasks which is irrespective of serotonergic vulnerability or integrity (Sambeth et al., 2007). Delayed recall impairments have been repeatedly reported for verbal (Harrison et al., 2004; Kilkens et al., 2004; Klaassen et al., 2002; McAllister-Williams et al., 2002; Riedel et al., 1999; Schmitt et al., 2000) and visual material (Booij et al., 2005b; Park et al., 1994; Rogers et al., 1999). Impaired performance has been reported for delayed verbal recognition (Harrison et al., 2004; Sobczak et al., 2002d) but only (a) as far as reduced speed of response (in the absence of reduced psychomotor speed) (Evers et al., 2005) and (b) at an observation time 24 hrs post depletion (Riedel et al., 1999). Most studies report no significant effect of depletion on verbal (Hughes et al., 2003; Park et al., 1994) and visual (Hughes et al., 2003; Matrenza et al., 2004; Park et al., 1994; Rubinsztein et al., 2001) recognition memory.

There is one critical factor to bear in mind regarding the effects of ATD on long-term memory and that is that ATD begins disrupting memory processes in the 30 mins post presentation of the index items (Schmitt et al., 2000). The aforementioned authors showed that when presentation, and thus learning,
occurred an hour before treatment, ATD did not affect memory consolidation. This means the optimal
time for items to be presented is at least four to five hours after treatment when depletion is maximal.

Traditionally, short-term memory has not been affected by ATD (Harrison et al., 2004; Riedel et al.,
1999; Shansis et al., 2000), but a recent pooled analysis of ATD data in a verbal memory task
suggested impairment on encoding and consolidation (Sambeth et al., 2007). Other studies have
suggested small but non-significant impairments (Hughes et al., 2003; Riedel et al., 1999; Schmitt et
al., 2000; Sobczak et al., 2002d). Furthermore, functional magnetic resonance imaging (fMRI) scans
taken during ATD have demonstrated encoding during a visual verbal episodic memory task
attenuates activation in the right hippocampus, whereas, retrieval has no effect on brain activity (Van
der Veen et al., 2006).

In older persons, there is an indication that memory may be affected by reduced tryptophan since the
study by Porter et al. (2005) found main effects in recall tests of verbal and visual memory.

Commentary

Definitive information regarding 5-HT enhancement is lacking; although SSRIs improve cognition in
people with depression. ATD has consistently induced impairments in verbal and visual memory
during tasks of recall. It is most likely the impairments are attributable to an effect on encoding and
consolidation rather than the retrieval aspects of memory.

4.3.3 5-HT and Executive Function

4.3.3.1 5-HT+ and Executive Function

TRP loading had not altered performance on the Controlled Oral Word Association task (COWA)
(Luciana et al., 2001), nor has paroxetine (COWA, Deakin et al., 2004, in patients with frontal variant
of frontotemporal dementia; Stroop, Kerr et al., 1992).

There were no effects on executive function in two SSRIs studies investigating cognition in healthy
older volunteers (Furlan et al., 2001), and in PD, escitalopram had no effect on executive function
(Weintraub et al., 2006).

4.3.3.2 5-HT- and Executive Function

TRP reducing studies have produced mixed performances by healthy adults on tasks of frontal
functioning. One study (Park et al., 1994) used the Tower of London (TOL) task of executive function
but interpreted an interaction between ATD and order as an effect on learning. Overall, ATD-related
performances include improvement (Emotional Stroop, Hayward et al., 2005; Stroop/Trails, Rowley et
al., 1997; Stroop/COWA, Schmitt et al., 2000), impairment (reversal learning on visual discrimination,
Rogers et al., 1999), or no difference (COWA, Allen et al., 2006; Stroop/COWA/Trails (reaction
time), Gallagher et al., 2003; Tower of London, TOL/COWA/Trails, Hughes et al., 2003; Kerr et al.,
1992; TOL, Murphy et al., 2002; Visual Discrimination, Park et al., 1994; TOL, Schmitt et al., 2000;
Hebb Digits/Corsi Blocks, Shansis et al., 2000).

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A similar inconclusive scenario holds for patient groups. There are reports of ATD being associated with main effects of improved (dose dependent effects on Neutral and Emotional Stroop, Booij et al., 2005b, in depressed or recently recovered depressed; Emotional Stroop, Hayward et al., 2005, in recovered depressed; Stroop and COWA, Riedel et al., 2002b, people with family history of depression; COWA, Schmitt et al., 2000), impaired (Letter Fluency, Booij et al., 2005b, in depressed or recently recovered depressed patients; Digit Span Backward, Porter et al., 2003b, in SDAT patients and controls) and no (TOL, Booij et al., 2005b, in depressed or recently recovered depressed patients; COWA, Porter et al., 2003b, in SDAT patients and controls; COWA, Porter et al., 2005, in older recovered depressed patients and controls) effects on performance.

4.3.3.3 Commentary

Evidence suggests enhancing the serotonergic system does not alter performance in executive function tasks; the situation during reduction is not as clear cut. Although Schmitt et al. (2006) claimed the impairments seen in long-term memory are not attributable to changes in executive function, it is difficult to see how such a call could be given when the above discrepant findings are taken into account. Sambeth et al. (2007) engaged the theory of Izquierdo et al. (1999) to circumvent the traditional explanation for working memory in explaining an ATD-induced impairment in immediate recall. Immediate recall of learned word lists is generally considered a short-term memory task and thus it assesses encoding. Because the immediate recall task of Sambeth et al. occurred after the 30 second duration of short-term memory, and because – as Izquierdo et al. hypothesise – short- and long-term memories involve separate mechanisms, the task is actually assessing consolidation and thus long-term memory traces. It may be, however, that two theories amalgamate. That is, the traditional theory that short-term memory progresses to long-term memory and the newer theory that short- and long-term systems are different. For example, in a recently published study, Izquierdo et al. (2007) demonstrated a time- and receptor-dependent correlation between the glutamate, AMPA, and dopamine D\textsubscript{1} receptors in the DLPFC and medial prefrontal cortex during both working and long-term memory, showing that the same cells activated during working memory (or what they synonymously call immediate memory) are activated during consolidation. Thus treatments that affect executive function or immediate memory can affect memory measured at a later time.

4.3.4 5-HT and Working Memory

4.3.4.1 5-HT+ and Working Memory

Animal literature has not generally considered 5-HT the prime operator in working memory, this role is given to DA and to some extent, ACh (Barch, 2004; Collins et al., 1998; Collins et al., 2000; Furey et al., 2000; Furey et al., 1997; Luciana & Collins, 1997; Luciana et al., 1992).

Verbal working memory was impaired in healthy adults after TRP loading (Digit Span Backwards, Luciana et al., 2001). One study using fenfluramine induced impairment in spatial working memory in humans which the authors concluded was due to 5-HT modulation of DA (Luciana et al., 1998).
4.3.4.2 5-HT- and Working Memory

No effects of ATD have been reported for spatial working memory in human studies (Harrison et al., 2004; Park et al., 1994; Porter et al., 2003b; Porter et al., 2005), however, ATD has been shown to affect verbal working memory. There was an impaired performance for all participants in the Digit Span Forwards (Porter et al., 2005) and Digit Span Backwards tasks (Porter et al., 2003b). This has not been seen in younger adults (Allen et al., 2006; Harrison et al., 2004).

4.3.4.3 Commentary

Evidence for the role of 5-HT in working memory is unclear. ATD studies, using younger participants – mostly university students – have consistently demonstrated that ATD has no effect on working memory. The two studies by Porter and colleagues showed working memory was affected by ATD, however, DigitsF was affected in one study (Porter et al., 2003b) but not in the other (Porter et al., 2005) and, reciprocally, the opposite pattern happened for DigitsB. The effect of ATD on working memory in older persons thus requires clarification.

4.3.5 Other

4.3.5.1 5-HT+ and Set Shifting

Administration of paroxetine to patients with frontotemporal dementia was associated with impaired ability to make a reversal shift but had no effect on ID or ED set shifting (Deakin et al., 2004).

4.3.5.2 5-HT- and Set Shifting

Performance on the WCST and a complex version of an ID/ED set shifting task, suggests ATD may impair reversal learning while leaving set shifting relatively unimpaired (Park et al., 1994; Rogers et al., 1999). However, this finding has not been supported in a more recent study using the IED from CANTAB, which found no ATD effects on set shifting or reversal learning (Talbot et al., 2006). The improvement in set shifting was not replicated also on the Go-No Go task used by Porter et al. (2003b) in healthy older adults.

Despite a trend towards slowed reaction time, no effects of ATD have been found in a probabilistic reversal learning task (Evers et al., 2005; on first test day only, Murphy et al., 2002). A recent neuroimaging study found improvement on the Stroop task, as evidenced by the decreased interference score for the incongruent colour (Evers et al., 2006b).

Commentary

By requiring a participant to make a response while inhibiting another response, tasks such as Stroop, ID/ED, and Vigil (Cegalis & Bowlin, 1991), involve the DLPFC and adequate levels of DA. Tasks that involve the reversal of a response activate cells in the OFC and require intact ACh and DA neurotransmitter systems (see Box 1). It is possible ATD would affect set-shifting in groups where these neurochemicals are deficient.
4.3.6 5-HT and Attention

4.3.6.1 5-HT+ and Focused Attention

There is insufficient data to make inferences about focused attention from 5-HT enhancing studies. Several studies with SSRIs in healthy young adults have found no effects in focused attention (Hindmarch & Harrison, 1988; Schmitt et al., 2002), only vicarious improvement based on response to irrelevant interference stimuli (Sobczak et al., 2003). One study with an older cohort reported improvement with fluoxetine but not with amitriptyline (Fairweather et al., 1993).

4.3.6.2 5-HT- and Focused Attention

In 5-HT reduction studies, however, there is persuasive evidence that 5-HT is implicated in focused attention. Several studies have reported improvement in healthy young adults during ATD (Ahveninen et al., 2002; Booij et al., 2005b; Gallagher et al., 2003; Schmitt et al., 2000). Others have found worsened performance (Ahveninen et al., 2002), or no effect (Rogers et al., 1999; Sobczak et al., 2002d) and vicarious effects (reaction time to incompatible stimuli) (Coull et al., 1995).

People with a family history of affective disorder are considered vulnerable to the effects of ATD; their performance on a task of focused attention improved during ATD (Riedel et al., 2002b).

4.3.6.3 5-HT+ and Sustained Attention

A TRP loading study found loading enhanced immediate vigilance when participants making fewer errors in a letter cancellation task (Luciana et al., 2001).

Several studies have used SSRIs to investigated enhanced serotonergic function in sustained attention. Although there are reports that serotonergic enhancers have not affected performance in vigilance tasks – with the exception of response time during administration of citalopram (Deijen et al., 1989; Harmer et al., 2002; Kerr & Hindmarch, 1996; Nathan et al., 2000) – even in healthy older persons (Furlan et al., 2001), many studies have found impaired performance (Ramaekers et al., 1995; Riedel et al., 2005; Schmitt et al., 2002). The effects seem to be specific to the SSRI used and the differential affinity these compounds have to different receptors and to other neurotransmitter systems. For example,

a. One study undertaken to investigate the association between serotonin and attention and contributing roles of specific serotonin receptors found combined treatment with escitalopram and ketanserin (a 5-HT2A receptor antagonist) impaired sustained attention, but not other types of attention; this did not occur, however, when the SSRI was combined with pindolol 5-HT1A receptor antagonist (5-HT1A receptor antagonist, Wingen et al., 2007).

b. Sertraline has been consistently shown not to affect sustained attention, possibly because of affinity to DA receptors; whereas citalopram with its specific serotonergic action impairs vigilance (Riedel et al., 2005).
c. The tricyclic amitriptyline impaired performance in several tasks of attention but as this compound has prominent anticholinergic effects as well as NA enhancing and antihistamine effects, the effect on performance may not be attributable solely to 5-HT.

4.3.6.4 5-HT- and Sustained Attention

No effects of ATD have been observed on vigilance tasks in healthy young adults (Harrison et al., 2004; Park et al., 1994; Shansis et al., 2000).

ATD has not been associated with altered performance in a vigilance task or in tasks that audited response time in older persons recovered from depression (Porter et al., 2005) or in SDAT patients (Porter et al., 2003b).

Commentary

Generally enhancement of 5-HT has no effect on focused attention but mixed effects on sustained attention. Reduction studies have found improved performance in focused attention but no effect in sustained attention. It may be that vigilance tasks involve both the serotonergic and dopaminergic systems in that simultaneous depletion by TRP and catecholamines has impaired this function in healthy controls (Harrison et al., 2004), while ATD on its own has no effect.

4.3.7 5-HT and Activity Rate

4.3.7.1 5-HT+ and Information Processing Speed

Information processing (or psychomotor) speed is used as an assessment of arousal as well as a vector for comparing cognitive load. Studies investigating reaction time with SSRI administration have found accelerated responses in a passive simple reaction time task with sertraline (Riedel et al., 2005) and an active choice reaction time task with citalopram (Hindmarch & Bhatti, 1988; Nathan et al., 2000), but nothing in the other SSRIs investigated. It is assumed from the bibliographies that these studies used the same task and it is assumed that movement time was enhanced in both, as opposed to decision making time. A recent study found that the SSRI citalopram and sertraline had no effect on simple or choice reaction time (Riedel et al., 2005). These studies highlight the problem of comparing results from studies employing different tasks with different variables because interpretations about the cognitive domain under investigation are confounded by variables other than treatment or group variables.

One study used a 5-HT$_{2C}$ agonist (mCPP) and reported increased reaction time in a simple reaction time task with no effects on movement time (Riedel et al., 2002a).

A TRP loading study showed a detrimental effect of TRP on time to completion in the grooved pegboard task assessing motor speed (Luciana et al., 2001).
4.3.7.2 5-HT- and Information Processing Speed

Six studies specifically investigating information processing speed have reported no main effect of ATD in healthy or patient groups (simple and choice reaction time, Harrison et al., 2004; visual inspection time and females only, Harrison et al., 2002a; simple and choice reaction time, Riedel et al., 1999; reaction time to ‘go’ signal, Rubia et al., 2005; simple and choice reaction time, Schmitt et al., 2000); likewise combined serotonin-catecholamine depleting (Matrenza et al., 2004). One study examining high cognitive demand in females only, found ATD increased the interference of negative words on color naming in the Emotional Stroop task (Evers et al., 2006a). Others have demonstrated increased response times when a task had a higher cognitive load (novel task only, Murphy et al., 2002) or assessed executive function (Sobczak et al., 2002d).

Two studies specifically reporting motor speed demonstrated improvement (Trails A, Gallagher et al., 2003; grooved peg board, Luciana et al., 2001). However, others suggest motor speed is not affected by ATD (TOL/Abstract Pattern Recognition, Booij et al., 2005b; Stroop variants, Evers et al., 2006b; Trails A and B, Hughes et al., 2003; movement time, Riedel et al., 1999; movement time Schmitt et al., 2000).

Commentary

It is unclear what the effect of serotonergic enhancement has on reaction time. There does not appear to be a significant difference in reaction time during ATD, however, it is possible that when cognitive load is higher, the response may be slower.

4.3.8 5-HT and Impulsiveness

It may be that reaction time tasks measure, not psychomotor speed, but impulsivity of responding. Impulsivity has been assessed with reductions in 5-HT activity, either through neurotoxin lesions (in rats, Harrison et al., 1997) or ATD (in humans, Booij et al., 2006; Walderhaug et al., 2002). Both have induced more impulsive responding in animal and human groups. Performance on tasks requiring response inhibition (e.g., choice reaction time tasks) are reported to be unaffected by ATD (Booij et al., 2005b; Cools et al., 2005; Evers et al., 2006b; Harrison et al., 2004; Luciana et al., 2001; Rubia et al., 2005); however, there is one study where ATD did produce an increase in impulsive responding (males only studied, Walderhaug et al., 2002).

4.3.9 5-HT and Consciousness

Studies investigating 5-HT activity in consciousness have tended to focus on the role of the monoaminergic and cholinergic activating systems in the states of sleep and wakefulness. 5-HT activity is increased during arousal and dampened during sleep (Wilkinson et al., 1991); and 5-HT enhancement with SSRIs has been shown to reduce arousal to monotonous tasks or environments (O’Hanlon et al., 1998). Reduction in 5-HT activity through lesioning heightened arousal in rats as measured by their rapid and impulsive responses (Harrison et al., 1997).
4.4 Summary

The activity of 5-HT is endemic in most regions of the brain. The serotonergic system communicates with other transmitter systems in the normal healthy animal and human brain in a finely tuned and highly orchestrated manner. In doing so, it is able to influence a myriad of endogenous actions and behavioural outcomes. There are gender and age differences in 5-HT synthesis and turnover, receptor numbers, and interactions with other neurotransmitter systems, which vary depending on brain region. These disparities may manifest in gender- or age-related behavioural differences.

Serotonin plays a major role in memory and the mental activity variable of attention. This seems to be largely through its inhibitory interactions with other neurotransmitter systems (Delgado et al., 1990). It is believed that a reduction in 5-HT may enhance cholinergic and other neurotransmitter systems responsible for cognitive processes. Animal research has already supported this hypothesis, but as yet there is very little definitive clinical evidence in either young healthy or patient groups. Knowledge of this in older persons is reprehensibly lacking.

It is important to increase our understanding of the role of 5-HT in behaviour because native or iatrogenic changes in serotonergic function interfere with cognitive, somatic or psychiatric states. There are several ways 5-HT function can be investigated, including reducing 5-HT levels in the brain. One safe and effective way is to do this is by lowering the availability of tryptophan with ATD.

ATD alters cognition in a number of ways, most notably with impaired long-term memory and global cognitive status, but improved focused attention. ATD has a mood lowering effect in people with a vulnerability to depression. The effects of ATD have not been examined in movement. The number of studies investigating gender or age and ATD has been negligible.

A pattern is emerging that differentiates the effects of ATD depending on the population being studied. It is hypothesised that certain groups may be more sensitive to the effects of ATD than others. In older persons this sensitivity may be related to underlying deficits in other neurotransmitter systems.
5 PARKINSON’S DISEASE

5.1 Introduction
Parkinson’s disease is a chronic and progressive disorder comprising a constellation of physical symptoms known as parkinsonism, along with a number of other features including cognitive and affective disturbances. The underlying pathology of PD indicates that a severe reduction in DA levels as a result of cell death in the substantia nigra induces changes in basal ganglia circuitry. Moreover, a specific and consistent pattern of pathology distinguishes PD even before the onset of symptoms (Braak et al., 2005).

DA replacement therapy initially ameliorates parkinsonian symptoms but in up to 80% of patients, chronic use eventually generates debilitating involuntary movements (i.e., dyskinesia), sudden unpredictable inefficiency (i.e., ‘on-off’ phenomenon), and shortening of the drug’s effective period (i.e., ‘wearing off’) (Miyawaki et al., 1997). Depression is a pathological feature of PD and not necessarily related to physical disability or psychological reaction (Tandberg et al., 1996). Also, there are a number of cognitive deficits associated with the disease and in many patients this progresses to dementia (Braak et al., 2005).

5.2 Description
The syndrome of parkinsonism is defined by a cluster of motoric symptoms. Primary parkinsonism (i.e., comprising PD with idiopathic or genetic aetiology) is one of four parkinsonian categories, the others being secondary parkinsonism (i.e., PD with environmental aetiology), parkinsonism-plus syndromes (e.g., progressive supranuclear palsy and dementia with Lewy bodies), and heterodegenerative disorders (e.g., Huntington’s disease and frontotemporal dementia). Because 20-30% of parkinsonism patients may have alternative causes for their symptoms (McKeith, 2000a), the most distinguishing differential feature of primary parkinsonism is its responsiveness to treatment with levodopa (i.e., the precursor of DA). This responsiveness is indicative of reduced pre-synaptic DA levels in the presence of intact post-synaptic receptor function.

The focus of this chapter is primary parkinsonism and is the PD referred to throughout this thesis. Based on current criteria, PD is diagnosed when bradykinesia (i.e., slowness of all voluntary movement and speech) and at least one of the following are present: rigidity, tremor, or postural instability (Calne et al., 1992; Hughes et al., 1992; Koller, 1992).

5.3 Epidemiology
PD is one of the most common neurological diseases in older persons, having an occurrence of 100-200 cases per 100,000. Its highest prevalence is in the seventh and eighth decades of life (McKeith, 2000a). Males, more than females, are more likely to develop PD by an estimated 73% in nearly every age group (Mayeux et al., 1992). The mean age at onset is 64.4 years (range 27 - 85 yrs) and the condition has a mean duration to death of 9 years (range 1 - 34 yrs, McKeith, 2000a).
5.4 Clinical Features

The most likely factors contributing to a diagnosis of PD are: an asymmetrical onset of symptoms, bradykinesia (i.e., slower hand, arm, and leg movements, slowness in making a facial expression), the presence of a resting tremor, and a response to levodopa medication. Other factors include autonomic and arousal disturbances, sensory, affective and cognitive complaints. However, the four cardinal signs of PD are motor: bradykinesia, rigidity, resting tremor, and postural instability.

5.5 Clinical Course

Progression is inevitable and without remission in almost all patients (Braak et al., 2006; Groth-Marnot, 2000; Ivory, 1994), with symptoms initially appearing intermittently or in response to stressors, worsening if untreated to a disabling immobility or disturbance of equilibrium. As the disease progresses men are likely to experience more severe parkinsonian motor features and women more levodopa-induced dyskinesia (Lyons et al., 1998).

Progression in PD follows a predictable set of stages. These have been described by Hoehn and Yahr (1967) and adapted in Starkstein and Merello (2002, p. 6), as described in Table 5-1.

Table 5-1 Modified Hoehn and Yahr (H & Y) staging of PD

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No signs of disease</td>
</tr>
<tr>
<td>1</td>
<td>Unilateral disease</td>
</tr>
<tr>
<td>1.5</td>
<td>Unilateral disease plus axial involvement</td>
</tr>
<tr>
<td>2</td>
<td>Bilateral disease, without impaired balance</td>
</tr>
<tr>
<td>2.5</td>
<td>Bilateral disease, with recovery on pull test</td>
</tr>
<tr>
<td>3</td>
<td>Mild to moderate bilateral disease; some postural instability; physically independent</td>
</tr>
<tr>
<td>4</td>
<td>Severe disability; still able to walk or stand unassisted</td>
</tr>
<tr>
<td>5</td>
<td>Wheelchair-bound or bedridden unless aided</td>
</tr>
</tbody>
</table>

Notably, these clinical stages of motor function have been correlated with stages in the neuropathological progression of PD (see section 5.7) and correlated with score on the Mini Mental State examination (Folstein et al., 1975) to suggest neuropathologic stage is as linked to non-motor symptoms, such as cognition, as they are to motor symptoms (Braak et al., 2005). If the working hypothesis for neuropathological staging is robust, it means characteristic, predictable and progressive biological events occur in patients which could manifest in similarly-natured behavioural changes.

5.6 Morphological changes

Pathophysiologically, parkinsonian motor signs are secondary to impaired DA function and result from selective and specific neuronal loss and cytoskeletal abnormalities. This includes the loss and depigmentation of the neuromelanin in DA neurons, an increase in glial cells, and the continual development of intraneuronal, ubiquitin and alpha-synuclein containing inclusions called Lewy bodies. There is also evidence that some patients have neuropathologic features more often associated
with SDAT (Braak et al., 1996). Clearly, these morphological changes will manifest in motoric, neuropsychiatric, and cognitive symptoms; and it is possible that by combining morphology, neurochemistry and neuropsychology, research will bring to light just what sort behaviour can be predicted from having PD.

Table 5-2 Neuropathological Changes Occurring at Each Stage in Parkinson’s Disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>Neuropathological Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Confined to lower medulla oblongata and anterior olfactory structures.</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Brainstem nuclei (e.g., parts of the lower raphe nuclei, reticular formation, and locus coeruleus). The clinical symptoms of PD are not yet apparent.</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Basal portions of the midbrain and forebrain (e.g., amygdala, tegmental pedunculopontine nucleus, cholinergic magnocellular nucleus of the basal forebrain – including Meynert’s nucleus). Aggregations are present in the cholinergic axons that pass through the external capsule towards the cerebral cortex. Lewy neuritis now appearing in the substantia nigra pars compacta and Lewy bodies in the melanised projection neurons of this nucleus – without neuronal loss as yet.</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Pathology extends now to the cerebral cortex. Inclusions appear in the anteromedial temporal mesocortex (i.e., the transition area between the allocortex and the neocortex that is highly susceptible to PD and SDAT). Lewy neuritis begin to appear in Ammon’s horn. It is at this stage that motor symptoms are present.</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Lewy body pathology intensifies and expands to the insular and cingulate mesocortex and the high-order association areas of the temporal neocortex.</td>
</tr>
<tr>
<td>Stage 6</td>
<td>Degeneration of melanuerons in vulnerable sites in the substantia nigra is nearly complete. There is still differential density of neocortical lesions based on cell type, however, pathology has extended to secondary and, sometimes, primary fields in the neocortex. Stages 5 and 6 are associated with the full range of clinical symptoms as a result of damage to the autonomic, limbic, and somatomotor systems.</td>
</tr>
</tbody>
</table>

Note: the stages are progressive in that each stage includes the pathology of the preceding stages and that this pathology is more severe. After (Braak et al., 2006).

Prior to the destruction of DA neurons, morphological changes have begun that will spread in an unrelenting, premeditated, and uniform manner from their induction in the medulla oblongata and anterior olfactory structures to the highest functioning regions of the cerebral cortex: the prefrontal cortex followed by the primary sensory/motor fields (Braak et al., 2004; Braak et al., 2006). These changes mark the progression of the disease through six neuropathological stages (Braak & Braak, 2000; Braak et al., 2003; Braak et al., 2004; Fahn, 2003; Braak et al., 2006). The stages described in Table 5-2 are premised on the assumption that Lewy body inclusions are present before the onset of
clinical symptoms and that pathologic processes increase in extent and severity with disease progression (Braak et al., 2006).

Lewy body inclusions comprise the thread-like Lewy neuritis which appear within cellular processes and Lewy bodies which appear in neuronal bodies. The appearance of Lewy neuritis precede Lewy bodies. They are present in only some cells types, which perhaps explains the regional pattern of the pathology (Braak et al., 2006).

5.7 Biochemical changes

A diagnosis of PD is traditionally made when cell loss in the substantia nigra interferes with activity in the striatum of the basal ganglia and movement is affected (Agid et al., 1987).

The biological basis of Parkinson’s disease is generally attributable to degeneration in the nigrostriatal dopaminergic pathways of the basal ganglia. However, other neurochemicals may also be involved including amino acids (e.g., GABA and glutamate), peptides (e.g., endorphins) and biogenic amines. The following section outlines some of the changes that occur during PD in the dopaminergic, noradrenergic and cholinergic neurotransmitter systems, while changes to the serotonergic system are discussed comprehensively in section 5.11.

5.7.1 Dopaminergic System

The main focus of the dopaminergic-related pathology is the cells of the SNpc that project to the putamen (Bernheimer et al., 1973), and to a lesser extent those in the ventral tegmentum area that project to the caudate nucleus in the basal ganglia (Uhl et al., 1985). This pattern has been observed in early-in-the-course patients in vivo with preserved caudate, but reduced \(^{18}\)F-dopa PET tracer binding in the putamen contralateral to the affected limbs (Morrish et al., 1995). Reduced uptake binding has been monitored as 50% loss in the putamen but compares to the post-mortem measurements of 60-80% loss in SNpc cells and 95% loss of putamen DA (Brooks & Piccini, 2006). Reductions in the uptake of the aforementioned tracer in the caudate do not occur until later when the disease has progressed (Brooks & Piccini, 2006). Visual inspection of tracer uptake in early-in-the-course PD indicates the reduction in DA terminal integrity – and thus, it is assumed, DA level – is limited to projections from the SNpc to the putamen, however, statistical parametric mapping (i.e., a technique for averaging activity in brain images and comparing between different groups) reveals additional motor cortex and anterior cingulate cortex (ACC) dysfunction (Brooks & Piccini, 2006). This neuroimaging data indicates early-in-the-course PD cannot be defined by basal ganglia dysfunction alone; an important point in support of the inclusion of pre-symptomatic stages in the staging system of Braak and colleagues (Braak et al., 2006; Braak et al., 2003; Braak et al., 2004). Lewy neuritis, the forerunner of Lewy bodies, appear in the substantia nigra pars compacta for the first time in Stage 3 when neuronal loss is yet to eventuate (Braak et al., 2006).
Box 2. Basal Ganglia

The basal ganglia are a collection of functionally related and interconnected nuclei in the basal forebrain and brainstem. They include the corpus striatum (caudate nucleus and putamen) and globus pallidus (interna, GPi; externa, GPe), plus nuclei of the diencephalon (thalamus; subthalamic nucleus) and mesencephalon (substantia nigra pars reticular, SNpr; substantia nigra pars compacta, SNpc; pedunculopontine nucleus).

The striatum is the main input structure of the basal ganglia and, as such, receives information from the cortex and thalamus and innervation from the dopaminergic neurons in the substantia nigra. The striatum is reciprocally connected with the substantia nigra, but sends most of its output to the globus pallidus. The GPi is the main output of the basal ganglia.

The SNpc sends and receives input from the striatum. The SNpc produces dopamine, which is critical for normal movement, and it is the cells in this structure that degenerate in Parkinson's disease causing the movement and, possibly, the cognitive and mood symptoms characteristic of this disorder.

The current models (Lichter, 2000) for basal ganglia activity comprise two distinct output pathways from the striatum, as shown in the diagram below. The direct pathway outputs from the striatum to the SNpr and GPi complex, then on to input the cortex via the thalamus. GABA is the inhibitory neurotransmitter for this pathway; cells in these structures inhibit GABA interneurons, resulting in a net excitation of the cortex. With the help of GABA, the indirect pathway outputs from the striatum to the GPe. Cells in these structures excite the subthalamic nucleus, which then uses glutamate to excite the GPi and SNpr. The effect is reduced excitatory outflow to the cortex.

DA innervates all parts of the striatum involved with both the direct and the indirect pathways. The direct pathway is modulated by excitatory D$_1$ receptors, while the indirect pathway is modulated by inhibitory D$_2$ receptors (Govoni et al., 2001). Thus DA level is crucial to the homeostatic regulation of the activities in the basal ganglia and the cerebral cortex.
When degeneration of the SNpc neurons does occur the reduced amount of DA available to the striatum and induces oppositional effects in the two basal ganglia pathways, which then cataracts to the cortex (see Box 2 for a description of the basal ganglia and these pathways). The indirect pathway becomes disinhibitory (i.e., overactive) and increases excitation to the drivers of the direct pathway, the GPi and SNpr, while the direct pathway, in becoming less active, reduces excitatory input to the cortex. The net result is reduced excitation of the cortex manifesting in hypokinetic parkinsonian symptoms.

Anatomical and electrophysiological studies in animals have shown that the direct and indirect striatopallidal connections are regulated by the nigrostriatal dopaminergic system (Alexander & Crutcher, 1990; Valls-Sole & Valdeoriola, 2002), while pathophysiological studies in PD patients confirm the involvement of the subthalamic nucleus and GPi in the pathology and pathogenesis of parkinsonian motor symptoms (Berardelli et al., 2001). PET studies have demonstrated reduced activity in the supplementary motor cortex (Playford et al., 1992), with increased metabolic activity following pallidotomy (Eidelberg et al., 1996), and functional studies have provided evidence for nigrostriatal dopaminergic dysfunction in the generation of abnormal motor cortex activity (Strafella et al., 2000; Ziemann et al., 1997).

Bradykinesia and rigidity are two outcomes of abnormality in subcortical and cortical motor pathways (Berardelli et al., 2001; Berardelli et al., 1983; Delwaide et al., 2000) and appear when the DA regulating this circuitry is reduced by 80% in the putamen and 60% in the substantia nigra (Bernheimer et al., 1973).

The pathology of PD targets DA producing cells in the substantia nigra disrupts communication in the nigrostriatal pathways of the basal ganglia. However, not all DA cells are affected in early-in-the-course PD. DA cells in the ventral tegmentum area project to the orbitofrontal (OFC), anterior cingulate cortex (ACC) and amygdala. 18F-dopa PET uptake in two of these areas (ACC and amygdala) has been shown to increase and subsequently normalize in early-in-the-course PD (Rakshi et al., 1999). PET and single photon emission computed tomography (SPECT) ligand markers – used to observe the integrity of the DA system via binding of dopa decarboxylase and pre-synaptic DA transporter (DAT) – have shown an upregulation in the former and downregulation in the latter, also in early-in-the-course PD, reflecting adaptation to preserve activity in the synapse (Lee et al., 2000b).

5.7.2 Non-dopaminergic Systems

A number of symptoms are attributable to the extranigral changes that also occur in PD. These include (a) changes to the density and distribution of Lewy bodies throughout the brain (Forno, 1987; McKeith, 2000b) and (b) cytoskeletal damage to the projection neurons of the cholinergic, serotonergic, GABAergic, and noradrenergic systems having lengthy axons (Braak & Braak, 2000; de Vos et al., 1996). Dysfunction of these neurotransmitter systems can lead to some of the motor and non-motor symptoms (for examples see, Cooper et al., 1992) and may provide a target for
pharmacological therapies, especially in those symptoms unresponsive to dopaminergic therapy (Bonnet, 2000). For example, autonomic symptoms result from deficits in the adrenergic and noradrenergic neural systems (Saper, 1998); cognitive impairment from dopaminergic, cholinergic, and noradrenergic deficits (Pillon et al., 1989); and depressive symptoms partially from dopaminergic denervation but also possibly reduced serotonergic neurotransmission (D'Amato et al., 1987).

5.7.2.1 Noradrenergic

Pathological changes occur during the disease process in neurons in the locus coeruleus, plus reductions in dopamine hydroxylase and metabolites (for review see, Brefel-Courbon et al., 1998; German et al., 1992). Based on the distribution of $\alpha_2$-adrenoceptor sites in the motor relays of the CNS, the limbic, thalamic, hypothalamic, and the PFC, noradrenergic deficiencies are likely to contribute to the motor, mood, and cognitive behaviours afflicted in PD.

5.7.2.2 Cholinergic

Muscarinic receptor antagonists are used to treat the motor symptoms of Parkinson’s disease based, in part, on the secondary relative overactivity of acetylcholine related to reduced striatal DA/ACh ratio (Bonnet, 2000). However, PD is also characterised by significant degeneration in this system and it is this damage that could account for the cognitive deficits seen in PD (Korczyn, 2001; Perry et al., 1985) There is a significant reduction in numbers of ACh neurons in the basal forebrain (Candy et al., 1983; Nakano & Hirano, 1984) and thus reduction is more pronounced than that which occurs in SDAT (Arendt et al., 1983). Moreover, AChE activity – which is correlated to working memory, executive function and attention performance – is significantly reduced in demented and non-demented PD patients compared to SDAT patients, as shown in Figure 5-1 (Bohnen et al., 2006; Bohnen et al., 2003).
5.8 Treatment

Treatment for motor symptoms in PD is usually based on DA replacement with levodopa proving to be the most potent therapeutic agent when administered in conjunction with a peripheral decarboxylase inhibitor (Fahn, 2003). These compounds enhance the synthesis of DA in the CNS because they allow more levodopa to cross the BBB when they inhibit the conversion of levodopa into DA in the periphery (Factor, 2007). The addition of a catechol-o-methyl-transferase (COMT) inhibitors increases the half life of levodopa by preventing its metabolism to 3-OMD (3-o-methyldopa).

One important issue for levodopa treatment is that when the medication is administered with a high protein meal, there is an overall reduction in its plasma level as a consequence of protein synthesis (Factor, 2007). Complications from long-term use in some patients and non-responsiveness of some symptoms to levodopa therapy means, on a cost/benefit basis, that DA agonists, MAO-B inhibitors, and anticholinergics (for a description of each refer to pp. 21-25, Starkstein & Merello, 2002).

Treatment for mood symptoms is usually based on SSRIs because of their relatively benign side effect profile in PD compared to other antidepressant drugs, like the TCAs and MAOIs; the latter have anticholinergic properties (Cummings & Masterman, 1999; Tom & Cummings, 1998). SSRIs are a structurally diverse class of antidepressants so individual drugs are likely to have different profiles.
There is some evidence that SSRIS are associated with extrapyramidal side effects. There are limitations with dopaminergic therapies (see section 5.9), but the DA agonist pramipexole is being investigated because it provides antidepressant benefits comparable to SSRIs; plus it significantly reduces motor symptoms (Lemke et al., 2006; Rektorova et al., 2003).

The limitation of the above strategies is that dopaminergic treatments fail to completely normalise cognition in early PD (Kulisevsky et al., 2000) and anticholinergics – used to treat motor symptoms – can result in frontal type cognitive deficits (Dubois et al., 1990), in particular memory, executive function, and global cognitive deficits (Tröster & Woods, 2007). Treatment for cognitive symptoms in PDD has been centred on cholinesterase inhibitors (e.g., rivastigmine, donepezil and galantamine), which are well tolerated, improving cognition and neuropsychiatric symptoms, as well as having little effect – or an improvement – on parkinsonism (Aarsland et al., 2003b; Aarsland et al., 2004b; Bullock & Cameron, 2002; Leroi et al., 2004; Liepelt et al., 2007; Minett et al., 2003; Tröster & Woods, 2007; Werber & Rabey, 2001) Amantadine (i.e., a NMDA receptor antagonist) may have a possible role in delaying the onset of dementia (Inzelberg et al., 2006), while memantine (i.e., a glutamatergic modulator) has been shown to improve cognition (for review see, Leroi et al., 2006) in PD.

5.9 Parkinson’s Disease and Movement

The model for PD as a basal ganglia movement disorder arises because patients with PD have difficulty initiating and coordinating smooth motor movements. The extrapyramidal symptoms of PD include bradykinesia, rigidity, tremor at rest, loss of postural reflexes, flexed posture, and the freezing phenomenon. Bradykinesia refers to slowness of movement and difficulty in initiating movement. There is also akinesia (i.e., poverty of movement), hypokinesia (i.e., poor movement, that is, smallness as well as slowness of movement) (Berardelli et al., 2001; Fahn et al., 1971; Starkstein & Merello, 2002) and hypometria (i.e., movements fall short of the intended goal). A myriad of other symptoms are linked to bradykinesia, akinesia, hypokinesia and rigidity, and they include micrographia (i.e., small, cramped handwriting), decreased eye blink rate, loss of arm swing, shuffling gait, hypomimia (i.e., immobile expressionless face), dysarthria (i.e., slurred impaired speech), hypophonia (i.e., soft speech), and sialorrhea (i.e., drooling). Patients may have blepharospasm (i.e., eye spasm with increased blinking) and a range of oculomotor abnormalities. They may also have structural degeneration in the form of scoliosis (i.e., sidewise curvature of the spine) and hand or foot deformities. Extrapyramidal symptoms attributable to prolonged levodopa therapy include dyskinesia and motor fluctuations.

Bradykinesia includes the underscaling of movement commands in internally generated movements. This means movement is undershot and patients end up making a lot of smaller steps to get wherever they want to go (Berardelli et al., 2001). Neuroimaging (e.g., PET and SPECT) studies have consistently localized the cause of bradykinesia and rigidity to reduced function in the terminals of the DA neurons in the putamen (Asenbaum et al., 1998; Benamer et al., 2000; Brooks et al., 2003;
Vingerhoets et al., 1997). Several studies using post-mortem samples and biomarkers for DA have also linked reduced DA in the putamen (Benamer et al., 2000; Brooks et al., 2003; Vingerhoets et al., 1997). Hyperactivity of the subthalamic nucleus and GPi causes inhibition of the motor cortex and the corticospinal tract (Albin et al., 1989; DeLong, 1990; Parent & Hazrati, 1995) and it is this output circuitry that most likely underscores bradykinesia and rigidity (Berardelli et al., 2001; Delwaide et al., 2000) and which, incidentally, has been the focus of surgery and electrical stimulation, enabling patients to minimize the need for dopaminergic pharmacotherapy (Valls-Sole & Valdeoriola, 2002).

A resting tremor of 3-5 Hz and a postural tremor of 4-8 Hz are symptomatic of PD. Although reduced DA has also been associated with resting tremor, the link is not supported by animal models of PD (Brooks & Piccini, 2006); nor is it supported by the unreserved efficacy of levodopa therapy (Koller & Hubble, 1990). The cause of tremor is not yet determined, but there is some suggestion 5-HT could be involved (Jacobs & Fornal, 1993) since SSRIs have been associated with reported aggravations to parkinsonian tremor.

Composite tremor, but not bradykinesia and rigidity, scores on the UPDRS have been significantly correlated with $5-HT_{1A}$ binding in the raphé nuclei of PD patients withdrawn from antiparkinsonian medication for 12 hours (Doder et al., 2003).

Current treatments for psychiatric symptoms that act on the serotonergic system have a motoric effect which suggests serotonin may have some role in movement. For example, SSRIs are associated with extrapyramidal side effects and clinicians are warned over their potential catalytic role in relation to the entire range of antipsychotic related motor dysfunction, especially in older patients (Cunningham Owens, 1999). Unlike first generation antipsychotics, second generation antipsychotics which rely on DA$_2$ and 5-HT$_{2A}$ antagonism are associated with a few extrapyramidal side effects (King & Waddington, 2004).

5.9.1 Conclusion
PD is recognised as a disease with significant motoric symptoms linked to reduced dopaminergic function. Recent research has suggested the serotonergic system may be involved with tremor.

5.10 Parkinson’s Disease and Mood
Depression is the most common non-motor symptom in PD (McDonald et al., 2003) with symptoms ranging from emotional incontinence through to mild (i.e., dysthymia) and major depression (i.e., moderate to severe symptoms). The depressive symptoms of anergia (i.e., lack of energy), motor retardation, and early morning awakening are not included in the diagnosis since these symptoms are also experienced by non-depressed PD patients (Starkstein et al., 1990c), but all the other psychologic and autonomic symptoms necessary for a clinical diagnosis of depression are present. The autonomic symptoms appear to be specific to depression in PD rather than an artifact of the condition itself (Levin et al., 1988).
It is difficult to determine an exact figure for the prevalence of depression in PD (Zgaljardic et al., 2003), largely because the symptom similarities between PD with depression and PD alone (e.g., motor retardation, insomnia, flat effect, anergia) and different types of assessments used across studies make evaluations difficult. While the frequency of depression in PD is generally considered to be around 40%, a large number of studies have estimated rates to be anywhere between 7% and 90%, with the lower rate found in epidemiological studies and the higher found in inpatient studies (Cummings, 1992; Starkstein & Merello, 2002). This discrepancy highlights one of the major issues in PD depression, which is the compounding nature of heterogeneous comorbidities. Both disorders have subtypes and the course of depression in PD will vary according to the characteristics of each subtype. For example, major depression in PD is associated with a significant decline in ‘activities of daily living’ (ADLs) and a faster disease progression (Starkstein et al., 1992). For men, more than women, it predicts impaired social and physical functioning (Cole et al., 1996).

Depression appears before the onset of motor symptoms, particularly in the younger cohort of patients (Cummings, 1992; Mayeux et al., 1981; Santamaria et al., 1986; Starkstein et al., 1989a; Starkstein et al., 1990b), implying that depression may not be just a psychological reaction to physical disability. This has been demonstrated in the findings of two studies. First, PD patients with depression, irrespective of ‘on’ or ‘off’ status, had more severe depressive symptoms than depressed rheumatoid arthritis patients having cyclic immobility (Tom & Cummings, 1998), and second, a large register study demonstrated the risk of getting depression was still increased a year after the initial diagnosis of PD was made (Nilsson et al., 2002). One group of researchers (Mayeux et al., 1984; Sano et al., 1990) has consistently argued against an association between major depression and disease severity, but other groups insist a connection exists (Brown et al., 1988; Gotham et al., 1986; Starkstein & Merello, 2002). By way of example, associations have been found between depression and the akinetic-rigid form of PD (Starkstein et al., 1998), between depression and fine motor skills (Kuhn et al., 1996a), and between depression and patients’ perception of their disabilities (Schrag et al., 2001).

There is a degree of uncertainty regarding the biological basis for depression in PD, but it is more likely that neuropathological changes in the brain rather than environmental or psychological factors are the cause (Tandberg et al., 1996). The biogenic amine hypothesis for depression is grounded in the premise that these neurotransmitter systems are dysfunctional in some way. Treatment with dopaminergic therapy, therefore, could be hypothesised to successfully alleviate symptoms in patients. Unfortunately, after an initial improvement, it provides no ongoing benefit (Mayberg, 2000; Mayeux, 1990; Sano et al., 1990) and, in fact, there is some indication that dopaminergic acting treatments may even exacerbate mood disturbances (Aarsland & Karlsen, 1999; Jankovic, 2000; Mayberg, 2000; Saint-Cyr et al., 1993).

It is possible that disruptions to frontocortical circuitry may be associated with depression in PD (Mayberg & Solomon, 1995). There are two possible explanations for this, especially since PET studies have found lower rates of metabolism in the frontal and temporal areas of depressed PD
patients (Mayberg et al., 1990; Ring et al., 1994; Tom & Cummings, 1998), and a disproportionate degeneration of DA neurons in the caudate and the ventral tegmentum area in depressed patients with PD and depressed patients with PDD, respectively (Mayberg et al., 1990; Torack & Morris, 1988).

The first explanation is based on the basal ganglia circuitry. Studies using the rabies virus as a tracer for neuronal connectivity have demonstrated 15% of the basal ganglia is devoted to the motor cortex and of this, 50% or less to motor control (Kelly & Strick, 2004). Half the basal ganglia output to the cortex is to non-motor areas in the PFC, infra-temporal cortex, and OFC (NIH). There is the possibility that – given its capacity to influence many areas of the frontal cortex – dysfunction in the basal ganglia will cataract to a wide variety of cortically-mediated behaviours. One of these cortical areas, the OFC is critical in major depression.

The second explanation suggesting DA neurons from the ventral tegmentum area account for depression in PD is that these neurons project to the OFC as well as the PFC. Mayberg et al. (1990) surmise that the mechanism for depression in PD could arise from an imbalance in the 5-HT/DA ratio. For example, both sets of neurons (i.e., SNpc and ventral tegmentum area) have projections to the OFC and the PFC but the major cortical outflow to the dorsal raphé nuclei and ventral tegmentum area originates in the OFC; degeneration of DA neurons could lead to dysfunction in the OFC which flows on with a secondary effect in the serotonergic neurons of the dorsal raphé nuclei. This idea is enticing, especially given that the CSF level of DA’s metabolite, homovanillic acid, does not correlate with mood and DA agonists have limited efficacy in treating depressive symptoms (Lichter, 2000); whereas, the opposite occurs for 5-HT’s metabolite and 5-HT agonists.

But in fact, there are widespread region- and transmitter-specific changes in PD, each of which may possibly be working, in isolation or in concert, to account for the diversity of motor, cognitive, and autonomic manifestations of PD (Halliday et al., 1990). Using SPECT, Murai et al. (2001) measured binding ratios for receptors of 5-HT and DA and found that, although striatal binding ratios – which reflect densities of DAT (i.e., dopamine transporters) – were correlated with motor symptoms as per Hoehn and Yahr stage (Hoehn & Yahr, 1967), 5-HTT (i.e., serotonin transporters) densities were correlated with the mentation, behaviour, and mood but not the cognitive items, in the non-motor subscale of the United Parkinson’s Disease Rating Scale (UPDRS) (Fahn et al., 1987). Likewise, a negative correlation has been found between the mood section of the UPDRS (section I. Mentation, behavior, and mood) and CSF levels of 5-HIAA (Liu et al., 1999).

Other lines of investigation have also encouraged the role for 5-HT in depression in PD. For example, (a) post-synaptic 5-HT function in PD was investigated with different 5-HT agonists on cortisol, prolactin, adrenocorticotropic hormone (ACTH), and growth hormone response, (b) serotonin mediation of the HPA axis was dysfunctional in depressed compared to the non-depressed patients (Kostic et al., 1996; Volpi et al., 1997a; Volpi et al., 1997b), (c) an improvement in mood after fluoxetine treatment has been significantly correlated with an increase (i.e., to normal levels) of
activity in the DLPFC (Mayberg et al., 1997), (d) depressed PD patients have greater neuronal loss in the dorsal raphé nuclei (Paulus & Jellinger, 1991), decreased 5-HT binding sites in the basal ganglia (Litvan, 2000), and lower CSF levels of 5-HIAA than non-depressed patients, especially those with major as opposed to dysthymic depression (Mayeux et al., 1984; Mayeux et al., 1986).

A number of factors may be involved in these findings however, which do not support a solitary role for serotonin in depression in PD. This is evidenced by the inability of Kuhn et al. (1996b) to replicate in de novo depressed and non-depressed patients, the 5-HIAA reduction found in two of the aforementioned studies. Also, the failure of PET and SPECT studies to show a relationship between medial raphé nuclei 5-HT\textsubscript{1A} binding or brainstem SERT binding and the presence of depression in PD (Kim et al., 2003; Sargent et al., 2000). Further, by the results from the pan-European PRODEST study in 1016 PD patients, showing 44% of depressed and treated patients still experience depression (Barone, 2007); also, the success of the dopamine agonist pramipexole in treating depression in PD (Barone et al., 2006). Despite several treatment algorithms (e.g., Poewe & Luginger, 1999; Tom & Cummings, 1998) and the use of SSRIs as first line of treatment in 51% of cases, there is still a dearth of empirical evidence for treating depression in PD (Veazey et al., 2005).

Although studies have consistently found a relationship between cognitive impairment and motor symptoms (Aarsland et al., 2003a; Levy et al., 2000; Williams et al., 2000) and not mood (Crucian et al., 2000; Crucian et al., 2001; Crucian & Okun, 2003; Mayeux et al., 1992; Mortimer et al., 1982; Starkstein et al., 1989c), there are some studies showing relationship between depression in PD and cognition. Tandberg et al. (1996) found patients with major depression – as indicated by their score on the Montgomery Asperger Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) – experienced, among other things, greater cognitive deficits and thought disorder. Patients with major depression have been found more likely to have lower scores on the Mini Mental Status examination (MMSE) (Folstein et al., 1975), irrespective of age, duration of illness, parkinsonian symptoms or performance maintaining ADLs (Starkstein et al., 1990b). One 5 year prospective study of 250 non-demented PD patients found depression was likely to predict the onset of dementia (Stern et al., 1993a).

The profile of cognitive deficits in depressed PD patients is a marker for dysfunction in PFC activity. Patients with major depression have generally exhibited poorer performances than non-depressed or dysthymic patients on executive function tasks (Boller et al., 1998; Starkstein et al., 1989c). A recent study (Stefanova et al., 2006) employing tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Robbins et al., 1994a) found depression preceded cognitive impairment and was extensively linked to cognitive function in early-in-the-course PD. An interesting outcome of this study, in light of results from the studies mentioned above, is that PD patients without depression exhibited executive dysfunction but patients with depression exhibited combined executive impairment, visuospatial and working memory deficits. These patients experienced mild to moderate motor symptoms as measured by the Hoehn and Yahr scale, which suggests depressed patients not
only experience impairment in cognitive functions associated with temporal regions, but that the severity of their depressive, and not their parkinsonian, symptoms account for these deficits.

5.10.1 Conclusion
Depression is common in PD and, depending on the severity of the symptoms, may be a predictor for incipient dementia. Major depression is likely to begin before the onset of motor symptoms, last more than a year, and be significantly related to reduced frontal metabolic activity and reduced CSF levels of 5-HIAA. It is more likely to occur in the akinetic-rigidity variant of PD, predict a faster decline in physical and cognitive function, be associated with more cognitive deficits, and be a real risk for the development of dementia. There is no clear treatment for depression in PD.

5.11 Parkinson’s Disease and Cognition
As previously discussed, degeneration in the nigrostriatal dopaminergic neurons of PD patients provokes aberrations in basal ganglia-cortical circuitry with motoric sequelae. Degeneration in PD, however, is not limited to a dysfunctioning nigro-putamen pathway and even in early PD, there appear to be neuropathologic changes that may manifest in cognitive change. It has been postulated that declines in cognitive function occur at stage 3 and that mild cognitive impairment, as described by Petersen (2001), proceeds on to dementia during the later stages of disease progression, (Braak et al., 2005).

Morphological changes have been demonstrated using the technique of statistical parametric mapping (e.g., from 18F-dopa PET images) which showed the accumulated loss of striatal, cingulate, and premotor and prefrontal DA storage as the disease progressed (Brooks & Piccini, 2006). These observations are important for understanding the cognitive deficits in PD because inter-related anatomical regions function via neuronal connectivity and adequate neurotransmitter levels are required to perform various executive functions, working memory, attentional, and mnemonic tasks (Cheesman et al., 2005; Rinne et al., 2000). Cognitive deficits in PD have largely traditionally been described as executive in nature, but also visuospatial and mnemonic.

The pathophysiology of Parkinson’s disease with no dementia (PDND) has been attributed to degeneration in the dopaminergic, but also non-dopaminergic neurotransmitter systems, and to pathological changes in the frontal cortex secondary to the dysfunction in subcortical afferents (Cooper et al., 1991). The pathophysiology of Parkinson’s disease with dementia (PDD) may include the above, but also a cholinergic deficiency in the cortex, SDAT pathology, and the presence of Lewy bodies in the cortex (Bosboom et al., 2004). It is generally associated with late stage PD and the widespread neuropathological changes that have occurred by this time (Braak et al., 2005).

5.11.1 Cognition in PD Patients without Dementia
It has been estimated that mild cognitive impairment is present in 55% of PDND patients (Janvin et al., 2003) and is recognised in early- as well as late-in-the-course PD (Mayberg & Solomon, 1995). Although, as mentioned above, some studies have suggested a link between depression and cognitive
status, others have consistently demonstrated – or reviewed (Mayeux, 1992) – strong correlations between motor symptomology and cognitive function (Aarsland et al., 2003a; Crucian et al., 2000; Crucian et al., 2001; Crucian & Okun, 2003; Levy et al., 2000; Mortimer et al., 1982; Williams et al., 2007), irrespective of cognitive impairment (Crucian et al., 2000). For example, a recent study (Williams et al., 2007) in 108 patients found the best predictors for cognitive impairment, as assessed by the Dementia Rating Scale (Mattis et al., 2002) and the MMSE, were bradykinesia, right-sided and axial symptoms.

Although associated with dopaminergic hypofunction, cognitive deficits are not necessarily dependent on the same neural structures and circuitry as motor symptoms, at least in the early stages (Cooper et al., 1992; Cooper et al., 1991). Moreover, pronounced cognitive dysfunction late-in-the-course is most likely attributable to neurochemical changes outside the dopaminergic system, as well as to changes in structural pathology (Tröster & Woods, 2007). Alexander et al. (1986) proposed five parallel but segregated circuits interconnecting defined regions of the brain to moderate motor, cognitive and behavioural functions. These circuits originate in the frontal cortex (e.g., supplementary motor, motor, DLPFC, OFC, and ACC regions) and project through the thalamus to topographically separate areas of the basal ganglia and back to the frontal cortex. Each circuit has the same number of structures and the same direct and indirect pathways in the basal ganglia, as described above. Information processing occurs within these circuits in response to the activities of dopaminergic, noradrenergic, cholinergic, and serotonergic neurons (Zgaljardic et al., 2003).

One of these, the dorsolateral prefrontal (DLPFC) circuit links the head of the caudate nucleus in the corpus striatum, the ventral and dorsomedial thalamic nuclei, and the area of the prefrontal cortex thought to be involved in executive behaviour (Cummings, 1993; Owen et al., 1999; Petrides, 2000b; Postle et al., 1999; Starkstein & Merello, 2002). While DA depletion in the putamen has been implicated in the motor symptoms of PD, depletion in the caudate nucleus may play a role in cognitive symptoms (Brown & Marsden, 1990). Maximal DA depletion has been detected in PD patients and as the caudate nucleus projects and receives information via frontostriato-thalamic circuitry (Goldman-Rakic et al., 1992; Lichter, 2000; Mehta et al., 2000), it could mean DA depletion greatly influences functions served by various PFC projection sites, e.g., executive function (Bedard et al., 1999; Brown & Marsden, 1990; Cropley et al., 2006). This could occur through disruption of DA mediated activity in the corpus striatum (Lewis et al., 2004; Owen et al., 1998a) or through degeneration of the ascending catecholaminergic neuronal systems within the frontal lobes (Dubois & Pillon, 1997; Starkstein & Merello, 2002).

The exact mechanism of DA’s role in the cognitive deficits of PD remains controversial, largely because the cognitive effects of dopaminergic agents are varied depending on the cognitive domain being tested (Bosboom et al., 2004; Bowen et al., 1975; Delis et al., 1982; Fournet et al., 2000; Girotti et al., 1986; Gotham et al., 1988; Malapani et al., 1994; Mohr et al., 1987) and because there is no consistent evidence that this therapy even affects cognition (Cooper et al., 1992; Molloy et al., 2006;
Cooper et al., 1992). It may be that the success of DA therapies in alleviating motor symptoms occurs because the putamen is heavily depleted by the time motoric symptoms appear, but caudate depletion sufficient to cause cognitive manifestations is found in only some patients (Brown & Marsden, 1990). Or perhaps, as Bosboom et al. (2004) suggest, mechanisms other than dopaminergic are responsible for cognition status in PD, especially since cognitive function has been shown to correlate with motor symptoms mediated by non-dopaminergic lesions (Aarsland et al., 2004a; Levy et al., 2000) or motor symptoms unresponsive to dopaminergic therapy (e.g., axial symptoms and gait disturbances) (Burn et al., 2003).

5.11.1.1 Executive Function and Working Memory

Different tasks used to assess executive function have consistently elicited impaired performances by PD patients, even from those in the early stages of the disease (Bowen et al., 1976; Cooper et al., 1991; Dubois & Pillon, 1997; Lees & Smith, 1983; Levin & Katzen, 1995).

Because executive functions involve the mental ability to control and manage cognitive processes (i.e., direct attention, create plans, form strategies, make decisions, solve problems), it means PD patients are likely to have problems with any “… task or situation for which no prior experience exists, which lacks explicit guidelines, which is highly effort demanding (i.e., extends beyond normal attentional resources), and which ‘forces’ the subject to develop his own plan(s) or action (formulate and switch novel mental sets, blend established sets in novel combinations, or both)” (Taylor & Saint-Cyr, 1995 p. 283). To some extent these deficits are akin to those found in frontal lobe patients (Owen et al., 1998b; Pillon et al., 2003), however, a study that specifically compared these two groups against controls found that, while frontal lobe patients exhibited poor performances on motor and span sequencing, the performance of PD patients was comparable to controls (Canavan et al., 1989). Also, because performances by PD patients on frontal tasks have shown extreme sensitivity to levodopa withdrawal (Cools et al., 2001; Cools et al., 2002), it is likely these deficits are underscored by DA dysfunction (Lange et al., 1992) and inefficiencies arising in frontostratrio-cortical circuitry. Robbins et al. (1994b) have suggested the mechanism for this effect may occur at the level of the caudate nucleus (Owen et al., 1996a) and hence the frontostriato circuit, or via mesocortical projections, especially, they say, in the light of the research by Goldman-Rakic (1995, 1998) implicating DA and the PFC in spatial working memory.

Animal and human evidence associates working memory in frontal regions with dopaminergic neurotransmission (for review see, Chudasama & Robbins, 2006). For example, working memory performance has either been improved or worsened following DA agonism or antagonism, respectively (Mehta et al., 2004a; Mehta et al., 2004b; Mehta et al., 2000; Nathan et al., 2002). Also, impaired performance (in marmosets) is associated with DA depletion in the striatum (Collins et al., 2000). Thus, it seems possible working memory performance is a product of the interaction between cortical and subcortical regions, and neurotransmission integrity.
A number of authors have highlighted a particular sensitivity of PD patients to working memory (Dalrymple-Alford et al., 1994; Dubois & Pillon, 1998) and, in particular, spatial working memory impairment (Cools et al., 2002; Owen et al., 1992; Postle et al., 1997), that may parallel the progress of their motor symptoms (Owen et al., 1998b). The literature points to a differential performance in spatial working memory with disease progression. Mild PD seems to be associated with dysfunction in tasks mediated by frontal brain areas (e.g., spatial working memory and strategic control), whereas severe PD seems more associated to decrements in mnemonic functions mediated by posterior cortical regions (Cools et al., 1999). This is especially so if patients are medicated with levodopa (Costa et al., 2003; Owen et al., 1997). Note, that impairment in verbal working memory has also been found (Lewis et al., 2003; Lewis et al., 2005).

The nature and heterogeneity of executive deficits in PD patients, however, raises a number of issues. First, the underlying cause of dysfunction is probably multifactorial; for example, as well as orchestrating movements, the corpus striatum is involved in attention and set maintenance (Dubois & Pillon, 1997; Owen et al., 1998a). Second, as Starkstein and Merello (2002) have suggested, executive dysfunction may be part of a more extensive cognitive decline. And third, executive deficits may be a characteristic of a subgroup only (Lewis et al., 2003), since they were not manifest in a group of high-functioning patients (Mohr et al., 1990).

5.11.1.2 Visuospatial Function

It is difficult to clarify the prevalence of visuospatial performance in PD because a number of domains have been subsumed under this one label namely, perception, memory, attention, orientation (Levin & Katzen, 1995). There is some evidence that performance in some of the visuospatial tasks is impaired in PD (Brown & Marsden, 1986; Canavan et al., 1990; Levin, 1990; Montgomery et al., 1993), but it is also possible that, after timing and motor components are taken into account, the explanation for deficits in this domain is determined by the degree of task demand on executive function or attention (Bondi et al., 1993; Brown & Marsden, 1990; Crucian et al., 2000; Levin & Katzen, 1995; Lichter, 2000; Raskin & Daily, 1992). This idea is particularly feasible given that performance deficits on visuospatial tasks were removed when covaried with performance on tasks assessing frontal function (Dubois & Pillon, 1998). Brown and Marsden (1990) reviewed a number of studies which encompassed a wide range of visuoperceptual and visuospatial tasks and say their results argue against a generalised visuospatial deficit. Taylor and Saint-Cyr (1995) concur, noting that there is no fundamental perception or visual discrimination disorder that justifies a visuospatial deficit and that PD patients experience problems only when a task requires them to use internally guided strategies, or their own body as a reference point for movement in space. However, a more recent review (Crucian & Okun, 2003) emphasised the complexity of visuospatial ability in PD and concluded deficits could not be attributable solely to executive function dysfunction (Crucian et al., 2000), but that other factors like gender were also important (Crucian & Okun, 2003).
5.11.1.3 Memory

Although memory deficits are evident in PD, Tröster and Woods (2007), note they are not considered characteristic of the neuropsychological profile in PD without dementia (PDND). However, mnemonic ability is impaired in PDND (Bondi et al., 1993; Brown & Marsden, 1990; Dubois et al., 1991; Hietanen & Teravainen, 1986; Raskin et al., 1990; Sullivan et al., 1989; Taylor et al., 1986), as evidenced by the difficulty these patients have in retrieving newly learned information. For example, mild impairment in free recall, but relatively intact recognition and cued recall (Tröster & Woods, 2007). Most studies find normal performances in tasks assessing verbal and visual recognition but significant deficits in tasks of free recall (Auriacombe et al., 1993); suggestive of a problem in the mechanism of retrieval rather than encoding (Knoke et al., 1998; Starkstein & Merello, 2002).

Neuropsychological deficits in PD have often been attributed to abnormalities in frontal lobe dysfunction (Bosboom et al., 2004; Ivory et al., 1999; Taylor et al., 1986; Taylor & Saint-Cyr, 1995; Zgaljardic et al., 2003), however, it is possible frontal and mesial temporal / hippocampal regions contribute to these deficits. The potential involvement of these regions was noted by Auriacombe et al. (1993) who demonstrated that verbal retrieval impairment in recall – but not recognition – underlies the deficit in PD in verbal fluency (category naming), a cognitive function usually associated with the PFC.

That verbal recall disturbance underlies cognitive ability in PD has been corroborated by studies showing patients have impaired retrieval strategy; they were less able than controls to organise the words for optimum recall (Hart et al., 1992; Taylor et al., 1990), less able to remember words in the absence of cues (Knoke et al., 1998), and less able to take advantage of external cues (Tweedy et al., 1982). In her review of the literature Lezak (1995) summarised PD mnemonic ability as an impairment in the short-term recall for words and unrelated verbal material, which improves to within normal limits with external aid (e.g., learning strategy or use of a recognition task).

Visual memory appears unimpaired when external aid is available, that is, when performing the task is less effortful (Flowers et al., 1984; Vriezen & Moscovitch, 1990). For example, pattern recognition memory was completely unaffected by the performances of either medicated or unmedicated patients at different stages of PD (Owen et al., 1995).

A comprehensive study of memory deficits in PD compared PDND with a medical control group having a similar level of physical disability and found no significant group differences on any of the memory tasks except when new verbal material was learned under incidental (but not intentional) learning conditions, and on tasks measuring remote memory and metamemory (Ivory, 1994; Ivory et al., 1999). After controlling for physical disability, verbal fluency, IQ, and mood, the authors suggested the impairments in PD were attributable to difficulties in allocating attention, formulating retrieval strategies, and learning under effortful conditions.
Importantly, the profile of memory deficits in PD is not homogeneous; that is, it is not confined to those traditionally labeled subcortical, rather, subgroups of patients fit different patterns of subcortical and cortical deficits (Filoteo et al., 1997). Performances in memory tasks are impaired when the task comprises a motor component (Brown & Marsden, 1990; Hietanen & Teravainen, 1986) and, interestingly, the pattern of deficits has been matched to motor lateralisation – verbal to left-sided and visuospatial to right-sided symptoms – even early-in-the-course (Blackwell et al., 2005).

5.11.1.4 Psychomotor Function

Bradyphrenia is a term used to describe psychomotor slowing, or slowed thinking time, and can be inferred from tasks assessing reaction time and planning in movement time. Some studies have demonstrated bradyphrenia in PD (Cooper et al., 1994; Mayeux et al., 1987), however, it is most likely this slowing is attributable to other factors, such as (a) concomitant depressive symptoms (Rogers et al., 1987; Smith et al., 1998b), (b) the cognitive demand of the task (e.g., choice versus simple reaction time, Zimmermann et al., 1992), (c) difficulties in planning a task response (Morris et al., 1988), or (d) the attentional elements of the task (Tachibana et al., 1997). Other studies have demonstrated no differences between PD patients and healthy controls in psychomotor speed (Davidson & Knight, 1995; Duncombe et al., 1994; Helscher & Pinter, 1993; Howard et al., 1994; Rafal et al., 1984; Spicer et al., 1994).

It is possible that reports of bradyphrenia in PD have arisen because information processing speed has been assessed with tasks that combine information processing speed (i.e., reaction time) with motor response speed (i.e., movement time). Starkstein et al. (1989b) compared event-related potentials (ERPs), reaction time and movement time in patients with severe motor fluctuations and found a significant decrement in P300 latency and movement time during the ‘on’, compared with ‘off’ phase, but none for reaction time and P300 amplitude, suggesting it is the motor element, not the psychomotor element, that influences task performance.

5.11.1.5 Attention

The literature provides conflicting evidence for attentional deficits in PD, possibly indicating that some types of attention are affected, but others not. Attention is an additional requirement in many cognitive tasks and, depending on the demands of the task, an inability to successfully allocate attention may interfere with performance on these tasks. In PD, simple tests of attention (e.g., sustained attention) remain intact, but those that are more complex and require a timed or internally guided response are likely to effect poor performances (Zakzanis & Freedman, 1999; Zgaljardic et al., 2003).

Shifting attentional set is markedly impaired in PD (Downess et al., 1989; Owen et al., 1998b). Compared to controls and other cognitively impaired neurological groups, non-medicated PD patients perform poorly in the IDS (i.e., intradimensional shift, when shifts are made to different exemplars of the same rule of perceptual dimension) and medicated patients with severe PD perform most poorly
during the EDS (i.e., extradimensional shift, when shifts are made to different perceptual dimensions). The better performance of medicated but severely afflicted patients in the IDS may reflect the benefit of dopaminergic medication targeting striatal structures, whereas their performance in the EDS may reflect the involvement of frontal structures.

5.11.2 Cognition in PD Patients with Dementia

PD patients with dementia form a sizeable subgroup of all PD patients and, depending on certain phenomenological features, are a cohort that a high number of PD patients will eventually join. A recent systematic review of studies has established the prevalence of dementia in PD as 24 to 31% of all PD patients, with PDD patients comprising 3-4% of all dementia patients (Aarsland et al., 2005). A prospective study in Norway found that after eight years, 78% of PDND patients had developed dementia (Aarsland et al., 2003a). The risk for dementia amongst PD patients is estimated to be two to six times more likely than for age-matched non-demented older persons, with risk being associated with an older age of PD onset, more severe motor symptoms, duration of disease, side effects of chronic medication, and low prodromal cognitive functioning (Bosboom et al., 2004). Neither age nor levodopa dose is correlated with cognitive status in PD, but EEG recordings of brain wave activity have linked reduced activity with global cognition as assessed by the MMSE (Caviness et al., 2007).

The cognitive deficits in PDD have been considered qualitatively distinguishable from those in SDAT, which has led some authors to subsume these disorders separately under two classes of dementia: PD as subcortical and SDAT as cortical dementia (Lezak, 1995). While some studies support this dichotomy, for example, Pillon et al. (1986) demonstrated patients with PDD had a greater executive dysfunction than patients with SDAT while the reverse occurred on delayed recall tasks; other studies demonstrate little difference in the neuropsychological profile of the two diseases. For example, Starkstein et al. (1996) assessed the cognitive function of 33 patients with PD and 33 with SDAT with a comprehensive test battery and concluded there were no significant differences between PDD and SDAT in any measure except a poorer performance by PDD patients on a visual discrimination learning task and an attentional set shifting task.

Neuropsychological studies have suggested that in PDD the observed memory impairment is not caused by damage in the hippocampus and related structures, as it is in SDAT, but to structures related to the functional use of the memory stores (i.e., the striatum and the prefrontal cortex, see Figure 5-2). This is because in the early stages of PDD, encoding and consolidation abilities are thought to be preserved, but retrieval impaired (Pillon et al., 2003). This is evident when patients have difficulty remembering something but less so if the task is a recognition task or they are given cued (Tröster & Woods, 2007). Starkstein and Merello (2002), however, have reported neuroimaging studies which consider SDAT and PDD as having comparable features, for example, neurofibrillary tangles and senile plaques (Bancher et al., 1993; Jellinger et al., 2002), hippocampal atrophy (using MRI, Laakso et al., 1996), lower regional perfusion with the tracer, hexamethyl propylene-amine-oxide (using SPECT, Starkstein et al., 1997), and a greater profile of metabolic deficits (using PET, Borgh et al.,
1997), as differentiated from healthy controls. From these studies, Starkstein and Merello concluded that one-third of PDD patients have SDAT neuropathology in the cerebral cortex, amygdala, and hippocampal areas combined with their PD neuropathology in the basal ganglia and thalamus. They also have Lewy bodies in the cerebral cortex, subregions of the hippocampus, amygdala and all major biogenic amine nuclei.

Figure 5-2 Areas of the brain in a PD patient where degeneration is thought to occur.
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Taxonomic debates of pathology aside, it is generally believed the neuropsychological profile of PDD is one of a progressive dysexecutive syndrome with memory deficits, in the absence of aphasia, apraxia or agnosia (Bosboom et al., 2004; Pillon et al., 2003). To assess executive function in PD, Henry and Crawford (2004) conducted a meta-analysis of 68 studies comprising 4644 participants (PD patients and healthy controls); they found semantic (i.e., category) fluency and, to a lesser extent, phonemic (i.e., letter) fluency was significantly impaired in PD, and that PDD patients demonstrated larger deficits than their PDND counterparts. Poor performance in tasks of verbal fluency – like the aforementioned – is considered a good predictor of cognitive status for future PDD (Pillon et al., 2003; Tröster & Woods, 2007), as it is for prospective SDAT (Henry et al., 2004).

As well as problems with executive functioning, PDD patients experience attentional deficits, fluctuating cognition, and hallucinations (perhaps exacerbated by chronic dopaminergic- and cholinergic therapy), in common with the clinical and pathological features they also share with patients having dementia with Lewy bodies (DLB) (Aarsland et al., 2001a; Ballard et al., 2002b;
Bosboom et al., 2004; Byrne, 1996; de Vos et al., 1996; Guo et al., 2005; McKeith & Mosimann, 2004; Richard et al., 2002; Tsuboi & Dickson, 2005).

Although controversial, clinical distinction between PDD and DLB can be made because DLB patients have less extreme parkinsonism and generally present with neuropsychiatric rather than cognitive symptoms (e.g., hallucinations, delusions, or fluctuating confusional states, Lennox & Lowe, 1996). Indeed, it is because of the psychosis, that DLB needs to be distinguished since antipsychotics are likely to generate a worsening of parkinsonian symptoms (McKeith et al., 1996).

Some of the neurobehavioural symptoms in patients with PDD or DLB may reflect underlying cholinergic dysfunction (Molloy et al., 2006; Perry et al., 1991), particularly those reflecting frontal executive function (Bohnen et al., 2006). PD, PDD, DLB, and SDAT patients have a diminution in cholinergic activity in the cerebral cortex but PDD and DLB patients have a more severe degeneration of cholinergic neurons in the nucleus basalis of Meynert, the location of cholinergic neurons which project to the cerebral cortex (Arendt et al., 1983; Asahina et al., 1998; Candy et al., 1983; Kuhl et al., 1996; Lange et al., 1993; Mesulam et al., 1983; Mori, 2005; Perry et al., 1985; Perry et al., 1993a; Piggott & Marshall, 1996; Tiraboschi et al., 2002).

Acetylcholine facilitates activation in different structures of the frontostriatal circuits involved in cognition and behaviour (Lichter, 2000). Reductions in choline acetyltransferase (ChAT) activity have been observed in the different structures of this pathway in patients with variations of parkinsonism and Lewy body disease (Langlais et al., 1993; Tiraboschi et al., 2000b; Ziabreva et al., 2006). Based on the observation that a marked difference in the ChAT/5-HIAA ratio occurred in hallucinating, compared to non-hallucinating Lewy body patients, an hypothesis has been advanced that visual hallucinations in this group may relate to a cortical imbalance in the cholinergic-monoaminergic system (Cheng et al., 1991; Perry et al., 1990a).

Dementia in PD is associated with both subcortical and cortical neuropathology, arising from cholinergic and catecholaminergic degeneration, which manifest in severe deterioration in executive performance and possibly attentional allocation.

5.11.2.1 Conclusion

There is cognitive impairment in PD which is consistently shown to correlate with severity and specific subset of motor symptoms. Cognitive impairment includes aspects of executive function, visuospatial tasks, some memory, and perhaps bradyphrenia. PDD is characterised by similar, but more severe deficits in these domains, but also with difficulties in allocating attentional resources, fluctuating cognition, and psychosis as concomitant symptoms. Definitive neuropathological correlates for dementia in PDD combine features of PD, DLB, and SDAT.
5.12 Serotonin in PD

Although the main pathology of PD is the degeneration of the DA producing neurons and the presence of Lewy bodies in remaining DA neurons, neurodegeneration and Lewy bodies are found in noradrenergic, serotonergic, and cholinergic systems (Shulz & Falkenburger, 2004).

There is some evidence of 5-HT abnormalities in PD patients (Hornykiewicz, 1998) and there is a possibility that 5-HT may play a role in the regulation of movement in this disorder, especially given that dorsal raphé neurons innervate all major parts of the basal ganglia (Lavoie & Parent, 1990; Meneses, 1998), which in the animal brain includes reducing activity in DA cells in the SNpc and effecting a reduction in DA release (Jacobs & Fornal, 1993; Ugedo et al., 1989). Abnormalities in markers for the serotonergic system (i.e., neuronal numbers and presence of Lewy bodies, tryptophan hydroxylase activity, serotonin level, and SERT binding) have been demonstrated in a number of morphological and neuroimaging studies and are reviewed comprehensively by Kish (2003). But by way of example, one study found a 40-50% decrease in the binding of [3H]citalopram, a serotonin reuptake inhibitor and pre-synaptic marker for serotonergic terminals (D’Amato et al., 1987); another found a 27% reduction in 5-HT$_{1A}$ binding potential in the raphé neurons in PD compared to healthy controls correlating with UPDRS composite tremor scores (Doder et al., 2003).

Other changes that occur in PD include increases in receptor binding sites in the basal ganglia, suggestive of an upregulation by these 5-HT receptors in response to a deficit in endogenous DA (Chen et al., 1998; Fox & Brotchie, 2000; Frechilla et al., 2001; Kim et al., 2003). Further evidence to support compensatory activity by the serotonergic system in response to reduced DA has been observed in animals after MPTP and 6-OHDA lesions produced hyperinnervation to serotonergic fibres and in PD where DA synthesised is striatal 5-HT terminals (Scholtissen et al., 2006c). Other changes to the serotonergic system in PD include reduced concentrations of 5-HT and increased concentrations of 5-HIAA in several brain regions, notably, the hippocampus and frontal cortex (Fahn et al., 1971; Scatton et al., 1983). Serotonin levels have been reported to be particularly low in the caudate nucleus, but also in the putamen, globus pallidus, substantia nigra, and frontal cortex (Bonnet, 2000; Kish, 2003). Apart from one study in the review of Kim et al. (2003), however, many of the aforementioned studies were surveying the brains of deceased patients; as it is most likely these patients were late-in-the-course, there is still much to learn about the health of the serotonergic system in early-in-the-course PD.

One study found levels of 5-HIAA were the same in PD patients compared to controls (Davidson et al., 1977), however, most studies have shown significant reductions in 5-HIAA levels in PD patients (for example, Kuhn et al., 1996b). This is particularly so for patients with the akinetic-variant as opposed to the tremor-variant of PD (Liu et al., 1999) and those with depression (Mayeux et al., 1988). The integrity of most 5-HT subtype receptors is preserved in the striatum and frontal cortex of PD patients, however, post-synaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor density is increased in the OFC and
temporal regions suggestive of an upregulation adaptation to lowered 5-HT concentrations. A number of experiments in animal models of PD have found 5-HT agents acting at these sites can have significant effects on motoric signs when used alongside dopaminergic agents (Bishop & Walker, 2003; Fox et al., 1998; Iravani et al., 2003; Oh et al., 2002; Scholtissen et al., 2006a). In humans, atypical antidepressants have been successful in avoiding extrapyramidal side effects through their antagonism of 5-HT$_{2A}$ as well as D$_2$ receptor sites (see section 4.1).

Imaging studies have reported significant reductions in 5-HT$_{2A}$ receptor density in the basal ganglia and premotor cortex area (Kroonenburgh et al., 2001) and in 5-HT$_{1A}$ binding in the raphé nuclei of Parkinson’s patients (Doder et al., 2003). Numbers for both these receptors have demonstrated increments in post-synaptic cerebral cortical regions, which may reflect their upregulation in response to reduced 5-HT concentrations (Frechilla et al., 2001; Scatton et al., 1983). Drugs acting at these two receptor sites have antidepressant, antipsychotic, anti- (levodopa induced) dyskinesia effects, implying activity at these sites may be important to psychiatric and extrapyramidal manifestations in PD (Chen et al., 1998; Naughton et al., 2000; Oh et al., 2002; Rosengarten et al., 2006; Schechter et al., 2002).

In PD patients, 5-HT supplementation or reduction has produced mixed effects. The use of 5-HT challenge agents is complicated by their different targets of action (i.e., on whether they exert agonist or antagonist effects), on which receptor they act, on which synaptic site, and whether the agent actually has multiple or opposing actions on the serotonergic system and on other neurotransmitter systems. For example:

a. The addition of pyridoxine to TRP supplementation worsened motor symptoms, but each compound on its own had no effect (Hall et al., 1972).

b. Parkinsonian motor symptomology and extrapyramidal symptoms have been described in depressed patients treated with SSRIs; however, this observation was not corroborated in a four treatment trial with these agents. The authors did suggest, however, there may still be a vulnerability of some patients to develop these symptoms (Dell’Agnello et al., 2001).

c. Nefazodone, a mixed SSRI/5-HT$_{2A}$ antagonist, improved motor symptoms (Avila et al., 2003).

d. Propranolol, on the other hand, has mixed β-adrenergic/5-HT$_{1A/1B/1C}$ antagonist properties but can relieve tremor (Koller et al., 2000; Tinajero et al., 1993).

e. ATD worsened motor symptoms – as measured by the Unified Parkinson’s Disease Rating Scale – in one patient with PD but so did placebo. The placebo response may be due to reductions in levodopa as a consequence of stimulated protein synthesis from the amino acid load plus the competition by tyrosine with other LNAAs to cross the blood brain barrier (McCance-Katz et al., 1992).
The difficulty of finding a clear relationship between 5-HT agents and dopaminergic-mediated motoric effects has led Miyawaki et al. (1997) to write that “...the relationship … does not appear to be a simple reciprocity between 5-HT and DA, and if 5-HT reuptake inhibition is associated with ‘more’ 5-HT, one cannot predict clinical sequelae that might be expressed from ‘less’ DA” (p. 302).

5.13 Summary
Parkinson’s disease is a degenerative condition which occurs generally, but not always, in older persons. Symptoms include a non-remitting and progressive physical handicap, along with possible affective, cognitive, and psychiatric manifestations. Degeneration in several, but largely dopaminergic, neurotransmitter systems underscore changes to neuroanatomical regions and neural pathways to precipitate these neurological, neuropsychological, and neuropsychiatric sequelae.

The cardinal neurological signs of PD are bradykinesia, rigidity, tremor, and postural instability, but there are a large number of adjunct movement-based symptoms that may accompany them.

Major depression may begin before the onset of motor symptoms, last more than a year, and be significantly related to reduced frontal metabolic activity and reduced CSF levels of 5-HIAA. It is more likely to occur in the akinetic-rigidity variant of PD, to predict a faster decline in physical and cognitive function, be associated with more cognitive deficits, and to be a significant risk for the development of dementia.

The primary impairment in PD is dysexecutive, with variable memory, visuospatial or psychomotor impairment occurring in tasks where more effortful performance is required. Patients with PD and dementia experience quantitatively greater executive dysfunction, memory and attentional deficits akin to those in SDAT, they may also have hallucinations and fluctuating cognition (i.e., fluctuating consciousness).

Because of the number and variety of symptoms in PD, a number of different neurotransmitter systems have been investigated for aetiological and therapeutic purposes. The serotonin system interacts with the dopaminergic system and both are known to undergo degenerative changes in PD.

There are three key issues arising from this chapter:

a. 5-HT is reduced in PD and this may be an adaptation to compensate for the sizeable reductions in DA. Further reduction of 5-HT may improve tremor.

b. Although this reduction in 5-HT may be adaptive, it may also be implicated in an increased risk of depression. Further reduction, as induced experimentally by ATD, may elicit depressive symptoms.

c. Some of the cognitive deficits associated with PD may be a function of changes to the serotonergic system and ACh loss. Further reduction may worsen these cognitive deficits.
6 METHODOLOGY

6.1 Study Design

The intention was to investigate the role of serotonin using the technique of acute tryptophan depletion (ATD) in persons with older age or Lewy body neurodegenerative disease in a series of studies having a similar design, but involving different groups. Study A examined data from healthy older persons to compare different ATD doses and as a pilot for a larger study named Study C; Study B was designed to compare PD patients with healthy control participants; and Study D to compare PDD and DLB patients with healthy control participants. A further study was conceptualised to investigate the effect of ATD in SDAT and DLB patients medicated with cholinesterase inhibitors.

Study A involved the collation and analysis of two data sets (from now on referred to as studies A1 and A2), collected for other studies in which the healthy older participants acted as control participants for patients with SDAT (A1) (Porter et al., 2003b; Porter et al., 2000) and recovered depression (A2) (Porter et al., 2005). Studies B, C, and D were parallel and overlapping experimental studies. Over the course of two years, data were successfully collected for the two testing days from only 4 patients with PDD or DLB. This meant a re-evaluation of the data set for the completion of the PhD within the degree time frame. This now comprises data from three studies: A, B, and C. Data for Study D is still being collected as part of an ongoing longer-term project and as such, this study is included in the methodology but case study data is presented in Appendix J. The investigation with ATD and cholinesterase inhibitors is yet to be realised because it is dependent on the results of Study D.

The studies employed a double-blind, placebo-controlled, randomised, counter-balanced, crossover within-subject design, with additional between-subjects components (Studies A, B, and C: male or female; Study B: patients or controls, male or female; Study C: young-old or old-old, male or female). Participant assignment to order of treatment was randomised using a block-design, and then counter-balanced to ensure equal numbers of participants were tested under each treatment condition on the two test days.

The treatment conditions comprised a placebo (i.e., control) arm of nutritionally balanced amino acid mixture and a depletion (i.e., active) arm of equivalent amino acid mixture deficient in tryptophan.

Patients and controls were matched for gender, age, and predicted verbal IQ.

6.1.1 Amino Acid Drink

The composition of the treatment drinks for all studies was based on that developed by Young et al. (1985), Miller et al. (1992), and Delgado and colleagues (1994; 1991). It comprised: alanine 5.27%, arginine 4.69%, cysteine 2.59%, glycine 3.07%, histidine 3.07%, isoleucine 7.66%, leucine 12.93%, lysine 10.53%, methionine 2.87%, phenylalanine 5.46%, proline 11.69%, serine 6.61%, threonine 6.23%, tyrosine 6.61%, and valine 8.52%, plus for placebo (2.2% tryptophan) or depletion (2.2% maltodextrin).
Even though it is a type of carbohydrate, the addition of maltodextrin to the active drink would not influence the effects of ATD on behaviour in the study. The reasons for this are:

a. This polysaccharide has weak hydrogen bonds which break apart very easily in the stomach giving it a similar absorption rate to glucose.

b. The addition of 63 g carbohydrate to the amino acid load has demonstrated instead a profound decrease in the TRP/LNAA ratio and TRP levels (for list of studies refer to p. 164, Blokland et al., 2004).

The ingredients were bought from SHS International Ltd, 100 Wavertree Boulevard, Wavertree Technology Park, Oakland, California and the 83.3 g doses were re-measured from 104.4 g mixtures by a hospital pharmacist. The amino acids are not on the Pharmaceutical Schedule (refer, PHARMAC, 2007) and are, therefore, classified as nutritional supplements in New Zealand.

In all studies, both placebo and depletion treatments were of identical composition, with the exception of L-tryptophan in the placebo and maltodextrin in the depleting mix. The amino acid drink was mixed immediately prior to ingestion and participants were instructed to swallow it as soon as possible (i.e., within 15 mins). The size (or dose) of the treatment was different across studies and this is detailed under the Design section in the corresponding Study chapters.

6.1.1.1 Flavouring

Methionine, cystine and arginine contain sulphur and have an unpleasant taste (Hood et al., 2005). Tryptophan is slightly bitter (Hartmann et al., 1974) and purportedly imparts an aftertaste similar to that of saccharin (Coppen et al., 1963). To cover the taste, the amino acids were mixed with 250 ml of water and flavoured with artificial flavouring and saccharin. The ingredients of the flavourings per 5 g sachet were:

Grapefruit: citric acid, flavourings (e.g., sugar, maltodextrin, flavouring preparations, carrier (E1450), artificial sweetener (E950), colour (E160a)). Lemon/lime: citric acid, flavourings (e.g., sugar, maltodextrin, flavouring preparations, carrier (E1450), artificial sweetener (E950)).

Nutritional information for the flavour sachets was:

Grapefruit: energy 65 kj (15 kcal), protein nil added, carbohydrate 1.4 g, fat nil added. Lemon/lime: energy 63 kj (15 kcal), protein nil added, carbohydrate 1.3 g, fat nil added.

6.1.1.2 Sugar-free Mints

Sugar-free peppermints were provided straight after the treatment to cover the taste of the drink. As the mints each contained a small amount of starch the participant was offered only two peppermints. Participants were given a choice of:
Chewing mints  Double “D” brand. Ingredients: maltitol, isomalt, vegetable fat, gelatin, emulsifier (e.g., soy lecithin), vegetable gum (414), salt, peppermint oil. Nutritional information per sweet: 0 carbohydrates, < 1 g protein, < 1 g total fat, < 5m g, sodium, 3.3 g maltitol syrup, 1.6 g isomalt.

Boiled mints  (a) Sweet ‘N Low brand. Ingredients: isomalt, citric acid, natural and artificial flavours, sodium bicarbonate, malic acid, sucralose. Nutritional information per sweet: 14 g carbohydrate, 0 protein, 0 total fat, 60 mg sodium, 14 g isomalt (e.g., sugar alcohol); (b) Jakemans (Confectioners) Ltd brand: isomalt, peppermint oil, sweetener (e.g., Acesulfame K).

6.2  Assessment
The following assessments, in alphabetical order, were included in all or some of the three studies. A list of assessments used in each study is included under the Procedure section of each study chapter.

6.2.1  Biochemical
Venous blood samples (10mls) were obtained by a qualified nurse phlebotomist at baseline (0 hrs), 4 hrs and 6.5 hrs (7 hrs in Study A) hrs after the treatment. Samples were placed on ice for approximately 15 minutes. Diaflo ultrafiltration membranes – obtained from Amicon Inc., Beverly, MA, USA – with a nominal molecular weight cut-off of 30 kilodaltons (kDa) that exclude plasma proteins were used in the centrifuge process. This entailed placing the plasma sample inside an ultrafiltration tower assembly – also purchased from Amicon – and centrifuging it at 3500 rpm in a fixed angle rotor at 37°C for 15 minutes. The resulting ultrafiltrate collection was then assayed using high performance liquid chromatography with fluorometric precision (Anderson et al., 1981).

Outcome Measures

Free TRP This is the amount of TRP in the blood that is not bound to albumin and which is free to cross into the BBB.

6.2.2  Mood
Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) (refer section 11.6.1.1)

The MADRS is a clinical measure used to assess mood and mood changes over time. The rating scale consists of ten items: Apparent Sadness, Reported Sadness, Inner Tension, Reduced Sleep, Reduced Appetite, Concentration Difficulties, Lassitude, Inability to Feel, Pessimistic Thoughts, and Suicidal Thoughts. It is assessed by interview on a 4-point Likert scale (defined scale steps: 0; 2; 4; 6 or between them: 1; 3; 5). The MADRS is used extensively worldwide as an observer instrument in clinical and psychopharmacological depression research to assess severity of depression after a categorical diagnosis has been ascertained (Muller et al., 2003).

No significant effects have been observed on the MADRS in four ATD studies (Aberg-Wistedt et al., 1998; Booij et al., 2005a; Porter et al., 2003b; Porter et al., 2005) but there was a significant lowering
of mood for currently depressed and treated patients as assessed by this rating scale at 6.5 hrs post the high dose treatment (100 g) but not low dose (25 g) treatment (Booij et al., 2006; Booij et al., 2005b).

Procedure

A trained administrator interviewed and assessed the participant on a Likert scale, which moves from broadly phrased questions about symptoms to more detailed ones, thus allowing a precise rating of severity. The administrator decides where the participant lies on the defined scale steps.

Outcome Measure

MADRS Total The score for each of the 10 items is tallied to give a total score of 60. A higher score reflects a lower mood. A cut-off score of 31 is used to differentiate moderate and severe depression (Muller et al., 2003).

6.2.3 Mood

Profile of Mood States (POMS) (McNair et al., 1992) (refer section 11.6.1.2)

The POMS is used to assess current mood state and consists of 67 adjectives (e.g., Tense, Miserable, Muddled, Listless) or short phrases (e.g., Sorry for things done, Ready to fight, Uncertain about things) which are self-rated by a participant on a five-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely). The items assess seven affect states: Fatigue-Inertia, Anger-Hostility, Vigor-Activity, Confusion-Bewilderment, Depression-Dejection, Tension-Anxiety, and Friendliness.

The POMS has been used to assess mood in a large number of ATD studies (for example, Benkelfat et al., 1994; Ellenbogen et al., 1996, 1999; Epperson et al., 2007; Evers et al., 2006a; Hayward et al., 2005; Hughes et al., 2004; Klaassen et al., 1999a; Klaassen et al., 1999b; Leyton et al., 2000b; Newhouse et al., 2002; Porter et al., 2000; Porter et al., 2005; Rubinsztein et al., 2001; Shansis et al., 2000; Sobczak et al., 2002a; Van der Veen et al., 2006; Yatham et al., 2001). Despite no significant change in the POMS during ATD for a group of older recovered depressed patients (Porter et al., 2005), patients with SDAT (Porter et al., 2000), and remitted depressed menopausal women (Epperson et al., 2007), it is possible the measure may be sensitive to mood changes in conditions DLB and PD as these conditions have concomitant symptoms of low mood (Ballard et al., 1995; Starkstein & Merello, 2002).

Procedure

The participant is asked to circle an adjective numbered 0 - 4 that best describes how he or she feels “right now”.

Outcome Measure

POMS Total The items are summed to make a total score out of 268; a higher score reflects a lower mood.
6.2.4 Movement

The Unified Parkinson’s Disease Rating Scale (UPDRS: Section III. Motor examination) (Fahn et al., 1987) (refer section 11.6.2.1)

The UPDRS is an assessment that enables the longitudinal course of Parkinson’s disease to be followed. The multidimensional items have been found to be both reliable and valid (Martinez-Martin et al., 1994). The UPDRS comprises five sections each evaluated by interview: Mentation, Behaviour, Mood, Activities of Daily Living, and Motor. Some sections require multiple grades assigned to each extremity. Total UPDRS scores range from zero (representing no disability) to 176 (representing total disability).

Procedure

The assessment is made by a trained administrator.

Outcome Measure

*UPDRS Total* This is the sum of all individual item scores. It is scored out of 176; a higher score represents more severe symptoms.

6.2.5 Neuropsychological

CAMbridge Neuropsychological Test Automated Battery (CANTAB) (Robbins et al., 1994a)

Computerised testing batteries have several advantages over pen and paper tests in that they (a) include precision, strategy, and speed as testing variables, (b) assess visual tasks with non-verbal responses, and (c) can be used with people having speech difficulties (e.g., PD patients). CANTAB was developed from animal behaviour paradigms then standardised – via a neural systems approach – against tasks that assess similar cognitive functions in humans. The tests have been validated in a large number of people and in different patient groups. Normative data was derived from over 2000 healthy people within a 16 - 80 year age range having an estimated IQ distributed across 4 NART bands (*CANTABeclipse*, 2007). Functional neuroimaging has extended understanding of the neural substrates involved in completing many of the sub-tests – for example, in Spatial Span and Spatial Working Memory – thus providing a useful confirmation of the neuroanatomical basis of the tests.

CANTAB was used in the studies because it has the potential to compare groups with different cognitive abilities, for example, SDAT and DLB (Sahgal et al., 1995) and SDAT and PD (Sahakian, 1990).

Procedure

Participants are required to sit approximately 30 cm away from a computer monitor and make responses by pressing a forefinger against a touch sensitive screen attached to the monitor. The touchscreen acts in the same way as the left button on a computer mouse. Every task uses a monitor with a blank black screen. The administrator gives the participant instructions for each task before it is
begun. Delayed Matching to Sample (DMS) has three practice presentations, SSP has one demonstration and one practice presentation, and SWM has one demonstration and two practice presentations.

**Motor Screening (MOT)**

MOT is a screening task for motor and visual disorders, and training for CANTAB and the touchscreen. It is also a measure for movement time. As previously mentioned movement time was shown to be slower after ATD (Schmitt et al., 2000).

**Procedure**

A series of crosses flash pink and green on the screen, as shown in Figure 6-1. The crosses disappear from the screen when they are touched correctly and their disappearance is accompanied by a change in audio tone. The participant is instructed to touch the crosses as they flash.

**Outcome Measures**

*MOT Accuracy* This is a measure of the mean distance between the centre of each cross and the location of the participant’s responding touch. Accuracy is measured in pixel units based on a screen resolution of 640 x 350 pixels; a lower score reflects a better performance.

*MOT Reaction time* This is the mean of all times taken by the participant to correctly identify and respond to each of 10 crosses as they appear on the screen. Reaction time is measured in milliseconds; a lower score reflects better (faster) response.

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**Figure 6-1 Motor Screening (MOT).**

Note: Two examples of crosses in the series to be touched.
**Pattern Recognition Memory (PRM)**

PRM assesses the ability to recognize abstract patterns. The test administration guide (*CANTABeclipse Test Administration Guide*, 2004) states that PRM gives a good indication of visual short-term recognition memory, which is impaired in conditions such as mild to moderate SDAT (Swainson et al., 2001). Performance was enhanced for females with SDAT during ATD (Porter et al., 2003b). Given the more severe cholinergic deficit in PDD and DLB, and the similar performance of DLB to SDAT patients during this task under normal conditions (Sahgal et al., 1995), it was thought this finding might be replicated during Study D. Performance by young healthy adults on this task during ATD has shown impairment (Rubinsztein et al., 2001), which reinforced the task’s inclusion in a study investigating ATD in healthy older adults.

**Procedure**

Twelve boxes each containing a different abstract pattern appear sequentially in the middle of the screen, as shown in Figure 6-2 (i) and (ii). The participant is instructed to look at the patterns within the boxes and remember them. When the sequence of patterns has completed, pairs of patterns are presented: one is a target pattern which is the same pattern as one of the index (original) patterns and one is a distractor (novel) pattern. The participant is asked to indicate the index pattern by touching this pattern on the touchscreen. A correct response generates a green tick, as shown in Figure 6-2 (iv), while an incorrect response generates a red cross, as shown in Figure 6-2 (ii).

**Outcome Measures**

*PRM Accuracy* This is the percentage of correct responses made by the participant; a higher score reflects a better performance.

*PRM Reaction time* This is the mean of all times taken by the participant to correctly identify and respond after the appearance of 24 pattern presentations. Reaction time is measured in milliseconds; a lower score reflects faster response.
Simultaneous and Delayed Matching To Sample (SMTS and DMS)

The DMS assess encoding, consolidation, and recognition aspects of visual memory by asking a participant to recognize complex visual designs during presentations of delayed matched patterns. With the inclusion of the simultaneous matching to sample (SMTS) task, it also assesses attention and perception (Sahakian, 1990; Sahgal et al., 1992). According to the manual for this task, the discrepancy between percentage scores on the simultaneous and delay conditions in this task gives a good indication of visual memory ability; with an increasing delay interval corresponding to an increasing load on memory. The complete task comprises two counterbalanced trials each having 5 simultaneous presentations and 5 presentations of the three delay intervals.

Although no difference has been observed between performances of PD patients and controls in this task (Lange et al., 1992), a statistically significant difference has been observed between DLB and SDAT patients. For example, the performance of DLB and SDAT patients was the same in the SMTS task, but the SDAT patients outperformed the DLB patients in the DMS task (Sahgal et al., 1995). Accuracy during a scopolamine challenge was impaired in the DMS task (Robbins et al., 1997) prompting the need to extend understanding with a further group of patients with severe ACh dysfunction.

Figure 6-2 Pattern Recognition Memory (PRM).

Note: i and ii = display examples of two index presentations; iii = an incorrect response; iv = a correct response.
There was no significant effect of ATD treatment found in the performance of SDAT patients and people recovered from depression (Porter et al., 2003b; Porter et al., 2005) across the different delay intervals.

Procedure

A red box containing an index pattern comprising four colours and four geometric shapes appears on the screen, as shown in Figure 6-3 (i). A set of four white boxes appear below the red box. They open to reveal one target pattern and three distractor patterns. Twenty of these sets are presented in either simultaneous, as shown in Figure 6-3 (ii), or delayed mode, as shown in Figure 6-3 (iii). The participant is asked to touch the pattern which matches the index pattern. A correct response generates a green tick, while an incorrect response generates a red cross.

Outcome Measures

SMTS Accuracy This is the percentage of correct responses when the target pattern and the three distractor patterns are on the screen at the same time; a higher score reflects a better performance.

SMTS Reaction time This is a mean of all times in which the participant responded correctly when the index, target, and distractor patterns are all on the screen at the same time. Reaction time is measured in milliseconds as the time between the complete appearance of the five patterns and touch; a lower score reflects a better (faster) response.

DMS Accuracy (0 ms, 4000 ms, 12000 ms delay) These are the percentages of correct responses in each delay interval; a higher score reflects a better performance.

DMS Accuracy (all delays) This is the percentage of correct responses from presentations in all delay intervals combined; a higher score reflects a better performance.

DMS Reaction time (0 ms, 4000 ms, 12000 ms delay) This is the mean of all times in which the participant responded correctly in each delay interval. Reaction time is expressed in ms; a lower score represents a better (faster) response.

DMS Reaction time (all delays) This is the mean of all times taken by the participant in all presentations with delay intervals combined. Reaction time is measured in milliseconds as the time between the complete appearance of the patterns and touch; a lower score reflects a better (faster) response.

DMS %Corr This is the percentage of all presentations in which the participant’s first response to the target pattern is correct.
Spatial Recognition Memory (SRM)

SRM assesses the ability to remember the spatial location of visual stimuli in the form of blank squares outlined in white. There are equivocal reports of ability in SRM; medicated PD patients with severe clinical symptoms (i.e., Hoehn and Yahr stage III-IV) were impaired on this task (Owen et al., 1993) but not patients withdrawn from L-dopa (Lange et al., 1992). Three experimental studies have induced SRM deficits in healthy adults; one used systemic sulpiride (D2 antagonist) to simulate the profile of cognitive deficits in PD (Mehta et al., 1999), another used tyrosine depletion (Harmer et al., 2001) to assuage SRM under conditions of reduced DA function, and a third used scopolamine to mimic the cholinergic deficits associated with SDAT by impairing both accuracy and reaction time on SRM (Robbins et al., 1997).

Procedure

Five blank squares appear sequentially at different locations on the screen, as per the example in Figure 6-4 (i). The participant is instructed to look at each box and remember its (index) location on the screen. When the series has been completed, pairs of squares are presented on the screen: one in the index location and one in a novel (distractor) location, as demonstrated in Figure 6-4 (ii). Four presentations of a series of five squares are held. The participant is asked to indicate the target location by touching the box in this location on the touchscreen. A correct response elicits a green tick, as
demonstrated in Figure 6-4 (iii), while an incorrect response generates a red cross, as demonstrated in Figure 6-4 (iv).

Outcome Measures

**SRM Accuracy** This is the percentage of correct responses made by the participant; a higher score reflects a better performance.

**SRM Reaction time** This is the mean of all times taken by the participant to correctly identify and respond to each of 20 box sequence presentations. Reaction time is measured in milliseconds as the time between the complete appearance of each target and distractor pattern and touch; a lower score reflects a better (faster) response.

![Figure 6-4 Spatial Recognition Memory (SRM).](image_url)

Note: i = an original presentation; ii = an original presentation paired with a distractor stimulus; iii = participant touches original stimulus and rewarded with tick; iv = participant touches incorrect stimulus.

**Spatial Span (SSP)**

SSP assesses immediate spatial memory and is similar to the Corsi block test in that it examines the ability to remember the order in which visual stimuli are presented. In this respect it is a non-verbal analogue of Digit Span (Fray et al., 1996).

Results from two studies with large samples of healthy persons (age range 8 - 79 years) showed performance on SSP declined with age (De Luca et al., 2003; Robbins et al., 1998). Men outperformed
the women in the former study. Porter (2005) found the performance of all older participants in their study was impaired in a verbal analogue of spatial span, Digit Span Forward, after ATD.

Spans in the SSP by PD patients withdrawn from L-dopa were shorter than when they were on the medication (Lange et al., 1992). Span length increased when DA levels were enhanced with the D₂ agonist, bromocriptine (Mehta et al., 2001). Patients with DLB have impairments in this task similar to those of SDAT patients thus reflecting the difficulty these patients have during a task involving hippocampal function (Sahgal et al., 1995). ATD has induced impairments on Digit Span Backward in older participants (Porter et al., 2003b).

Procedure

Nine white squares appear on the screen and change color one by one (i.e., sequentially ‘light up’), as shown in Figure 6-5 (i) and (ii). The participant is instructed to indicate the order in which the squares change color. The participant completes three presentations of each span, in which progressively more squares change color, with span increasing from three through to nine squares. If, by the third presentation of a span the participant fails to correctly remember the order of the color changes the test is self-terminated.

Outcome Measures

**SSP Span** This is the longest span correctly recalled by the participant. The maximum score is nine; a higher score reflects a better performance.

![Figure 6-5 Spatial Span (SSP).](image)

Note: examples of two sequentially highlighted boxes within a span.

**Spatial Working Memory (SWM)**

SWM assesses spatial working memory and self-initiated strategy. Working memory is the ability to keep relevant spatial information active while formulating an appropriate response and then following through with an accurate execution of this response.
Performance on SWM was shown to decline with increasing age when older persons made more within-search errors (i.e., returning to a box already opened in the same search sequence) (De Luca et al., 2003) or between-search errors (i.e., returning to open a box in which a token has already been found in a previous search) than younger adults (De Luca et al., 2003; Robbins et al., 1998). In the former study men made fewer errors than women. Compared to age and IQ matched controls, patients with frontal lobe damage and PD show impaired performances in this task (Robbins et al., 1994b). DLB patients make more mistakes (i.e., ‘between search errors’, see below) than both SDAT patients and controls during this task, possibly suggesting the task is sensitive to the more severe frontal pathology of the DLB patients (Sahgal et al., 1995). Scores in the task were also impaired for PD patients after administration of sulpiride (degree was dose-dependent) possibly indicating a vulnerability of PD patients in SWM tasks (Mehta et al., 1999). After conducting a number of studies using this task in unmedicated and medicated patients at different stages of PD, Owen et al. (1998b) have concluded “…there is an apparent increase in severity and broadening of spatial memory impairments as patients show increasing clinical disability” (p. 163).

Studies in younger healthy adults investigating the effects of ATD on working memory have given conflicting results, some suggesting improved function (Rowley et al., 1997; Schmitt et al., 2000) and others finding no effect (Park et al., 1994). No observed difference of ATD has been shown in older adults, either in patient or healthy comparison groups (Porter et al., 2003b; Porter et al., 2005).

Procedure

A number of boxes (i.e., three up to nine) are displayed on the screen. Each opens upon touch to reveal either a blank square or a blue token. The participant is asked to search through the boxes on the screen to discover where the computer has hidden a token. When a token is found the participant is instructed to place it in the column on the right hand side of the screen in the ‘home’ position, as shown in Figure 6-6 (i) and (ii). They are told that once a token is found within each presentation, the box in which it is hidden will not hide a token again. The test does not self terminate and the participant has to find every token and place it in the correct order, in the ‘home’ position, in every presentation before the task is completed. Task difficulty is manipulated by increasing the number of boxes appearing in a presentation (i.e., hiding targets behind four, six, or eight boxes).

Outcome Measures

There is one type of error in this task and it relates to which box the participant touches and in which order a participant touches it before a trial is completed. The computer monitors the participant’s sequence of touches and records the search errors.

*SWM Between errors (4x box, 6x box, 8x box):* This is the number of times within each presentation of 4, 6, and 8 boxes that the participant revisits a box in which a token has previously been found; a lower score reflects a better performance.
SWM Between errors (all delays): Between errors is a composite score of the sum of all errors in every level; a lower score reflects better performance.

![Figure 6-6 Spatial Working Memory (SWM).](image)

Note: i = The first box correctly placed in the 'home' position with the second box touched ready to be moved 'home'; ii = the fourth box placed in the 'home' position with the fifth box touched and ready to go 'home'.

Cognitive Drug Research (CDR) battery (Simpson et al., 1991)

The Cognitive Drug Research (CDR) system is a computerised battery of cognitive tests which has been used over the past 17 years in numerous studies and in a variety of conditions, including (non-specified) severe dementia (Simpson et al., 1991), SDAT (Ballard et al., 2001; Barker et al., 1998; Wesnes et al., 1990) and Parkinson’s disease (Ballard et al., 2002b; Bronnick et al., 2006; Mosimann et al., 2003). The CDR library comprises a wide variety of tests assessing different domains of cognitive function and an individualised study battery is compiled which caters to the needs of each study. All the tests have a visual component because presentations are made on a computer screen. In the case of Word Presentation, the presentation is accompanied by an auditory and recital component.

The CDR has been used to assess the effects of combined monoamine depletion in healthy younger women (Matrenza et al., 2004) with accuracy and reaction time being impaired during depletion in the sustained attention task of Digit Vigilance; there were no effects of treatment on any of the other CDR tasks of memory or psychomotor speed.

Procedure

For the purposes of the current research, the CDR battery had the following components: Word Presentation, Immediate Word Recognition, Digit Vigilance, Simple Reaction, Time Choice Reaction Time, and Delayed Word Recognition. Participants are required to sit at a computer monitor and hold an external two-button (YES or NO) response box in their hands. Responses are made using opposing thumbs or fingers (left on the NO button for a response of ‘no’ and right on the YES button for a response of ‘yes’). There is a training session for the battery before testing and instructions are again given to the participant before each task is begun.
Word recognition performance is not generally impaired in older persons (Fleischman et al., 2004; Huppert, 1991), however, the Immediate Word Recognition and Delayed Word Recognition tasks were included because recognition memory – like verbal recall – involves encoding and consolidation. ATD has not generally impaired performance on verbal recognition tasks, as exemplified by the non-significant difference in women’s performances in this CDR task after ATD (Harrison et al., 2004). However, Schmitt (2000) in healthy younger adults, and Scholtissen (2006b) in PD and healthy older participants, found ATD impaired performance on delayed verbal recognition tasks. The pooled analysis of Sambeth (2007) showed delayed verbal recognition memory on the RAVLT was impaired in women having the placebo drink (with the amino acid load) but not during ATD. These aforementioned findings justified the inclusion of the recognition task in the present research.

The Simple Reaction Time task is used as a measure of focused attention and psychomotor speed. Likewise, the Choice Reaction Time task, which, with the additional advantage of discrimination and response inhibition components, is a useful assessment for measuring the variability of attentional performance across time, in particular fluctuating consciousness in DLB patients.

Evidence of attentional performance in younger adults after ATD is equivocal, but there is a suggestion ATD improves focused attention (Gallagher et al., 2003; Schmitt et al., 2000). There does not appear to be an ATD-induced effect in attentional performance in SDAT (Porter et al., 2003b).

**Word Presentation (WP)**

WP comprises stimulus words for Immediate Word Recognition (IWR) and Delayed Word Recognition (DWR).

**Procedure**

Fifteen solid white words (index words), matched for frequency, appear in the centre of a blue screen at a consistent interval. The participant is instructed to read the words aloud together with the administrator and to remember them. By reading the words aloud the administrator ensures the participant has attended to the word.

**Immediate Word Recognition (IWR)**

IWR is administered directly after WP. Participants are required to distinguish target words (i.e., the index words from WP) from distractor words. Distractor words are matched with index words both for length, number of syllables, ‘imageability’, and frequency of words in the language. The CDR has up to 50 equivalent lists of words and the same participant receives different lists on different occasions. Lists are selected at random and exclude previously presented lists. IWR assesses the ability (i.e., speed and sensitivity) to discriminate novel from previously presented words immediately following their presentation.
Procedure

The participant is instructed to identify and respond as quickly as possible to a target word by pressing the YES button on the response box and to identify and respond to a distractor word by pressing the NO button on the response box. There are equal numbers of target and distracter words.

Outcome Measures

**IWR SI** The SI (sensitivity index) combines the accuracy scores of the target and the distractor words into one variable, with an adjustment for chance responding. That is, it combines the ability to correctly recognise the targets with the ability to correctly reject the distractors. The SI was chosen as an outcome measure for both IWR and DWR because the variables, ‘target hit’ and ‘false alarms’, tend to show mirror effects in recognition memory (Glanzer & Adams, 1985; Glanzer et al., 1993). The SI is expressed as a percentage; a higher score reflecting better discrimination accuracy.

**IWR Reaction time** The mean of times taken by the participant to correctly identify and respond to a target or distractor word after it appears on the screen. Reaction time is measured in milliseconds as the time between the appearance of a target or distractor word and correct button press; a lower score reflects a better (faster) response.

**Delayed Word Recognition (DWR)**

Delayed memory is the ability to register, store and retrieve information over time. DWR assesses the ability (i.e., speed and sensitivity) to discriminate novel from previously presented words after an interval of 15 minutes from when the word was presented during WP. In all other respects it follows the same format.

Outcome Measures

**DWR SI** This is assessed as per IWR.

**DWR Reaction time** This is assessed as per IWR.

**Digit Vigilance (DV)**

Sustained attention allows a participant to maintain readiness and keep track of external events over a period of time. DV is a measure of sustained attention, intensive vigilance and the ability to ignore distraction. If a participant’s attention wanders then targets would be missed. With DV there are no original and new stimuli as such and a participant’s performance is covered in the ‘percentage’ and ‘false alarms’ components, that is, if you miss a target and don't press, it comes off the accuracy; if you hit when you shouldn't, it is a false alarm.

Procedure

Throughout the task, a solid white index digit is displayed on the right side of a blank black screen. Next to this index digit, in the middle of the screen, a continuous stream of 15 randomly dispersed
target and 75 serially unordered distractor digits appear at a rate of 80 per minute. The target digit is the same as the index digit, while the distractor digit is not. The participant is required to identify and respond as quickly as possible using the YES button when a target digit appears on the screen.

Outcome Measures

**DV Accuracy** This is the percentage of times the participant correctly presses the YES button in response to one of 15 target digits; a higher score reflects a better performance.

**DV Reaction time** The mean of the times taken by the participant to identify and respond to a target digit. Reaction time is measured in milliseconds as the time between the appearance of a target or distractor digit and correct button press; a lower score reflects a better (faster) response.

**Simple Reaction Time (SRT)**

SRT assesses alertness, power of concentration, and speed of reaction to an expected event.

Procedure

The participant is required to identify and respond to the appearance on the screen of a solid white ‘yes’ word by pressing the corresponding YES button on a response box as quickly as possible. There are 50 stimuli in total appearing at irregular delay intervals. New words are not presented until a response has been made.

Outcome Measures

**SRT Accuracy** This is the percentage of times the participant identifies and responds to a ‘yes’ word by correctly pressing the YES button; a higher score reflects better attention.

**SRT Reaction time** The mean of the times taken by the participant to identify and respond to a ‘yes’ word, by correctly pressing the YES button. Reaction time is measured in milliseconds as the time between the appearance of the word and correct button press; a lower score reflects a better (faster) response.

**Choice Reaction Time (CRT)**

CRT assesses alertness, power of concentration, stimulus discrimination, and response organisation, and psychomotor speed. It is similar to SRT but additionally measures the processing time required to identify, select, and respond to two index words (‘yes’ and ‘no’). It has been used to assess attention or fluctuating consciousness in SDAT and DLB patients at three observation points across a one hour time period (see, Walker et al., 1999).

Procedure

Fifty stimuli appear on the screen with each being selected randomly with an equal probability. They appear at irregular intervals. The participant is required to respond as quickly as possible to the visual
presentation of the words ‘yes’ or ‘no’ by pressing the corresponding YES or NO button on the response box.

Outcome Measures

*CRT Accuracy* The percentage of times the participant identifies and responds to a target word by correctly pressing the YES button for the word ‘yes’ and the NO button for the word ‘no’; a higher score reflects a better performance.

*CRT Reaction time* The mean of the times taken by the participant to identify and respond to a ‘yes’ or ‘no’ word by correctly pressing the corresponding YES or NO button. Reaction time is measured in milliseconds as the time between the appearance of the word and correct button press; a lower score reflects a better (faster) response.

**Controlled Oral Word Association test (COWA) (Benton & Hamsher, 1976; Benton & Hamsher, 1989; Spreen & Strauss, 1991)**

The COWA test is considered an index of executive abilities (Pachana et al., 1996). It is a standardised test of verbal fluency and measures the ability to spontaneously generate words that begin with a certain letter within a set time. The COWA consists of three word-generating trials based on letter combinations, for example, ‘F’, ‘A’, ‘S’.

Several studies reviewed by Spreen and Strauss (1998) provide mixed results on age effects in the COWA, however, the reviewers report that, in general, age effects are shown only for people in the higher age ranges. Although Benton et al.’s (1981) original data corrected the scores of persons over 55 years by adding 3 points, Benton wrote later (1994) that performance on the COWA did not seem to change with normal ageing until the age of 80 years. His claim was supported earlier by a study investigating the effects of age, gender, verbal intelligence, and education on COWA scores in 199 healthy adults (age range 39-89 years) which found no age effects, but gender and cognitive effects; women outperformed men (Bolla et al., 1990). A glance at the table of normative data for COWA in Spreen and Strauss (1998) shows females score more than males in persons over 16 years and at all education levels.

The task has proven useful in detecting dementia (Eslinger et al., 1985) and in differentiating persons in the pre-clinical stages of SDAT and vascular dementia (Jones et al., 2006). Difficulty in generating words has been associated with PD (Dalrymple-Alford et al., 1994) and this finding has been corroborated by results from a longitudinal study (Azuma et al., 2003). Irrespective of cognitive ability, PD patients generate fewer words than SDAT patients (Stern et al., 1993b), with McKeith (2000a) reporting two thirds of PD clinic attendees having reduced verbal fluency scores. Results from a meta-analysis of PD patients’ performance on the COWA, however, concluded that although PD patients show impairment relative to controls, the effect of the difference for non-depressed, non-demented patients is small (Harrison et al., 2002c). The performance of PD patients with dementia,
however, is worse, even when age and MMSE status are accounted for (Bayles et al., 1993). A longitudinal study which showed verbal fluency is able to predict the onset of dementia a year later, (Jacobs et al., 1995) suggested the impairments in non-demented patients could, in fact, be attributed to patients with pre-clinical dementia.

ATD studies have reported equivocal results in COWA performances depending on the group being investigated. Studies with SDAT or remitted depressed patients have observed no significant effects of treatment on COWA performance (Porter et al., 2003b; Porter et al., 2005). Another study (Schmitt et al., 2000), found ATD improved the performance of healthy younger adults; given performance does not appear to change until the eighth decade, it may be that ATD would improve the performance of PD and DLB patients, and healthy older persons as well.

Procedure

The participant is instructed to listen to a letter read aloud by the administrator and then say as many words that begin with that letter as possible within a 60 s time frame. Using the letter ‘B’ as an example, the participant is asked not to produce proper nouns like “Brian”, “Brylcream”, or “Brisbane”, or to generate words with grammatical variations to a previously generated word like “beat” and “beating”. The participant is asked if the rules are understood and if not, the participant and administrator together generate new word examples of the non-permitted words beginning with the letter “B”.

Outcome Measures

**COWA Total** This is a tally of the words generated; a higher score reflects a better performance.

**COWA Verrors** This is a tally of violation errors, that is, proper nouns or grammatical variations; a lower score reflects a better performance, in that the participant is able to generate words that fit the inclusion criteria while ignoring words that are not permitted.

**COWA Rerrors** This is a tally of the word repetitions; a lower score reflects a better performance, in that the participant is able to generate words and keep track of words already generated.

**Digit Ordering Test (DOT)** (Werheid et al., 2002) (refer section 11.6.3.3)

The DOT is an analogue of the Digit Span test (see below). It consists of six sequences of digits increasing in span from three to eight digits. Each presentation comprises two different sequences of digits, one of which contains a repeated digit. The task uses the same rate and discontinuation rule (i.e., when two responses are incorrect, the task is aborted) as Digit Span, but differs with an instruction to order the digits. The participant is instructed to repeat each sequence of digits in ascending order immediately following its presentation by the administrator.

DOT is used as an auxiliary to the Digit span test because it is a relatively pure measure of the executive and manipulative components of working memory. PD patients have produced reduced
scores in DOT (Cooper et al., 1991), suggesting DOT may be sensitive to detecting working memory deficits in PD.

Procedure

After giving instructions and providing the participant with a practice, the administrator recites each sequence of numbers at a rate of one per second. The participant immediately repeats the sequence back to the administrator in ascending order.

Outcome Measures

**DOT Total** This is the total number of sequences the participant correctly recites in ascending order. It is scored out of 12; a higher score represents better performance.

**Digit Span (Wechsler, 1955, 1981) (refer section 11.6.3.2)**

Digit Span Forward (DigitsF) is used to assess short-term verbal and auditory memory and attention (Lezak, 1995) and by including an additional trial with an active manipulation of digits, Digit Span Backward (DigitsB), Digit Span also assesses working memory (Groth-Marnot, 2000). Each trial comprises seven pairs of random number sequences of increasing span (DigitsF: from three to nine digits; DigitsB: from three to eight digits). The WAIS-III (Wechsler, 1997) scores the trials per number of spans, that is, out of 14 for each trial.

A recent review of demographic data for the WAIS-III, reported strong declines in performance with age in Digit Span (Heaton et al., 2003). Spreen and Strauss (1998) reviewed studies having both large and small decrements in scores with increasing age, and the Table 11-9 in their book on p.155 shows a general decline in scores with age from 16 years to 95 years across all educational levels.

DigitsF is a measure of attention and is thus a useful assessment to use in PD (Lezak, 1995), and especially in PDD and DLB since cortical ACh activity has been correlated with Digit Span scores (Bohnen et al., 2006). ATD has induced significant impairments in Digit Span Forward and Digit Span Backward, irrespective of group (Porter et al., 2003b; Porter et al., 2005).

Procedure

After giving instructions and providing the participant with a practice, the administrator recites each sequence of numbers at a rate of one per second. The participant has two opportunities for each span length to repeat back a sequence in either the same order for DigitsF, or reverse order for DigitsB. The task is terminated when the participant fails two attempts to repeat or reverse the span.

Outcome Measures

**DigitsF Total** This is the total number of sequences the participant correctly recites in the same order as the administered order. It is scored out of 14; a higher score reflects better performance.
**DigitsB Total** This is the total number of sequences the participant correctly recites in reverse of the administered order. It is scored out of 14; a higher score reflects better performance.

**Modified Mini-Mental State examination (3MS) (Teng & Chui, 1987) (refer section 11.6.3.4)**

The 3MS is an assessment of general cognitive status. It comprises 15 items: Date and Place of Birth, Registration, Mental Reversal, First Recall, Temporal Orientation, Spatial Orientation, Naming, 4-Legged Animals, Similarities, Repetition, Read and Obey, Writing, Copying Pentagons, Three-stage Command, and Second Recall. It is used for diagnosis or research purposes because it is a reliable measure for discerning cognitive severity and cognitive change over time (Folstein et al., 1975; Tombaugh & McIntyre, 1992). Performance on 3MS was impaired after ATD compared to placebo in SDAT patients (Porter et al., 2000) and there was a significant interaction of group by ATD in a study of recovered depressed patients and controls (Porter et al., 2005). No effect of ATD has been observed in healthy older persons.

**Procedure**

The administrator asks the participant to provide autobiographical and current dates and locations, manipulate numbers and letters, memorise and repeat back a list of words, name two objects displayed by the administrator, generate lists of words from a category, recite and write a sentence, copy two interlocking pentagons, and follow a verbal command.

**Outcome Measure**

**3MS Total** Each item is scored according to a set schedule of scores. The item scores are summed to provide a total score out of 100; a higher score reflects better global cognitive status.

**Mini-Mental State examination (MMSE) (Folstein et al., 1975) (refer section 11.6.3.4)**

The MMSE is a quick and clinically relevant assessment of general cognitive status embedded in the 3MS. This means that both the 3MS and MMSE scores can be derived from a single administration (Tombaugh, 2005). One of the main purposes of the MMSE in medical and neuropsychological research is to measure change in cognitive status. Performance on this assessment was impaired after ATD in medicated but remitted depressed patients (Porter et al., 2005). No effect of ATD has been observed in healthy older persons.

**Procedure**

MMSE is administered with the 3MS.

**Outcome Measure**

**MMSE Total** Each item is scored according to a set schedule of scores. The item scores are summed to provide a total score out of 30; a higher score reflects better global cognitive status. A score of < 24 corresponds to dementia.
One Day Fluctuation Assessment Scale (ODFAS) (Walker et al., 2000) (refer section 11.6.3.5)

Fluctuating cognition (or alternatively labeled, confusion, consciousness, attention) is an important clinical feature of DLB, with a frequency rate of 80-90% (Byrne, 1996; McKeith et al., 1992b). The ODFAS is a scale designed to assess changes in consciousness over various observation points. It comprises seven items: Falls, Fluctuation, Drowsiness, Attention, Disorganised Thinking, Altered Level of Consciousness, and Communication. The ODFAS has validity against other electrophysiological and neuropsychological markers of fluctuation (refer Walker et al., 2000).

Procedure

A rater or informant assesses a patient’s consciousness across the day prior to assessment. It takes approximately 15 mins to complete the scale.

Outcome Measure

ODFAS Total Each item is scored in a binary manner based on whether it was observed during the observation period; plus, where appropriate, a grade (i.e., 1, 2, or 3) for severity level. Scores are summed to provide a severity score out of 21.

Rey Auditory Visual Learning Test (RAVLT): Recall trials only (Rey, 1964) (Refer section 11.6.3.6)

The RAVLT assesses immediate and delayed verbal recall, and verbal recognition memory. It also elicits retroactive and proactive interference tendencies with distraction and interpolated activity components (Lezak, 1995). The initial tasks take 15 minutes to administer with a delay task administered 30 minutes later.

Procedure

Participants are read a list (A) of 15 words at a constant rate and asked to recite these back to the administrator in any order. The list is repeated four times, the participant being asked on each occasion to recite as many words as possible. A distractor list (B) is read and participants asked to recite as many words as possible from this. Without repeating List A, the participant is asked again to remember as many words as possible from this list. After thirty activity-filled minutes, the participant is asked again to remember the words from list A. The RAVLT also comprises a recognition trial in which 50 words containing all items from both lists A and B, plus semantically and phonetically similar distractor words. The recognition trial is administered after the delay trial.

Outcome Measures

RAVLT Trials I - V, and Trial VI Each of these is a tally of the words the participant correctly recites from List A; a higher score reflects a better performance.

RAVLT List B This is the tally of the words the participant recites from the interference list; a higher score reflects a better performance.
**RAVLT Trial VII** This is a tally of the words recited from List A after a 30 min delay; a higher score reflects a better performance.

**Rey Visual Design Learning Test (RVDLT): Immediate recall trial only (Rey, 1964)**

The RVDLT is a brief assessment of immediate visual recall and recognition memory. It comprises 15 geometric forms, each on a stimulus card which the participant draws on completion of the series presentation.

**Procedure**

The procedure for this task matches that for the RAVLT except the participant is shown 15 geometric patterns printed on separate cards. They are presented at a rate of two seconds per card and at the end of each presentation the participant is asked to recall and draw as many patterns as possible. The procedure is repeated five times. Unlike the RAVLT, there is no distractor set of designs or a delayed recall, but there is a recognition trial in which participants are shown 30 designs and asked to indicate which comes from the set already viewed.

**Outcome Measures**

*RVDLT Trials I - V* This is a tally of the patterns recalled from the initial presentation of the stimulus patterns and correctly drawn for each of five trials is tallied: a higher score reflects a better performance.

**Verbal Fluency Performance Test (VFPT) (Bryan et al., 1997)**

The VFPT is a working memory task and is similar to the COWA except it requires words to be generated, not based on an initial letter, but excluding a specified letter. It relies on creative and strategic retrieval. Like the COWA, it requires speed of information processing as well as performance monitoring on the part of the participant, to check the rules are being adhered to.

**Procedure**

A participant is asked to generate as many words as possible in two 60 second trials that do not contain a specified letter. In the first trial subjects are required to produce as many words as possible not containing ‘E’ and, in the second trial, words not containing ‘A’.

**Outcome Measure**

*VFPT Total* This is a tally of the words generated across the two trials; a higher score reflects a better performance.

**Visual Analogue Scale (VAS) (refer section 11.6.4.1)**

It is known that side effects can occur from ingesting the amino acid drinks, but quantification of them is seldom published. Because they subside in one or two hours they do not affect assessment performances, however, they can be uncomfortable or unpleasant for the participant. Vomiting may
affect the depletion effect of ATD. Severe adverse effects may influence a participant’s premature withdrawal from the study.

The VAS is a subjective rating of behaviour, physical state, or both. Five states (i.e., Hunger, Sleepiness, Nausea, Happiness and Irritability) are rated by the participant. The VAS was positioned before the POMS at baseline (0 hrs), 4.5 hrs and approximately 6.5 hrs post treatment, because as a smaller task with a similar purpose and format, it was considered a practice and prompt for the longer POMS.

Procedure

The participant is asked to circle the number alongside each physical state that best describes how they are feeling “right now”.

Outcome Measure

Each item has a four-point Likert scale:

0 = Not at all; 1 = A little; 2 = Moderately; 3 = Quite a bit; 4 = Extremely.

Visual Object and Space Perception test (VOSP) (Warrington & James, 1991) (refer section 11.6.3.7)

The VOSP test comprises a number of tasks to assess visual perception. Perceptual functions include awareness, recognition, discrimination, patterning, and spatial orientation.

Patients with PDD and DLB have both been shown to have visuoperceptual deficits, with DLB patients faring worse than controls in the Fragmented Letter and Silhouette Identification tasks of the VOSP and significantly worse than SDAT patients on the Fragmented Letter task (Calderon et al., 2001; Mosimann et al., 2003).

Shape Detection Screening Test

The Shape Detection Screening Test is used as an initial screening and figure-ground perception task. It comprises a set of 20 black and white speckled patterns printed on white card in a flip-style booklet. Ten of the patterns have a degraded ‘X’ printed within a visually complex background. The remaining patterns do not have an ‘X’. It was included in the studies as a screening device.

Procedure

After the administrator has given instructions for this task, the participant is shown two practice items and asked to indicate whether there is an “X” or “no X” in each pattern. They are then asked to repeat this procedure for 20 more patterns.
Outcome Measure

*Total* This is a tally of correct responses to the degraded pattern; a higher score reflects better visual perception. The manual for this task recommends a cut-off score for pass-fail of 15 which means a participant scoring below 16 does not continue with the remaining VOSP tasks.

**Fragmented Letters**

Fragmented Letters comprises a series of 20 white cards in a flip-style booklet. Each card has a letter of the alphabet printed on it in black ink. This letter is degraded by 70%.

**Procedure**

After the administrator has given instructions for this task, the participant is asked to distinguish and name a degraded practice letter ‘B’ followed by a degraded practice letter ‘F’. These two practice letters are degraded by only 30% and the task is abandoned if the participant is unable to name or identify these practice items. The administrator follows this with 20 sequential presentations of different letters.

**Outcome Measure:**

*VOSP IncomLetters Total* This is a tally of the correct responses to the degraded letters; a higher score reflects a better visual perception and the manual for this task recommends a cut-off score for pass-fail of 16.

**Silhouette Identification**

Silhouette Identification comprises a series of animal silhouettes followed by a series of silhouettes of inanimate objects printed in solid black on white card in a flip-style booklet. The shapes in the silhouettes are stereotypical rather than realistic in style and are orientated at an oblique angle. There are 15 silhouettes in each trial and 30 items in total.

**Procedure**

After giving instructions for this task, the administrator asks the participant to identify (by name or with gestures) the animal or inanimate objects. The administrator presents 15 animals followed by 15 objects in a sequential manner with the participant making a response to each one in turn.

**Outcome Measure**

*VOSP Silhouettes Total* This is a tally of the correct responses to all silhouetted shapes; a higher score reflects better visual perception. The manual for this task recommends a cut-off for pass-fail of 5 failures in each silhouette type.
6.2.6 Physical State
Height, Weight, and General Wellbeing

Height and weight are recorded to use during the biological analyses and blood pressure is taken to check the participant’s general health at the commencement of the testing session.

Procedure

A booking phone call is made for the first test day to investigate any changes to the participant’s medical history since the screening session. Records were assessed at baseline on the first test day.

6.2.7 Screening
Cambridge Mental Disorders of the Elderly Examination (CAMDEX): Cognitive Section B (CAMCOG) (Roth et al., 1986)

CAMCOG forms a mini neuropsychological battery within the CAMDEX (Roth et al., 1986) interview. Along with a structured clinical interview and a structured interview with a relative or informant, it is used to diagnose mild cognitive impairment or dementia. It consists of 14 out of the 19 MMSE items plus 43 items covering additional aspects of cognitive function in the form of eight subscales which include: Orientation, Language, Memory, Attention, Praxis, Calculation, Abstract Thinking, and Perception.

Procedure

An experienced clinician or interviewer examines the participant on a number of cognitive tasks. Some of the responses are recorded so that spontaneous speech and content of language can be systematically assessed at a later time.

Outcome Measure

A cut-off score for CAMCOG is not recommended as screening for dementia is also dependent on age, gender, education, and social class (Huppert et al., 1995).

The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) (refer section 11.6.5.2)

The manual for the MINI states that it is a brief structured interview for the major Axis I psychiatric disorders in DSM-IV which can be used by either clinicians or lay interviewers. It is used in research to include or exclude potential participants with a major psychiatric disorder. It comprises a set of questions requiring yes or no answers in 16 categories each of which corresponds to a diagnostic category.
Procedure

The interviewer asks the participant a question from the assessment and ticks the appropriate ‘yes’ or ‘no’ box depending on the response. All questions must be rated and the interviewer may ask for examples to ensure accurate coding.

Outcome Measure

An interviewer with experience in diagnosing psychiatric disorders judges whether the participant has a disorder or not.

National Adult Reading Test (NART) (Nelson, 1982) (refer 11.6.5.3)

The NART provides an estimate of pre-morbid verbal intellectual (PVIQ) ability and Spreen and Strauss (1998) assert that it is one of the most reliable tests in clinical use. There is no significant difference in scores based on age or gender. It comprises a list of irregularly spelled (e.g., ‘ache’, ‘naive’, ‘thyme’) and generally unfamiliar words (i.e., ‘assignate’, ‘demesne’, ‘campanile’) which can only be pronounced correctly if someone has prior familiarity with them (Lezak, 1995). The words are short, so a participant does not have to analyse a complex visual stimulus. Also intelligent guesswork will not provide the correct pronunciation (Spreen & Strauss, 1991). It is used in research to match healthy controls with patients groups having cognitive impairment for IQ.

Procedure

The participant is asked to slowly and clearly read aloud, one word at a time, a list of 50 words from a laminated card, 25 words on each side, flowing over two columns on both sides of the card. Pronunciation guides accompany the test and these vary according to the language of the participant. Responses in Australia and New Zealand can be rated using the pronunciations given from the Macquarie University of Australia rather than in the NART manual.

Outcome Measure

PVIQ The number of incorrect pronunciations are tallied and converted to WAIS-R PIQ using a table provided in the manual.

6.3 Practice Effects

The CDR battery had a training session to avoid practice effects. This was administered within 30 m of the treatment on the first experimental day. It served to make the participants feel comfortable with the computer because many older people are not familiar with these devices. Some CANTAB and pen and paper tasks had practice sessions prior to each assessment. MOT, PRM, and SRM from CANTAB, plus some pen and paper, assessments however, did not. This raises the issue that performance on a task can vary depending on whether a person is unfamiliar or familiar with the task. That is, (a) the first session may have a novelty component, which either enhances or hinders performance or (b) ‘training’ from the first session enhances performance in the second session.
Practice sessions and reliable repeat versions of tests are employed to avoid these effects. Based on the experience of Porter (2003b) with SDAT patients, practice sessions for all cognitive tasks were not adopted because it was felt the protocol was already demanding and time consuming and that adding further sessions would likely make the study too taxing for dementia participants, thereby inserting a possible increase in dropout rates. Instead a number of measures were used to avoid a systematic bias of practice effects:

a. *Repeat testing procedure*. Reliable repeat test versions were used for CANTAB (DMS, PRM, SMTS SRM); CDR (IWR, DWR, DV, SRT, and CRT; COWA (FAS and CFL); Digit Span (Forward and Backward); DOT (DOT-A and DOT-B).

b. *Practice session procedure*. 3MS and MMSE did not have repeat test versions, but their administration at the screening session served as a practice session. All CDR tasks had a practice session as did some CANTAB tasks (DMS, SMTS, SSP, SWM).

c. *Statistical procedure*. Order was examined as a variable in the repeated measures analysis of variance to see if an interaction between treatment and order was present. Order was defined as the order of treatment administration, that is, placebo first or placebo second. Interaction effects mean the order in which the treatment is given interacts with the test day order to affect performance. That is, practice effects would be magnified if the ATD drink was administered on the second test day when learning and ATD combined: ATD affects learning. Conversely, attenuated effects would be magnified if ATD was administered on the first test day when the task was novel (Hughes et al., 2003).

### 6.4 Statistical Analysis

SPSS for Windows Release 13 (SPSS, Chicago, Illinois) was used for the statistical analysis. Before the analysis, all variables were examined for accuracy of data entry and missing values. The ‘Exclude cases pairwise’ option was chosen, so as to exclude a person only when data is missing for a specific analysis. Generally data were analysed using repeated measures analysis of variance (ANOVA) with ATD and placebo entered as within-subject factors and group or gender as between-subject factors. All other details for analysis are given in specific study chapters, e.g. sections 7.3.3, 8.5, and 9.3.2.

**Clinical and demographic variables** were investigated with a Mann-Whitney U test (for MADRS), chi-square test (for gender), and independent samples t-test (for age, PVIQ) to see if the groups in each study were significantly different on any of these variables. The level of significance was set at $\leq .05$ throughout, however significant results of $< .01$ and $< .001$ will be displayed in the tables.

**Assumptions** (a) The Levene’s test was used to check equality of variances in the independent samples t-test – when the significance value was larger than .05, equal variances were assumed but if it
was ≤ .05, it was not assumed – and the reported t-values reflected this; (b) The normal assumptions of a between-subject design for analysis of variance (normal distributions and homogeneity of variance) were observed; (c) In case the sphericity assumption (i.e., that the group correlations are the same across conditions in the within-group design) was not met, reported p values were corrected using the Huynh-Feldt correction factor. When the critical values in the $F$-table were too small this assumption is not met and there will be an increase in Type I errors. There are several ways of correcting for this, and the Huynh-Feldt correction was chosen because it is less severe and less conservative than the Lower-bound correction or the Geisser-Greenhouse correction, respectively.

**Bonferroni** corrects for multiple comparisons and takes into account the selected significance value. Given the large number of variables in Study B and Study C, there was a chance one of the variables would be significant by chance. As it was decided a priori to interpret the results only with respect to observed patterns, the Bonferroni was not used. This meant that some of the isolated results will not be interpreted.
7 STUDY A: A POOLED ANALYSIS OF THE EFFECTS OF ACUTE TRYPTOPHAN DEPLETION AND DOSE ON MOOD AND COGNITION IN THE HEALTHY ELDERLY

7.1 Introduction

The technique of acute tryptophan depletion (ATD) has been used in a number of studies to investigate the role of serotonin in mood and cognition in healthy young adults and in groups of patients with conditions involving abnormalities of the serotonergic system. A predictive pattern of mood effects has emerged from these studies with either resistance in healthy persons, mood lowering in persons at risk for depression, or a return of depressive symptoms in treated depressed patients. Studies investigating ATD in cognition in young adults, have consistently suggested ATD impairs verbal memory (Harrison et al., 2004; Kilkens et al., 2004; McAllister-Williams et al., 2002; Riedel et al., 1999; Schmitt et al., 2000; Sobczak et al., 2002d). There is little consistent evidence for a similar pattern in executive function. In fact, the opposite, with several studies reporting improved performances in focused attention and executive function (Coull et al., 1995; Gallagher et al., 2003; Murphy et al., 2002; Schmitt et al., 2000).

In contrast to studies in young adults, individual studies in older adults have not replicated the memory impairments but have demonstrated impairments in working memory (Porter et al., 2003b; Porter et al., 2005). Moreover, mood effects in younger persons with remitted depression were not replicated in older persons recovered from depression (Porter et al., 2005). These discrepancies raise questions about the resilience of the serotonergic system in older people. They also raise questions about the methodology of ATD studies. For example:

a. Heterogeneity and type II errors. Working memory may be impaired in groups of older persons but the samples used in previous studies have not been large – or homogeneous – enough to show this. The nature of cognitive variability in older, even healthy older, persons compared to younger adults (Ylikoski et al., 1999) means the likelihood of missing something in this older cohort is increased.

b. Restricted age ranges. Unlike some studies in younger persons (Booij et al., 2005b; Klaassen et al., 2002; Riedel et al., 1999), studies in older persons have had restrictive age ranges. This limits the inferences that may be made with respect to the age effect during ATD.

c. Different neuropsychological testing batteries being used in different studies. Most cognitive tasks are not necessarily function specific and may assess a number of functions at one time. For example, CANTAB’s SWM comprises planning, strategy, attention, perception, motor control, visuospatial memory, as well as working memory. Thus, differently named tests which purport to assess the same function may, in fact, be assessing different functions. This means making comparisons between studies is unreliable.
d. The psychometric properties of tasks. Different properties mean some tasks are able to pick up effects in the group being studied while others are not. It was previously hypothesised that a likely reason for the impairment in executive function and working memory tasks in older persons was because performance in these types of tasks is reduced in the older persons (De Luca et al., 2003) in general and, therefore, this group was more susceptible to pharmacological manipulation such as ATD.

e. Age-related changes being gender biased. The effects of ATD may be more pronounced in females who may have a higher turnover of 5-HT and a greater degree of reduction in 5-HT synthesis during ATD (Nishizawa et al., 1997). Given the effect that reduced oestrogen secretion at menopause may have on 5-HT function and on cognitive function (Schmitt et al., 2005; Sherwin, 1996) this is an important consideration when comparing groups across age and gender.

f. Studies varying according to the size and exact composition and quantity of amino acids used for the treatment. For example, studies have used doses of approximately 50, 70, 80, or 100 g and, while most ATD drinks comprised 15 amino acids (Young et al., 1985), others comprised only 7 (Neumeister et al., 2002) or 8 amino acids (Hayward et al., 2005). The degree of 5-HT depletion may therefore vary across studies.

It would be valuable to examine the effect of ATD in older persons in order to clarify some of the inconsistencies found relative to younger adults. Ideally, this would involve a fresh study using a large group of mixed gender participants from a wide age range. In this manner a comprehensive testing battery could be designed to observe behavioural effects in a large number of domains and a number of confounds would be controlled to avoid some of the methodological issues mentioned above. It was planned to do so (e.g. Study C) however, prior to commencing this, previously collected data was available to reanalyse in a pooled analysis. A pooled analysis is different to a meta-analysis. To answer the hypothesis, a pooled or mega-analysis combines the raw data from several studies into one analysis; a meta-analysis, on the other hand, aggregates studies and corrects for various statistical artifacts. A pooled analysis has been used previously with data from the present study, in order to examine the effects of ATD on episodic memory in one cognitive task (Sambeth et al., 2007). Age and gender were included in this study as between-subject variables. The main finding from that pooled analysis was that ATD – congruent with other ATD studies – was associated with impaired delayed word recall. However, it was also associated with impaired immediate recall and recognition. Moreover, the effects of ATD were more pronounced in females and, despite an age-related decline in cognitive performance, were independent of age. This suggests learning and memory deficits also occur in older persons after ATD.

Data existed from two studies using different ATD doses in older persons. Some of the assessments in these studies were the same and thus provided a pool of data with which to examine mood and
cognition. The present study pooled the data from these studies to investigate mood and cognition in healthy older persons and to specifically compare the effects of dose, gender, and age.

### 7.2 Aim

The aim of Study A was to extend the observations in younger adults by combining data to make a group of healthy older persons over 60 yrs of age. This pooled analysis had the advantage of using a larger set of data than had been used previously in the two individual studies, such that age and gender could be examined more robustly. It also had the advantage that a wider range of cognitive functions could be studied than in the analysis of Sambeth et al. (2007). It is known that ATD induces depressive symptoms in some groups of people; this susceptibility may extend to adults of older age. The larger data set meant mood and cognition could be investigated more robustly.

One further aim was to directly compare the effects of two different strength drinks. There have been reports of gastrointestinal complaints in some studies (Riedel et al., 1999) and it was thought an examination of the trade off in dose between lessening complaints and optimising treatment effects would prove valuable for further studies.

**Hypothesis 1** ATD would impair memory, particularly in females having the higher dose.

The rationale for this hypothesis was that tryptophan is important in the acquisition of new information; also, because females metabolise tryptophan differently from males, females will perform more poorly on the RAVLT after ATD than males, relative to placebo.

### 7.3 Method

Study A was a pooled analysis (i.e., mega-analysis) comprising data from the healthy control participants of two separate studies (study A1 and study A2): (A1) a study with SDAT patients published as Porter et al. (2000) and Porter et al. (2003b); (A2) a study with older persons recovered from depression published as Porter et al. (2005). The design of each study is outlined in section 6.1.

Both studies had received ethical approval from the Newcastle and North Tyneside Local Research Ethics Committee, Newcastle upon Tyne, United Kingdom.

#### 7.3.1.1 Screening

Recruitment, screening, and exclusion followed a similar procedure to Study B.

#### 7.3.1.2 Treatment and ATD Dosages

In Study A1, a 52 g amino acid drink was given to all participants. This was based on the finding that 5-HT receptor numbers decline with ageing (Meltzer & Reynolds, 1999), the hypothesis that this may indicate an increased sensitivity in older persons to the effects of ATD and the fact that tolerability of the 100 g drink had not been established. The 50 g drink was well tolerated so in Study A2, males received 104.4 g and females 83.3 g. Henceforth, the dose in Study A1 will be referred to as the low dose and that in Study A2 will be referred to as the high dose. This distinction is based on the weight
of the ATD mixture. Merens and van der Does (2007) have suggested using plasma TRP concentrations to distinguish the effects of a low from a high dose instead of using the amount and content of ATD mixture. These authors say this would avoid the confusion arising in studies reporting effects from a low dose when this dose was in effect a high dose. The present study aligns with the response of Cowen et al. (2007) in that individual variability and time of sampling may confound the use of plasma concentrations, and that the term low dose be used for doses substantially less than the conventional 80-100 g drink.

All amino acid drinks were mixed up with 300 ml water and flavouring. The preparation of the drink was as described by Schmitt et al. (2000).

Participants attended the research unit of the Stanley Research Centre, School of Neurosciences and Psychiatry, Division of Psychiatry, Leazes Wing, Royal Victoria Infirmary, Newcastle-upon-Tyne, UK.

In all other respects the Procedure was the same as outlined for the control participants in Study B, except the assessment battery was different.

7.3.2 Apparatus
7.3.2.1 Computer Hardware

PC with Windows 98 operating system; Touchscreen Magic Touch by Keytec, Inc.

7.3.2.2 Computer Software

Cambridge Neuropsychological Automate Battery (CANTAB): CANTAB for Windows (Robbins et al., 1994a)

7.3.3 Assessment

Adaptations or comments regarding the assessments are as follows.

7.3.3.1 Biochemical

Biological assessment followed the procedure outlined in Study B, except the last blood sample was taken at 7 hrs not 6.5 hrs post treatment.

7.3.3.2 Mood

Mood was assessed with the MADRS and with the same assessment scheduling as occurred in Study B (see section 8.5.2). A screening cut-off score of < 8 was used in Study A1 and < 10 in Study A2 for inclusion purposes.

7.3.3.3 Neuropsychological

For RAVLT, only the outcome measure Trials I-V was included, because data for Study A1 was incomplete.
7.3.4 **Assessment Order**

The following assessments were administered in the following sequences:

**Screening**

MADRS, CAMCOG; MINI; MMSE; NART.

**Test Days**

Rating: MADRS.

Neuropsychological: Study A1: 3MS; Digit Span Forwards and Backwards; MOT; SWM; RAVLT; SMTS: DMS; RVDLT; COWA; PRM; SRM. Study A2: 3MS; Digit Span Forwards; RAVLT; RVDLT (delayed recall and recognition trials omitted); SMTS; DMS; RAVLT (delayed); Digit Span Backwards; COWA.

7.3.5 **Analysis**

Biochemical, mood, and cognitive responses were analysed using the general linear model repeated measures analysis of variance (ANOVA) with treatment (ATD or placebo) as a within-subject variable. Age was entered as a covariate, as age differed slightly across the two studies.

Dose and gender were entered as between-subject variables. Order was not entered into the ANOVA for two reasons:

a. Although order has been examined in other studies, it was felt the inclusion of another factor, along with treatment, dose, and gender, would create a level of complexity that would have been essentially uninterpretable.

b. This study was a preliminary analysis leading to further studies, thus rationalising the exclusion of one potentially confusing factor.

The biochemical data is presented as a percentage change from baseline. Because one of the main outcome variables for this study was the effect of dose, the results are presented as the percentage difference in mean scores between baseline level and 4 or 7 hours. This format to display the results was a simpler way of presenting the results than histograms with eight bars each.

Mood data is also presented as percentage difference between baseline level and 4 or 7 hours.

Neuropsychological variables are presented as percentage difference between ATD and placebo.

7.4 **Results**

All participants fulfilled the criteria for the inclusion of healthy controls outlined in Study B.

Thirty six healthy participants volunteered for the two studies (the low-dose study, A1: n = 17; the high-dose study, A2: n = 19). All were aged between 60 and 81 years of age. One female withdrew from Study A1 during the first visit due to nausea and two participants (one male and one female)
from Study A2 declined to return after the first visit. Thus, 33 participants completed both experimental days, of whom 18 were male and 15 were female.

The mean age was 70.4 yrs (SD = 5.61, range = 60 - 81). Eight males and eight females had the low dose. Ten males and seven females had the high dose.

Comparison of demographic and baseline data between the two studies is shown in Table 7-1. Groups were well matched between studies except on age, which was significantly younger in the high dose study (t$_{31}$ = 3.05; p = .005).

### Table 7-1 Study A. Means and Standard Deviations for Demographic Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low dose study</th>
<th></th>
<th>High dose study</th>
<th></th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>73.13</td>
<td>4.9</td>
<td>67.82</td>
<td>5.1</td>
<td>*</td>
</tr>
<tr>
<td>PVIQ</td>
<td>107.8</td>
<td>8.4</td>
<td>112</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Baseline CAMCOG</td>
<td>99.2</td>
<td>3.5</td>
<td>99.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Baseline MADRS</td>
<td>1.94</td>
<td>2.2</td>
<td>1.06</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>n</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/f)</td>
<td>8/8</td>
<td>10/7</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; * = independent samples t-test; CAMCOG = Cambridge Mental Disorders of the Elderly Examination Cognitive Section; MADRS = Montgomery-Asberg Depression Rating Scale; PVIQ = predicted IQ (from the NART: National Adult Reading Test).

#### 7.4.1 All Measures

The significant main effects of treatment and interactions between treatment and other variables are shown in Table 7-2. The means and standard errors for all main effects and interactions are presented in Appendix G.

#### 7.4.1.1 Missing Data

Biochemical data from two participants were missing: one male having the low dose and one female having the high dose.

#### 7.4.1.2 Biochemical

There was a significant (a) main effect of treatment, (b) interaction of treatment by dose, (c) interaction of depletion by time, and (d) interaction of treatment by dose by gender on free TRP, as shown in Figure 7-1 and Table 7-2. The variable %change 0-4hrs, represents the percentage difference in free TRP levels between baseline and 4 hrs; the variable %change 0-7hrs, represents the percentage difference in free TRP levels between baseline and 7 hrs.
Figure 7-1 Change (%) in free TRP levels 0-4 hrs for gender and dose.

Table 7-2 Study A. Effects of Treatment (ATD vs Placebo on All Variables)
Repeate d Measures ANOVA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Treatment</th>
<th>Treatment</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>x Dose</td>
<td>x Gender</td>
</tr>
<tr>
<td>Free TRP</td>
<td>1.26</td>
<td>0.01 ***</td>
<td>34.72 ***</td>
<td>1.51</td>
</tr>
<tr>
<td>Free TRP</td>
<td>1.26</td>
<td>1.36 ***</td>
<td>16.05 ***</td>
<td>0.61</td>
</tr>
<tr>
<td>MADRS</td>
<td>1.28</td>
<td>0.03</td>
<td>1.51</td>
<td>0.00</td>
</tr>
<tr>
<td>MADRS</td>
<td>1.28</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>3MS</td>
<td>1.28</td>
<td>0.03</td>
<td>0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>DigitsF</td>
<td>Total</td>
<td>1.24</td>
<td>0.33</td>
<td>2.20</td>
</tr>
<tr>
<td>DigitsB</td>
<td>Total</td>
<td>1.28</td>
<td>2.13</td>
<td>0.99</td>
</tr>
<tr>
<td>SWM</td>
<td>Between</td>
<td>1.28</td>
<td>1.20</td>
<td>1.25</td>
</tr>
<tr>
<td>VFPT</td>
<td>1.28</td>
<td>0.12</td>
<td>0.58</td>
<td>0.79</td>
</tr>
<tr>
<td>COWA</td>
<td>1.28</td>
<td>0.03</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>RAVLT</td>
<td>Trials I-V</td>
<td>1.28</td>
<td>0.96</td>
<td>0.85</td>
</tr>
<tr>
<td>RVDLT</td>
<td>Delayed</td>
<td>1.28</td>
<td>1.88</td>
<td>0.00</td>
</tr>
<tr>
<td>SMTS</td>
<td>Trials I-V</td>
<td>1.28</td>
<td>0.49</td>
<td>1.85</td>
</tr>
<tr>
<td>SMTS</td>
<td>Accuracy</td>
<td>1.28</td>
<td>0.49</td>
<td>0.42</td>
</tr>
<tr>
<td>SMTS</td>
<td>Latency</td>
<td>1.28</td>
<td>0.72</td>
<td>2.59</td>
</tr>
<tr>
<td>DMS</td>
<td>Accuracy</td>
<td>1.28</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>DMS</td>
<td>Latency</td>
<td>1.28</td>
<td>2.67</td>
<td>2.15</td>
</tr>
</tbody>
</table>

* p ≤ .05, *** p < .001.

Note: COWA = Controlled Oral Word Association test; DigitsB = Digits Span Backward; DigitsF = Digit Span Forward; DMS = Delayed Matching to Sample; 3MS = Modified Mini Mental State examination; MADRS = Montgomery-Asberg Depression Rating Scale; RAVLT = Rey Auditory Verbal Learning Test; RVDLT = Rey Visual Design Learning Test; SWM = Spatial Working Memory; SMTS = Simultaneous Matching to Sample; TRP = tryptophan.

7.4.1.3 Mood

There was no significant main effect of treatment on MADRS, nor interactions of treatment by time, or any other variable. Only statistically significant results for individual assessments are referred to in the text.
7.4.1.4 Neuropsychological

The significant main effects of treatment and significant interactions of treatment with gender and dose are shown in Table 7-2. There were no main effects of treatment on the neuropsychological variables. There were no independent effects of age, significant interactions of age by treatment, or 3-way interactions of age by treatment by dose, or gender.

Memory

There was a significant interaction between treatment, dose, and gender on RAVLT I-V ($F_{1,28} = 5.74$, $p = .024$). A marked reduction in performance during ATD was in the females receiving the high dose, while the other three conditions revealed no clear changes (Figure 7-2).

![Figure 7-2 Study A.Difference between treatment scores for gender and dose on RAVLT Trials I-V.](image)
There was a significant interaction between treatment, dose, and gender on SMTS Accuracy \((F_{1,28} = 5.66, p = .024)\). While there was a reduction during ATD in females, but an increase in males, receiving the high dose, there was also an increase in females, but no change in the males, receiving the low dose (Figure 7-3).

![Figure 7-3 Difference between treatment scores for gender and dose on SMTS Accuracy.](image)

**Working Memory**

There was a significant interaction between treatment, dose, and gender on DigitsF \((F_{1,24} = 4.20, p = .05)\). The greatest reduction in performance during ATD was in the females receiving the high dose, but females given the low dose showed an increase on this measure (Figure 7-4).

![Figure 7-4 Difference between treatment scores for gender and dose on DigitsF.](image)
Executive Function

There was a significant interaction between treatment, dose, and gender on the COWA ($F_{1,28} = 4.40, p = .045$). Females receiving the high dose and males receiving the low dose had a lower score during ATD, whereas the high dose males and the low dose females showed no marked change (Figure 7-5).

![Figure 7-5 Difference between treatment scores for gender and dose on COWA.](image)

7.5 Discussion

Study A was the first to examine the effects of ATD on mood and cognitive function in a group of healthy older persons. A range of assessments were administered to 33 healthy older persons in two separate studies; one using a low dose (52 g) and one using a high dose (104 g for males and 83 g for females); 18 of the participants were male and 15 were female. The data from these studies were pooled to form one set of results.

The principal findings for the present study were:

a. A reduction in free TRP that was similar in males and females with both doses. However, the increase in free TRP following placebo was greater following the higher dose, while the increase following the low dose placebo was present in females only, as shown in Figure 7-1.

b. No significant effects of treatment on mood.

c. A differential effect of dose and gender on a number of neuropsychological measures: DigitsF, total words recalled on the RAVLT trials 1-V, SMTS, and COWA. The pattern of this interaction in DigitsF and RAVLT was the same, with the greatest difference between ATD and placebo being in females having the higher dose; in each case scores were lower during ATD, as shown in Figure 7-2 and Figure 7-4. There
was an increase in scores on SMTS for females receiving the lower dose. There was a worsening of scores on COWA in females receiving the higher dose but this was not as great as that seen in males receiving the lower dose.

7.5.1 Biochemical
There was an interaction of treatment by dose by gender on free TRP levels. This was probably accounted for by the greater increase in free TRP level in males receiving the high dose placebo drink and the most likely explanation for this finding is that males in Study A2 received the largest drink (104.4 g) and therefore the largest dose of TRP (see section 7.3.1.2). This suggests the males did not compensate for the greater TRP load in the placebo drink with a more rapid peripheral metabolism, or any other factor. The fact that free TRP was equally reduced in males and females receiving either dose of the TRP free mixture suggests that in these cases, peripheral protein synthesis during the procedure could only increase by a certain amount and thus reached a ceiling above which it would not increase, regardless of the additional load of amino acids. However, it is possible that the larger drink may have enhanced the competitive advantage of the other large neutral amino acids (LNAAAs) in crossing the blood brain barrier. The competitive rivalry between LNAAAs for a transport molecule is one of the mechanisms that underpin the ability of ATD to reduce 5-HT levels in the brain. If more amino acids were crossing into the brain at the expense of TRP, there would be less TRP in the brain available for 5-HT synthesis. Unfortunately LNAAAs were not measured in this study, so dose comparison of the TRP/LNAA ratio is not possible.

7.5.2 Mood
Congruent with results from studies in healthy younger adult participants, mood was not significantly altered by ATD in healthy older persons of either gender. This indicates that despite changes in the serotonergic system which may occur as part of the ageing process, older persons are no more vulnerable to the mood effects of ATD than healthy younger persons, suggesting no particular increase in 5-HT–mediated vulnerability to depression.

7.5.3 Cognitive
The most significant finding for the neuropsychological hypotheses was that episodic memory and working memory were both impaired during ATD but in a domain, dose and gender dependent manner; females having the high dose were more impaired, generally across tasks, but sometimes there was a relative improvement in females (e.g., DigitsF, SMTS) when a low dose was used. An unexpected finding was the worsening of executive function from the males having the low dose. These results, along with the other significant result – that females receiving the low dose improved in visual memory – are discussed forthwith.

A similar pattern of response was observed in the RAVLT immediate word recall and DigitsF tasks, suggesting a role for gender effects in memory and working memory. In both domains, the greatest effect was an ATD-induced reduction in the scores of females having the higher dose. The significant
gender effect is in keeping with previous studies which suggest females have a greater vulnerability to
the behavioural effects of ATD (Booij et al., 2002; Sambeth et al., 2007). A recent pooled analysis
(including the RAVLT data from this study) showed the effect of ATD in immediate word recall was
greater in females and that this effect occurred irrespective of age (Sambeth et al., 2007). The possible
explanation for this risk may be inherent since several neuroimaging studies have demonstrated males
have an approximately 50% greater rate of 5-HT synthesis (Nishizawa et al., 1997; Okazawa et al.,
2000; Sakai et al., 2006), while females have a greater concentration of 5-HIAA in the cerebrospinal
fluid (Asberg et al., 1976; Traskman et al., 1981); the inference being that females have a higher
turnover of 5-HT. The findings in the present study suggest – although reduction in free TRP levels
did not vary with gender – females may be more vulnerable to the effects of ATD. Also, although TRP
levels were the same for both doses, it is likely the extra load of amino acids was not mopped up into
protein synthesis or the LNAAs (including TRP) from the extra load were not being catabolised in
some way.

An effect on immediate word recall despite the absence of an independent effect on delayed word
recall suggests an effect on learning – encoding and initial consolidation. There are three stages in
memory: encoding, consolidation and retrieval, with serotonin having a particularly sensitive role in
the initial stages (Buhot et al., 2000). This has been demonstrated consistently in a number of studies
when ATD was associated with impaired performances in delayed verbal memory tasks (Harrison et
al., 2004; Riedel et al., 1999; Schmitt et al., 2000; Van der Veen et al., 2006). The recent pooled
analysis of Sambeth et al. (2007) found an overall effect on recall performance on the first repetitions
of word lists, that is, immediate memory, which the authors suggested was possibly attributable to
impaired consolidation, as well as encoding. This is because the presentation of each word list
occurred outside the 30 s duration expected to account for encoding (for definition see, Izquierdo et
al., 1999). The effect of ATD on both immediate and delayed aspects of verbal memory seems to
occur at the encoding and consolidation stages and thus involves the medial temporal lobes, including
the hippocampus (Kohler et al., 2000; McAllister-Williams et al., 2002; Van der Veen et al., 2006).
The findings in the present study on immediate verbal memory accord with this proposal.

However, another possibility is that, since the word list is repeated before each trial, scores for the
RAVLT Trials I-V could also be assessing working memory. In juxtaposition with an effect on
DigitsF, the RAVLT result may mean that alternatively, or additionally, ATD impairs working
memory. The multiplicative nature of many neuropsychological tasks means it is sometimes difficult to
tease out the exact mechanism being measured by a task. To date, the only ATD studies showing
effects on working memory have been the two from which the present data were sourced. In
cognisance of this and the more recent literature on immediate verbal memory (Hayward et al., 2005;
Sambeth et al., 2007), it seems more likely the effects on RAVLT are related to verbal memory.

Previous studies have repeatedly demonstrated an effect of ATD in delayed verbal recall. No such
effect was observed in the present study. In particular, there was no effect of ATD on delayed recall of
words, a variable that has been consistently impaired in previous studies with younger adults. Perhaps the most likely reason for the non-significant finding is that the performance of older persons on this measure is more variable than that of younger adults, the implication being that the increased variance made statistical significance less likely. The absence of an interaction of ATD by age may be explained simply by the relatively narrow age range. As such the study does not answer the question of whether normal ageing poses an increased vulnerability of the 5-HT system to be challenged in this manner. The pooled analysis of Sambeth et al. also did not find an interaction between ATD and age on word learning, which suggests ageing may not affect the response to ATD. However, this really needs to be examined empirically and with a number of tasks assessing a broad range of cognitive functions in an older group of variable age.

7.5.3.1 Visual Memory

It is difficult to interpret the result on SMTS, which is contrary to that generated in the aforementioned assessments. The finding is particularly problematic because ATD studies have not generally demonstrated an effect of ATD on short-term memory in younger adults and when an effect has been found – as in the Sambeth et al (2007) study – it is one of impaired not enhanced performance. This finding is difficult to explain and may represent an anomaly, which may not have occurred if the Bonferroni correction had been used.

7.5.3.2 Executive Function

The pattern of performance on COWA is likewise difficult to interpret. Participant performances in previous ATD studies have been equivocal; some studies have demonstrated improvement (Riedel et al., 2002b; Schmitt et al., 2000; Stewart et al., 2002), but others have found no change (Allen et al., 2006; Gallagher et al., 2003; Hughes et al., 2003; Porter et al., 2003b; Porter et al., 2000; Porter et al., 2005). COWA is an executive function task and thus assesses the integrity of the PFC; the consensus from the literature review (see section 4.3.3.3) is that ATD does not generally affect performance in frontally guided tasks. Because the outcome measure for this task was the difference between ATD and placebo scores, the greater impairment in males receiving the low dose may have been driven by either the ATD or the placebo treatment. Thus, the interaction effect on this task may present an anomaly for the same reasons noted in section 7.5.3.2.

7.5.4 Strengths and Critique of Present Study

The potential advantages for the present study were firstly, that a larger number of subjects allowed analysis of factors such as gender with less risk of type II error and secondly, that in using two different dosages in similar groups within the same setting, the effects of amino acid dose were able to be analysed and compared. However, there are some important limitations to this study which need to be addressed. Specifically,

a. Although the technique of pooling data from separate studies is useful, there are a number of factors not taken into account in the statistical analysis which may
confound the result. This was that the variable dose may have been confounded with study. That is, the different characteristics of the studies: duration of testing schedule; ordering of the assessments within the schedule; time of year; and physical, social and personality variables. For example, participants in Study A1 were administered tasks of attention and memory that were not administered in Study A2.

b. The loss in statistical power occurring as a result of interaction effects. Dose was found to be a determining factor in ATD effects thus differentiating the groups based on dose limited the sample size and immediately negated any power from the larger sample size.

c. The low and high dose groups were not matched a priori on all important variables. Potential confounds were identified and countered as follows. First, differential group baseline performances in cognitive status: groups were matched on CAMCOG scores plus possible differences were taken into account by the within-subject design. Second, a significant difference between mean group age: age was entered as a covariate in the statistical analysis; however in practical terms, the difference was small (i.e., five years) and there were no age effects associated with any of the cognitive variables.

d. The age range was restricted to 21 years. One study in remitted depressed patients included people from an 18 - 65 year age range and it would be preferable to have an equally wide range from an older group, especially given the greater likelihood of age differences between the young-old and the old-old (Hedden & Gabrieli, 2004).

e. LNAA levels were not measured and, therefore, the TRP/LNAA ratio was not calculated. TRP competes with other LNAAAs to enter the brain via a specific transport molecule and the ratio is vital in determining 5-HT synthesis. It is likely, despite similar TRP concentrations, that the higher dose reduced central TRP to a greater extent than the lower dose. However, since no assessment of the LNAAAs was made, this can only be inferred.

f. The neutrality of the placebo drink; that is, the greater free TRP level in males having the high dose placebo drink. In a study using the same technique in the same centre, the TRP/LNAA ratio was calculated in adults suffering from schizophrenia who had received 104.4 g drinks of identical composition. There are differing effects of placebo on the TRP/LNAA ratio and the likelihood that these will not lead to an over- or under-estimation of ATD effects is discussed in section 3.5.3. The balanced amino acid placebo drink is probably a neutral manipulation; however, this does not necessarily reflect the situation in the present research.
Study A analysed a large number of neuropsychological variables and did not use a correction for this in order to determine whether a domain specific pattern emerged, as outlined in section 6.4. The findings on DigitsF and RAVLT may constitute a pattern which is consistent within this study and within the literature. The findings on COWA and SMTS, however, are not consistent with the pattern in this study, nor are they consistent with the literature.

7.6 Summary
This was the first study to examine the effects of ATD, at variable doses, in a group (n = 33) of healthy older participants. The study examined a number of methodological inconsistencies arising from previous studies while investigating the interactions of treatment with age and gender. The main finding, that females receiving a high dose of ATD have impaired memory, may be attributable to the encoding and consolidation stages of memory. Although the findings for other neuropsychological variables were largely non-significant, the results of this study encouraged a further investigation of the effect of ATD in older persons, in particular older females. This investigation would include a larger number of each gender in order to effect more robust comparisons. The treatment dose would be driven by the results from the present study, as would the selection of assessments. These would include a broader range of mood and cognitive assessments. It is germane that such a study be completed because – as the results of this small study demonstrate – the response of older persons to ATD is not the same as that for younger adults.
8 STUDY B: THE EFFECTS OF ACUTE TRYPTOPHAN DEPLETION ON MOOD, MOVEMENT AND COGNITION IN PARKINSONS’ DISEASE

8.1 Introduction

The serotonergic system has been investigated in Parkinson’s disease (PD) but the precise nature of this role is unclear (for review see, Scholtissen et al., 2006c). It is hypothesised alterations in this system may be involved in some of the behavioural symptoms experienced by patients with this condition (Hanagasi & Emre, 2005; Leentjens et al., 2006). Serotonergic hypofunction may arise as an adaptive response to lowered DA levels to maintain the homeostatic ratio of these two neurotransmitters (Mayeux, 1990). A consequence of this may be an increased risk for depressive symptomology. Conversely, a relative hyperactivity in the serotonergic system unadjusted to reduced cholinergic ratio may be associated with cognitive deficits.

PD is characterised by a number of severe and subtle biological changes that manifest in overt ways of behaving. The hallmark of PD, in pathological terms, is the severe reduction of dopaminergic neurons and the downstream function arising in brain regions served by these neurons. DA levels in the basal ganglia have been measured post-mortem and correlated against motor behaviour so that a 60-80% reduction in cells in the substantia nigra pars compacta leads to a 95% reduction of DA in the putamen (Brooks & Piccini, 2006). In living patients with early-in-the-course PD, the onset of motor symptoms corresponds to a 50% loss of DA terminals in the putamen manifesting in dysfunction in the motor cortex and anterior cingulate cortex (Brooks & Piccini, 2006; Morrish et al., 1995). Other neuropathological features of the disease which contribute to the behavioural changes include the number and location of Lewy bodies, amyloid plaques and neurofibrillary tangles.

Neuroimaging data have demonstrated that the correlation of neuropsychological performance and DA levels in the caudate nucleus – the structure in the basal ganglia that projects DA to the frontal cortex – is crucial to performance in executive function, spatial working memory and immediate verbal memory tasks (Rinne et al., 2000). Although this does not exclude other more generalised cognitive impairment in PD, motor and depressive symptomology also suggest early-in-the-course PD may not be confined to a lesion model of basal ganglia DA levels. For example, clinical evidence points to a possible role of 5-HT in (a) movement, to the extent that tremor scores have been correlated with reduced 5-HT\textsubscript{1A} receptor binding potential in the dorsal raphe nuclei (Doder et al., 2003) and some depressed PD patients develop extrapyramidal symptoms when treated with SSRIs (Dell’Agnello et al., 2001), also that 5-HT\textsubscript{2} antagonist are used to treat psychosis because they are associated with fewer extrapyramidal side effects than other antipsychotic medications (Cunningham Owens, 1999); and (b) mood, to the extent there are abnormalities in the serotonergic systems (Kish, 2003) plus a high prevalence of major depression in PD (Sano et al., 1990), and (c) possibly cognition, to the extent changes in 5-HT activity may impact on the release of neurotransmitters in severely compromised dopaminergic and cholinergic systems (Court & Perry, 1991).
The biological basis for depression in PD is uncertain, but because the natural history of depression does not parallel the progression of physical symptoms it has been suggested vulnerable patients may be susceptible to an independent process of depression (Remy et al., 2005). It begs the question also, whether vulnerable individuals are susceptible to developing both depression and PD, because two Danish register studies of 164,385 and 211,245 patients, respectively, found PD patients were more likely to develop depression than their counterparts with other chronic and disabling diseases, regardless of age or gender. Moreover, people hospitalised with major depressive disorder have over twice the likelihood of developing PD as osteoarthritis (Nilsson et al., 2001; Nilsson et al., 2002). There is a common pathophysiological pattern between depression and PD or a sensitivity of the same individuals to develop these conditions.

Neuropathological differences have been observed between depressed and non-depressed PD patients, with depressed patients having greater frontal lobe dysfunction and more extensive involvement of dopaminergic, noradrenergic and serotonergic systems (Cummings & Masterman, 1999; Mayberg & Solomon, 1995; Tandberg et al., 1997). All three monoamine neurotransmitter systems are innately and intimately connected and disruption in the serotonergic system (see section 5.11), either on its own or in conjunction with the dopaminergic system, may be a contributing factor to some of the behavioural changes that occur in PD (Cummings & Masterman, 1999).

Serotonin activity is reduced in PD as a consequence of neuronal degeneration, reduced 5-HT and 5-HIAA concentrations, possible slightly reduced SERT binding potential, and alterations in tryptophan hydroxylase activity and receptor activity (Kish, 2003). The integrity of this system may be challenged even more in patients with depression (Mayeux et al., 1988), as evidenced by the large number of studies showing reductions in CSF levels of 5-HIAA in depressed but not non-depressed patients (for list see, Mayberg & Solomon, 1995).

A serotonergic hypothesis has been proposed for depression in PD (Mayeux, 1990) suggesting reduction in serotonergic tone as a compensatory adaptation to reduced dopaminergic activity (Mayeux et al., 1984). Leentjens et al. (2003) suggest this is a plausible explanation for the development of depression so commonly preceding PD (Starkstein & Merello, 2002; Starkstein et al., 1990b) because there are compensatory mechanisms already operating long before the manifestation of clinical symptoms. However this hypothesis has yet to be established.

An extension of the hypothesis could be made into the cognitive domain based on the proposed role of 5-HT in learning and memory (McEntee & Crook, 1991). Most people with PD have a mild cognitive impairment which may go on to dementia (Aarsland et al., 2003a). This is especially so for patients having a history of major depression (Starkstein et al., 1990a; Starkstein et al., 1992). Cognitive impairments have been linked to degeneration in the cholinergic system – which in PD is more severe than the degeneration seen in Alzheimer’s disease (SDAT) (see section 2.3.1.1) – and although the pathological aetiology of PD is well recognised, the explication of the link between ‘hardware’
malfuction, neurotransmitter activity and behavioural outcomes is still limited (Brooks & Piccini, 2006).

It is possible 5-HT’s role in inhibiting ACh may account for the cognitive deficits when the cholinergic system is compromised as occurs in neurodegenerative disorders such as SDAT and PD (see Figure 5-1). It is expected that reductions in 5-HT level – and as a consequence the modulation of the transmitter on the cholinergic system – may improve cognition. Previous evidence in SDAT patients (Porter et al., 2003b; Porter et al., 2000) has not supported this and thus the issue needs to be re-examined in a group having a severe cholinergic deficit, and in healthy controls.

There is no current medication for effective treatment of cognitive deficits in PD. Cholinesterase inhibitors are the most widely used strategy in SDAT and are generally well tolerated by PD patients, but more evidence is required regarding their efficacy in PD (for a review of studies see, Aarsland et al., 2004b). There is considerable research into the role of the serotonin system in animal models of cognitive dysfunction and ongoing efforts to develop agents acting on the 5-HT system which may be therapeutic in these conditions. In particular, 5-HT1A antagonists have been trialed but as yet none are clinically available (Schechter et al., 2002).

Changes to the dopaminergic, noradrenergic, serotonergic and cholinergic systems may be responsible for motor symptoms, depressed mood and cognitive deficits in PD. Serotonin has an inhibitory action on these neurotransmitter systems (Robbins, 1997) which may become hyperactive if degeneration in the other systems outstrips the capacity of mechanisms to correct themselves. If homeostatic ratios between 5-HT and DA, ACh, or NA were able to be re-established, it may be that a restored balance would manifest as improved movement, mood or cognitive outcomes. One way to investigate this is to lower 5-HT levels with acute tryptophan depletion (ATD) and observe the behaviours affected by this challenge.

8.2 Aim

The aim of the present study was to investigate the effect reduced 5-HT activity would have on movement, mood, and cognition in Parkinson’s disease. The reduction was effected experimentally using the technique of acute tryptophan depletion. The behaviours were assessed by a number of standardised assessments of movement, mood and cognition. The hypotheses for the study were:

**Hypothesis 1** ATD would improve aspects of motor function in PD patients compared to healthy controls.

The rationale for this hypothesis was that serotonergic enhancing agents (e.g., SSRIs) induce parkinsonism, while agents that inhibit serotonergic activity (e.g., olanzapine) reduce the incidence of extrapyramidal symptoms such as bradykinesia and rigidity.
**Hypothesis 2** ATD would lower mood in PD patients compared to healthy controls.

The rationale for this hypothesis was based on experimental evidence that ATD induces significant mood symptoms in people who are vulnerable to depression (Booij et al., 2003). People with PD have a disease-related vulnerability to major depression, thus predisposing them to mood lowering effects during ATD. This was demonstrated in one PD patient with remitted depression who experienced a return of depressive symptoms during ATD (McCance-Katz et al., 1992).

**Hypothesis 3** ATD would worsen global cognitive status in PD patients compared to healthy controls.

The rationale for this hypothesis arose from a study investigating ATD in SDAT (Porter et al., 2003b). The authors of this study attributed this effect to the severe cholinergic deficit observed in these patients. If this is the case, then ATD is likely also to worsen global cognitive function in patients with PD because this patient group has a more severe cholinergic deficit than the group having SDAT. Hence the study was powered in order to detect a clinically significant change in global cognitive status (see section 8.5.6.1).

### 8.3 Method

Two groups were studied: one group of patients with PD and one group of healthy age, gender and IQ matched control participants.

The study received ethical approval from the Canterbury Ethics Committee, Christchurch, New Zealand.

#### 8.3.1 Screening

Healthy controls were recruited via a letter (Appendix A) sent to organizations, clubs, and societies having older persons as members. The list of these clubs was sourced from the Christchurch City Council website. Letters sent to these groups were accompanied by a short media release (Appendix B) which could be inserted into a newsletter or newspaper. The media release was published in two newspapers (Mace, 2005a, b). Volunteers were also recruited from an existing volunteer database held at the Van der Veer Institute and by word of mouth.

Parkinson’s disease patients were recruited by specialist neurologists in Christchurch (e.g., Professor Tim Anderson and colleagues). All patients met the United Kingdom Parkinson’s Disease Society Brain Bank Criteria as per Hughes et al. (1992) and had had a physical examination and the usual neurological investigations within the past year.

Each volunteer was sent an Information Sheet (Appendix C) explaining the rationale for the study and what was involved with testing. Upon reading the Information Sheet, all volunteers were asked to phone the administrator who arranged a home visit in order to – among other things – ascertain the volunteer’s suitability for the study. Demographic data, medical history and a range of screening assessments were completed with the volunteers, at which point they were asked to sign a consent form (Appendix D).
Educational level was also established at this time. Because many participants in the oldest age groups had left school at an earlier age or attended classes / obtained qualifications that did not match those in the younger age groups it was decided to use the model proposed by Willshire et al. (1991). Instead of years of education, the model categorises educational status as shown in Table 8-1.

Table 8-1 Description of Education Levels

<table>
<thead>
<tr>
<th>Educ Stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Some primary school</td>
</tr>
<tr>
<td>2</td>
<td>Some secondary school</td>
</tr>
<tr>
<td>3</td>
<td>Some secondary school + trade qualification</td>
</tr>
<tr>
<td>4</td>
<td>Secondary school completed</td>
</tr>
<tr>
<td>5</td>
<td>Tertiary education begun</td>
</tr>
</tbody>
</table>

8.3.1.1 Screening Exclusion Criteria

Personal Medical History

It was important to exclude participants with serious health issues for safety reasons, but also because some have specific associations with the serotonergic system. These include: (a) a history of affective or other psychiatric disorder, because most incriminate the 5-HTergic system in some way, (b) eating disorders, because they are associated with 5-HT hypofunction (for review see, Naughton et al., 2000), (c) nutritional deficiency e.g., pellagra, because of the aetiological deficiency of TRP metabolite, niacin, (d) coeliac and Crohn’s disease because, nutrient absorption from the gastrointestinal tract is impaired and depression is a common co-morbidity (refer, Sainio et al., 1996 for discussion), (e) other serious gastrointestinal dysfunction, because 5-HT is linked to motility and anxiety in IBS (Kilkens et al., 2004; Shufflebotham et al., 2006), (f) endocrine dysregulation, because cortisol induces the TRP catabolic enzyme (Altman & Greengard, 1966), (g) HIV, because some symptoms are linked to low TRP levels (Fuchs et al., 1990), (h) serious cardiovascular disease, because there is a link between platelet dysfunction, depression, and cardiovascular disease (Musselman et al., 2002; Nemeroff & Musselman, 2000), (i) diabetes, because metabolism is altered in diabetes (Allegri et al., 2003), (j) kidney disease, because the kidneys are involved in TRP metabolism in two ways: they eliminate TRP derivatives on the one hand, and they produce several enzymes taking part in TRP metabolism mainly via the kynurenine pathway on the other, (k) alcoholism, because 5-HT modulates mood and alcohol urges in people with alcohol dependence (Pierucci-Lagha et al., 2004) plus alcoholism is associated with damage to serotonergic neurons (Chen et al., 1991; Halliday et al., 1993), and (l) drug addiction, because some recreational drugs have 5-HT effects e.g., MDMA, LSD.

Family Medical History

Family history was considered only as far as mood was concerned. No volunteer was excluded on the grounds of having a first degree relative (i.e., parent, sibling, or child) with major depression.
Personal Cognitive History

All volunteers were excluded if they had a MMSE score of < 27. In a key paper focusing on the MMSE in geriatric psychiatry, Brayne (1998) wrote that, because each population’s performance was closely linked to education and cultural context, a uniform cut-off point in the MMSE was not possible for older populations. Instead, a variety of cut-off points are suggested depending on the context, for example, 17/18 for clear-cut cases, 21/22, 23/24, and even 25/27. The cut-off point in the present study was based on the criteria used by Aarsland et al. (2002) who used a 16 - 26 for their study with cognitively impaired PD patients; also by Volkow et al. (2002) who excluded control participants with MMSE scores < 27 for their PET study investigating brain metabolism in SDAT patients.

Personal Medication History

To avoid possible confounding of the results, all volunteers were excluded if they were currently taking, or had ever taken, serotonergic medication. Studies have found that prior exposure to an antidepressant SSRI is likely to predict mood response to ATD in depressed people (Booij et al., 2002) and although this was not demonstrated in healthy persons (Barr et al., 1997), it was felt prudent to exclude people with this medication history. No volunteer was excluded for taking non-serotonergic medication, including anticholinergic. This step was taken for logistical reasons; it is difficult to recruit large numbers of drug naïve older persons.

8.3.1.2 Medication

PD patients were to continue taking their medication on both test days. Along with levodopa, this medication included various antiparkinsonian drugs which have effects on the CNS. The evidence regarding the effect of pharmacologic treatment on cognition in PD is equivocal. Some studies have found levodopa improved performances on a number of tasks, while others have found this effect short-lived (Levin & Katzen, 1995). This, and the lack of consensus also with anticholinergics, is probably due to methodological confounding. One study compared the performances of early-in-the-course patients who were drug naïve, or treated with dopamine, anticholinergics, or both, on verbal and visuospatial memory tasks and found no significant difference between any of the four groups (Levin et al., 1991). Although it may have been preferable to include only drug naïve patients, it was not possible to recruit enough patients fitting this criterion for the present study. In case it was thought the actions of different medications would influence any outcome measure in the present study, the following helpful information was extracted from Tom and Cummings (1998):

a. Movement The levodopa and carbidopa combination enhances DA synthesis by providing more substrate; amantadine acts presynaptically by stimulating an increase in the release of DA and inhibiting its reuptake; pergolide and bromocriptine act directly at post-synaptic DA receptors; selegeline inhibits MAO-B to decrease the catabolism of DA and other catecholamines; anticholinergics, like bentropine, decrease tremor.
b. Mood Pergolide has no antidepressant action; amantadine alleviates depressive symptoms in elderly patients without PD who don’t meet criteria for major depression; benztropine alleviated baseline depression in one PD patient when it was added to a levodopa-carbidopa and fluoxetine regime; selegeline’s antidepressant activity is attributed to its MAO-A and MAO-B inhibiting properties when it is administered in higher doses than usually given for parkinsonian symptoms. Szabadi and Bradshaw (2004) note selective MOA-B inhibitors, like selegeline, are ineffective as antidepressants.

8.3.2 Treatment

Male participants received 104.4 g of each treatment and female participants were given 80% of this dose. The 83.3 g mixture given to females was based on a 20% reduction of the male dose based on the premise that females have a nearly 20% lower average weight (Ellenbogen et al., 1996). The high dose was informed by the effects this dose had on cognition in Study A.

Both amino acid drinks were mixed up with 250 ml water and flavouring. Initially the preparation of the drink was as described by Schmitt et al. (2000); however, from the third participant on it was mixed by hand with a whisk thereby avoiding the thick froth which participants disliked.

8.3.2.1 Treatment Order

Participants were randomly allocated to treatment order based on a block design. That is, participants were stratified on diagnosis with ID numbers being arranged in permuted blocks of size 4 to balance for test day sequence. Both participant and administrator were blind to the treatment order and the blind was not broken until the end of the study when data for all participants were collected. Double blinding was achieved with the help of a third person (Saskia von Stockum) who provided the drink in unmarked containers. Saskia had access to containers labeled for the two treatments and knowledge of what participants were to receive on each test day. She selected one pot of each treatment, removed the commercial labels so that no-one would know what treatment a pot contained and re-labeled each pot with a participant’s ID# and the date of his or her test days. She then placed the container in the clinic ready for the test days.

8.3.3 Apparatus

8.3.3.1 Computer Hardware

Two computer laptops were used in these studies: a Compaq N1020 with Windows 2000 operating system and a Compaq nx5000 with Windows XP Professional operating system. Both computers had a sound card and speakers, and graphics card capable of supporting Microsoft DirectX® drivers; Touchscreen Magic Touch by Keytec, Inc.; CDR response box supplied by Cognitive Drug Research Ltd., Gatehampton Road, Goring-on-Thames, RG8 OEN, UK.
8.3.3.2 Computer Software

CAmbridge Neuropsychological Automated Battery (CANTAB): CANTABeclipse v2.0 system (Robbins et al., 1994a); Cognitive Drug Research (CDR) battery (Simpson et al., 1991); the touchscreen was set up as a mouse with the mode set to ‘Click on touch’, to single behaviours (no double clicks, drags or other modifiers), and to silent during screen touching. The Touchscreen was integrated with the CANTABeclipse.

8.3.4 Assessments

The neuropsychological battery for Study B was designed in such a way that comparisons could be made between the non demented patients in this study and those in another parallel study with patients having dementia with Lewy bodies (DLB) and Parkinson’s disease with dementia. As such, some of the assessments were specifically included to target cognitive symptoms of the dementias. For example, fluctuating consciousness in DLB was to be assessed with the One Day Fluctuation Assessment Scale and the Choice Reaction Time task. Other tasks, that perhaps could have been included, were omitted because they were considered too fatiguing for dementia patients to undergo amidst a selection of other assessments; the idea was to assess as broad a range of functions as possible within the shortest period of time. The decision of what assessments to include and what to exclude was based on fatigability and previous experience. One task that has been impaired in some studies with younger adults is the Rey Auditory Verbal Learning Task (RAVLT, Rey, 1964). A pooled analysis (Sambeth et al., 2007) of data from 211 participants published after the current studies were commissioned, found ATD induced impairments in the immediate and delayed recall components of the RAVLT. Given the significant dementia of DLB patients and that potential fatigue could result in floor effects, the RAVLT was not included in the battery. A shorter verbal recognition task was included which was hypothesised to still be sensitive to ATD effects on memory encoding and consolidation in this group.

General information about the assessments is to be found in Chapter 6 Methodology but specific information about the assessments for this study is outlined in sections 8.3.5 to 8.3.9.

8.3.5 Mood

MADRS

Two items (i.e., Reduced Appetite and Reduced Sleep) were removed from the MADRS because they would not change across the course of the testing. It meant the percent change scores on the MADRS could be compared to percent change scores on the MADRS in depression studies. The adjustment was similar to those made by Booij et al. (2005b) in the MADRS and Smith et al. (1997c) in the HAM-D (Hamilton, 1960). It meant the MADRS total now ranged from 0-48.
POMS
Two changes were made during the analysis in accordance with the instructions in the manual (McNair et al., 1992). These were: (a) all positive adjectives (i.e., Friendly, Clearheaded, Lively, Considerate, Active, Energetic, Sympathetic, Helpful, Cheerful, Good Natured, Alert, Trusting, Activated, Carefree, and Vigorous) were deleted and (b) the weighting on 'Relaxed' and 'Efficient' was reversed so that 0 = 4, 1 = 3, 3 = 1, and 4 = 0. The direction of POMS scoring now matched the MADRS and at each observation, a higher score indicated more mood symptoms. The POMS was accompanied by a list of alternate words and phrases, used to standardize inquiries from the participant regarding the meaning of the POMS items. The list of alternate words was sourced from Albrecht and Ewing (1989) and was based on the words a person with a year eight education would easily understand.

8.3.6 Movement

UPDRS
The Motor Examination (items 18-31) only was used. The Motor Examination is scored across 27 items each with a 5-point Likert scale. It is scored out of 108. Also, a subscore of composite tremor scores (i.e., axial and limb, resting, and action tremor scores) was devised to investigate an association between ATD and tremor, as per the composite used by Doder et al. (2003).

8.3.7 Neuropsychological
The neuropsychological computer batteries CANTAB and CDR were initially randomised and then purposefully counterbalanced for diagnosis, gender and age. This meant each group set (patient/control, male/female, 50/60/70/80th decade) had half its members receiving CANTAB on the first test day and CDR on the second; and vice versa for the second test day.

SMTS and DMS
Only one trial of 20 pattern presentations was administered based on the arduousness of forty presentations for the patients with the DLB patients in the concurrent Study D.

COWA
The letters ‘C’, ‘F’, and ‘L’ were used for the parallel version. These letters balanced with the number of words that could be generated per minute with the letters “F”, “A”, and “S”. These particular sets of letters were used because, in elderly patients, the letter ‘A’ has a test-retest reliability coefficient below the coefficient of the other two letters, ‘F’ and ‘S’ (Snow (1988), cited in Lezak, 1995). The choice of the CFL combination was based on the work by Benton and Hamsher (1976) who analysed which letters best matched ‘F’, ‘A’, and ‘S’ for verbal associative frequency.

CRT
The a priori decision regarding time for CRT was observed; CRT was analysed as per section 8.5.
8.3.8 Physical State
The VAS was positioned before the POMS at baseline, 4.5 hrs and approximately 6.5 hrs post treatment because, as a smaller task with a similar purpose and format, it was considered a prompt for the longer POMS. The VAS item ratings were entered into the ANOVA, with treatment and time as factors. A significant interaction effect of treatment by diagnosis meant the VAS item was entered as a covariate in the analyses of dependent variables.

8.3.9 Assessment order
The following assessments were administered in the following sequences.

Screening
MADRS; 3MS and MMSE; MINI

Test Days
Rating Scales: MADRS; UPDRS; VAS; POMS.
Neuropsychological Tasks: CDR: CRT; Depending on randomisation either CANTAB (MOT, PRM, SRM, SDMTS, SSP and SWM) or CDR (WP, IWR, SRT, DV, CRT, and DWR); 3MSE and MMSE; Digit Span (Forward and Backward); DOT; VOSP; Depending on randomisation either CDR or CANTAB; COWA; CDR: CRT; ODFAS.

8.4 Procedure
Participants attended the Van der Veer Institute for Parkinson’s Disease and Movement Disorders in Christchurch, New Zealand on two test days at least one week apart.

Participants were not required to follow any particular diet prior to the test days because it was felt a long-term restrictive diet would be too difficult for older persons – or patients with dementia from the concurrent comparative study (Study D) – to adhere to. They were required to fast from the evening meal onwards and – aside from ingesting the amino acid drink – to continue until the end of the test day. This protocol was chosen instead of the 24 hr low protein diet and the ingestion of low TRP foods during the test days used by other studies for the following reasons:

a. Low TRP diets of 200 mg and 700 mg per day for 10 days were shown to reduce total plasma by only 15% to 20%, with no significant change in free plasma levels (Delgado et al., 1989); free plasma levels give a better indication of the amount of TRP available for entry into the brain than total plasma levels (see section 3.3).

b. It is known that carbohydrates raise peripheral insulin levels – thus, increasing protein synthesis and the uptake of the branch chain amino acids (i.e., leucine, isoleucine, and valine) into muscle – and that this process raises the TRP/LNAA ratio (Blokland et al., 2004). It has been demonstrated that as little as 5% protein in a meal can block the ability of carbohydrate to raise the TRP/LNAA ratio (Teff et al., 1989), so it is
unlikely this ratio would have been affected by giving the participants food during the test day, especially considering the large load of amino acids they ingested. However, because it is difficult to know the exact effect food has on TRP availability in the brain and what this effect is in older persons, food was not offered in the study.

c. There is not a great difference between baseline plasma levels of free and total TRP in low protein dieters and overnight fasters. In older persons the baseline free TRP levels averaged 7.47 µmol/L after an overnight fast (Porter et al., 2005) and averaged 8.88 µmol/L after a 24 hr low protein diet (Epperson et al., 2007); total plasma levels averaged 46 µmol/L (Sobczak et al., 2002a) and 46.10 µmol/L (Epperson et al., 2007).

Patients took their usual medication with water at 7.00 am after the overnight fast on both test days. This allowed at least some absorption of levodopa to the brain before the treatment (i.e., 2 hours later) and, thus allowed participants to maintain motor function during the test day. Moreover, the overnight fast avoided the potential for high protein foods to reduce levels of circulating levodopa. The load of ingested amino acids in both treatments was expected to increase protein synthesis and thus reduce concentrations of the circulating precursors of levodopa – tyrosine and phenylalanine – however, during ATD the circulating levodopa was likely to increase competition for access to the CNS with the remaining TRP (Leenders et al., 1986; Young, 1986). This meant ATD effects could be greater in medicated patients than non-medicated.

Following the overnight fast, participants arrived at the clinic at 8.30 am where, for the first day, height, weight and blood pressure were measured. Participants were asked to arrive in the morning because plasma TRP reaches its minimum in the morning and peaks in the late evening (Feigin et al., 1971; Wurtman et al., 1968). As the evening is also the time of lowest tryptophan pyrrolase activity (Pakes, 1979) depletion was done as early in the day as reasonable to take advantage of every possible physiological means of reducing 5-HT.

A 10 ml blood sample was taken at baseline, 4 hrs and 6.5 hrs post treatment. Before treatment at 9.00 am participants completed, in the following order, four rating scales of general mood (MADRS), movement (UPDRS), physical (VAS), and mood state (POMS). These were followed by a training session on a computer with the CDR battery. During the subsequent 4.5 hrs, participants were asked to remain as rested as possible by reading a newspaper or book and listening to the radio, audiotape or CD. They were able to go to the toilet. At 4.5 hrs post treatment the four rating scales were re-administered. This was immediately followed by the neuropsychological battery which was carried out between 4.5 and approximately 6.5 hrs. Cognitive tasks were administered at this interval from treatment firstly, to allow absorption and biotransformation of the amino acids and secondly, because cognitive effects are purported to be stronger if stimuli are learned after ATD (Meeter et al., 2006). The four rating scales were re-administered after the cognitive testing, followed by a third administration of the CDR Choice Reaction Test. After the assessments were completed the
participants were given a light meal of mixed protein and carbohydrate to restore a healthy amino acid balance (i.e., protein repletion) and to reverse any effects of the tryptophan depletion before they left the research unit. They were assessed and transported home. The ODFAS was completed by the administrator at the end of each test day. Contact was made within 24 hours to check participants.

All participants participated voluntarily and gave written informed consent. They received a voucher by way of thanks for each test day of participation. Participants were organised into two study groups: controls and patients with Parkinson’s disease.

8.5 Analysis
The following section outlines the a priori decisions made regarding participant numbers and also, biochemical, mood, movement and neuropsychological assessment and analysis. Specific adaptations or comments regarding the assessments follow this section:

a. The number of participants required to effect the most robust comparisons is not necessarily determined by having exactly the same number of controls to patients, but the maximum number of controls possible within defined parameters. For the present study these parameters were established to be a similar gender ratio, followed by a group mean difference of < 5 points for PVIQ score and < 3 yrs for age. When this was achieved in both genders, the genders were to be combined into the two participant groups and t-tests performed to see if the patient and controls groups were matched for PVIQ and age.

b. The variables diagnosis, order (placebo first or placebo second), and gender were entered as between-subject variables. If there was no significant main effect of order the analysis was re-run with order omitted. When this occurred, cells (for example, in Table 7-2) under the columns headed Treatment x Order are left blank.

c. When a test had a level of difficulty (e.g., DMS: 0 s, 4 s, and 12 s delays; SWM: 4 box, 6 box, 8 box) or when a test was administered at different times of the day (e.g., mood and movement: 0 hrs, 4.5 hrs, 6.5 hrs; CRT: beginning, during, and post battery) level or time were entered as further factors. If there were no significant interaction effects for these variables with treatment, the analysis was re-run without them, that is, with only treatment as the within-subject variable. If there was a significant interaction of treatment by level or time, the analysis was re-run for each level or time to examine the pattern of difference. This meant a dependent variable could have 3 (treatment, diagnosis, gender), 4 (treatment, diagnosis, gender, order or level or time), or 5 (treatment, diagnosis, gender, order and level or time) factors entered into the analysis.

d. Significant effects are presented in the graphs, with the exception of DMS in Figure 8-7 and SWM Between errors in Figure 8-13. This means that a significant main effect...
will be graphed as ATD against placebo; it will not be graphed showing the separate performances of each group as well.

e. The graphs for order effects in section 8.6.1.10 were ordered and coloured in such a way as to make the explanation of order effects as uncomplicated as possible. This meant, in a systematic manner, that (a) the order could be read first, since the first visit for each group was on the left – and the second visit on the right – of each pair of histobars, (b) ATD bars were coloured yellow and placebo coloured grey so that the colouring of the histobars for group having placebo first of each was reversed for the group having placebo second, (c) any practice or novelty effect could be seen at a glance since a better score on the second visit for both sets of bars suggested a practice effect and a better score on the first visit suggested a novelty effect, and (d) the interaction of treatment with these order effects could be read next by noting the difference in the scores of both treatments for each group.

8.5.1 Biochemical
The TRP/LNNA ratio was not obtained for financial reasons. Although the TRP/LNAA ratio is an indicator of the competition of TRP with LNAAs at the blood brain barrier – and thus the amount of TRP available for 5-HT synthesis – free TRP level may also be used. Free TRP level is a close correlate of central 5-HT depletion and this is the measure used in the present study to approximate 5-HT depletion (for examples of studies see, Biggio et al., 1974; Moja et al., 1989).

8.5.2 Statistical
The procedure for statistical analysis is outlined in section 6.4. That is, biochemical, mood, movement and cognitive function responses were analysed using the general linear model repeated measures analysis of variance (ANOVA) with treatment (ATD or placebo) as a within-subject variable and diagnosis (PD or control) or gender (male or female) as a between-subject variable. Free TRP level at baseline on both test days was compared with a paired samples t-test to see if there was a significant difference in the levels at this time. An independent samples t-test was done for free TRP level (ATD day) to see if there was a significant difference between the groups.

8.5.2.1 Power Calculation
Based on previous studies in older patient groups (Porter et al., 2000; Porter et al., 2005), the primary outcome of this research was score on 3MS. The study was powered to have 80% power to show a mean difference of 5 points (SD_{diff} = 5) in the PD group n = 16, at a two-tailed significance level of .05.

8.6 Results
There were 55 people who fulfilled the inclusion criteria and participated in this study: 20 PD patients and 35 healthy controls. All women in the present study were menopausal and not on hormone replacement therapy. All participants completed both test days.
8.6.1.1 Matching

Matching was not one-to-one; data were selected from a pool of data collected from a group of healthy elderly whose data were used in Study C. Groups in Study B were matched, in order, on gender ratio, PVIQ, and age. As the gender ratio for PD patients was 3:2 (M = 12/F = 8), 21 males and 14 females were selected for the larger pool of healthy adults in the following manner.

Males: when the male having the highest PVIQ score was removed the mean difference for controls and PD patients on PVIQ was < 5 points (M = 3.66, SD = 7.94; t_{31} = 0.988, p = .33). The mean difference in age was < 3 yrs (M = 0.59, SD = 5.07; t_{31} = 0.159, p = .87).

Females: Seven female controls needed to be removed from the original dataset to balance the 3:2 gender ratio that existed for the PD patients. The PVIQ was lower for PD patients, so the six female controls having the highest PVIQ were removed; the lowest PVIQ was also removed to enable a mean age difference of < 3 yrs. This meant the mean difference for controls and PD patients on PVIQ was < 5 points (M = 1.45, SD = 3.1; t_{20} = 0.45, p = .66). The mean difference in age was < 3 yrs (M = 2.68, SD = 8.79; t_{16} = 0.823, p = .42).

8.6.1.2 Demographic

There were no significant differences between the groups on all demographic variables and baseline scores, as shown in Table 8-2, except for the MADRS: patients had a lower mood (M = 1.75, SD = 2.02) than controls (M = 0.4, SD = 0.85; z = -2.8, p = .005).

Table 8-2 Study B. Means and Standard Deviations for Demographic Variables

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>67.8</td>
<td>5.72</td>
</tr>
<tr>
<td>PVIQ</td>
<td>106.5</td>
<td>8.81</td>
</tr>
<tr>
<td>Educ Stage</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.1</td>
<td>0.85</td>
</tr>
<tr>
<td>MADRS</td>
<td>1.75</td>
<td>2.02</td>
</tr>
<tr>
<td>H &amp; Y</td>
<td>2.18</td>
<td>0.78</td>
</tr>
<tr>
<td>Age Onset (yrs)</td>
<td>61.5</td>
<td>9.28</td>
</tr>
<tr>
<td>Time from diagnosis (yrs)</td>
<td>6.75</td>
<td>6.23</td>
</tr>
<tr>
<td>Side Onset (n) Left (5)   Right (14) Unsure (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| n | Gender (m/f) | 20/8 | 35/14 | 21/14 |

Note: M = mean; SD = standard deviation; f = female; m = male; H & Y = Hoehn and Yahr score; MADRS = Montgomery-Asberg Depression Rating Scale; MMSE = Mini Mental State examination; PVIQ = Predicted Verbal IQ score (from the NART: National Adult Reading Test). All comparisons are independent samples t-test except * = Mann-Whitney U test; ** = chi square.
8.6.1.3 Family Depression History

Of the 55 participants, 20% of the PD patients and 23% of the controls had a family history of depression, as defined by at least one first degree relative having at least one episode of depression (including bipolar disorder type II). This definition of family history was chosen because studies have found small mood effects during ATD in healthy first-degree relatives of patients having depression or bipolar disorder (Klaassen et al., 1999b; Sobczak et al., 2002b). Small mood effects were induced in healthy males with a multigenerational family history of affective illness (Benkelfat et al., 1994) but not in healthy females (Ellenbogen et al., 1999).

8.6.1.4 Medication Status

All patients were taking medication at the time of testing: 16 on combination therapy (i.e., levodopa and decarboxylase inhibitor); 14 on a dopamine agonist; four on a peripheral – but not CNS acting – DA antagonist for nausea; four on anticholinergic medication; and seven on a dopamine-anticholinergic combination. The numbers listed for each drug in Table 8-3 include two patients who were taking two anticholinergic medications each (i.e., amantadine and benztropine; amantadine and disipal); one of these patients was also taking a MAO-B inhibitor. This meant a total of nine patients taking anticholinergic medication.

Table 8-3 Study B. Number of Patients Taking Different Parkinsonian Medication

<table>
<thead>
<tr>
<th>n</th>
<th>Trade name (generic)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Sinemet (Sinemet)</td>
<td>Levodopa-carbidopa</td>
</tr>
<tr>
<td>4</td>
<td>Madopar (Levodopabenserazide)</td>
<td>Levodopa-benserazide</td>
</tr>
<tr>
<td>4</td>
<td>Domperidone (Domperidone)</td>
<td>Peripheral DA antagonist</td>
</tr>
<tr>
<td>3</td>
<td>Lisuride</td>
<td>DA agonist</td>
</tr>
<tr>
<td>2</td>
<td>Rotigotine</td>
<td>DA agonist</td>
</tr>
<tr>
<td>1</td>
<td>Ropinerole (Ropinerole)</td>
<td>DA agonist</td>
</tr>
<tr>
<td>8</td>
<td>Pergolide (Pregolide)</td>
<td>DA agonist</td>
</tr>
<tr>
<td>7</td>
<td>Amantadine (Symmetryl)</td>
<td>DA agonist + anticholinergic</td>
</tr>
<tr>
<td>1</td>
<td>Disipal (Orphenadrine)</td>
<td>Anticholinergic</td>
</tr>
<tr>
<td>1</td>
<td>Kemadrine (Procyclidine)</td>
<td>Anticholinergic</td>
</tr>
<tr>
<td>2</td>
<td>Benztropine (Benztropine)</td>
<td>Anticholinergic</td>
</tr>
<tr>
<td>5</td>
<td>Selegeline (Selegeline)</td>
<td>MAO-B inhibitor</td>
</tr>
</tbody>
</table>

8.6.1.5 Somatic

The amino acid drinks were well tolerated with no serious side effects. No participant vomited but irrespective of treatment, 30% of participants experienced nausea during the morning of both test days and 19% experienced diarrhoea on the day following both test days. Table 8-4 records the somatic effects for each gender for each treatment arm. There was a significant interaction of treatment by gender on nausea ($F_{1,49} = 4.37, p = .04$). Females reported more nausea after ATD ($M = 0.43, SE = 0.13$) compared to placebo ($M = 0.15, SE = 0.1$), whereas males reported less after ATD ($M = 0.22, SE = 0.1$) compared to placebo ($M = 0.3, SE = 0.17$).
### Table 8-4 Study B. Number of Participants Experiencing Somatic Complaints

<table>
<thead>
<tr>
<th></th>
<th>Nausea Placebo t(m/f)</th>
<th>Depletion t(m/f)</th>
<th>Diarrhea Placebo t(m/f)</th>
<th>Depletion t(m/f)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PD</strong></td>
<td>3 (1/2)</td>
<td>3 (1/2)</td>
<td>1 (0/1)</td>
<td>2 (1/1)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>6 (4/2)</td>
<td>4 (2/2)</td>
<td>4 (2/2)</td>
<td>3 (2/1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>9 (5/4)</td>
<td>7 (3/4)</td>
<td>5 (2/3)</td>
<td>5 (3/2)</td>
</tr>
</tbody>
</table>

Note: t = total; m = male; f = female.

### 8.6.2 All Measures

The significant effects are shown in Table 8-5. The means and standard errors for all main effects and interactions are presented in Appendix H.

#### 8.6.2.1 Missing Data

Participants’ were excluded from the analysis on particular dependent variables if their data were incomplete: this is represented in the degrees of freedom for each variable. These are listed as Variable (group: n): Biochemical (patient: 2; controls: 6); POMS (patients: 3; controls: 3); SWM Between errors (control: 2); CRT Accuracy and Reaction time (patients: 1; controls: 1); VAS (control: 1).

#### 8.6.2.2 Biochemical

Measurements are expressed in nanograms per millitre (ng/ml). It is important to note that the minimum measurement of TRP obtainable with the chromatography apparatus – used in the present study – was 500 ng/ml. All measurements below this level were classified as this minimum and thus reductions in TRP levels may be lower than the data suggest.

At baseline, there was no significant difference in free TRP levels between the ATD ($M = 1765.97$, $SD = 142.62$) and placebo days ($M = 1850.00$, $SD = 124.09$; $t_{48} = 0.86$, $p = .39$). There was no significant difference between patients ($M = 1989.47$, $SD = 858.23$) and controls ($M = 1787.10$, $SD = 819.65$; $t_{48} = 0.83$, $p = .41$) at baseline on the ATD test day.

There was a main effect of treatment ($F_{1,40} = 156.07$, $p = .000$) with free TRP level being lower after ATD ($M = 933.750$, $SE = 48.509$) than after placebo ($M = 3474.015$, $SE = 233.927$).

There was a significant interaction of treatment and diagnosis after ATD ($F_{1,40} = 4.19$, $p = .047$). The 75% difference between ATD and placebo levels for patients was greater (ATD: $M = 986.67$, $SD = 76.66$; placebo: $M = 3943.03$, $SE = 369.69$) than the 71% difference for controls (ATD: $M = 880.83$, $SD = 59.46$; placebo: $M = 3005.00$, $SE = 286.73$), as shown in Figure 8-1.
There was a significant interaction of treatment by time ($F_{1,40} = 107.63, p = .00$). During ATD this represented a 71% reduction in free TRP from baseline ($M = 1765.97, SE = 142.62$) to 4 hrs ($M = 519.94, SE = 9.75$) which remained at 71% to 6.5 hrs ($M = 515.34, SE = 11.54$); during placebo, this represented an 189% increase in free TRP from baseline ($M = 1850.00, SE = 124.09$) to 4 hrs ($M = 5354.09, SE = 343.25$) and a 74% increase to 6.5 hrs ($M = 3217.96, SE = 321.21$), as shown in Figure 8-2.
### Table 8-5 Study B. Effects of Treatment on All Variables
Repeate[...]

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>Treatment F</th>
<th>Treatment F</th>
<th>Treatment F</th>
<th>Treatment F</th>
<th>Treatment F</th>
<th>Treatment F</th>
<th>Treatment F</th>
</tr>
</thead>
<tbody>
<tr>
<td>freeTRP</td>
<td>1.40</td>
<td>156.07 ***</td>
<td>419.00 *</td>
<td>0.01</td>
<td>3.86</td>
<td>107.63 ***</td>
<td>4.04 *</td>
<td></td>
</tr>
<tr>
<td>MADRS Total</td>
<td>1.51</td>
<td>0.40</td>
<td>0.00</td>
<td>0.98</td>
<td>2.97</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>POMS Total</td>
<td>1.41</td>
<td>0.60</td>
<td>0.56</td>
<td>25.52 ***</td>
<td>0.33</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPDRS Total</td>
<td>1.51</td>
<td>0.30</td>
<td>0.96</td>
<td>0.98</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOT Accuracy</td>
<td>1.51</td>
<td>1.67</td>
<td>2.48</td>
<td>0.12</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOT Reaction</td>
<td>1.51</td>
<td>2.17</td>
<td>15.37 ***</td>
<td>0.32</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MADRS</td>
<td>1.47</td>
<td>0.78</td>
<td>8.60 **</td>
<td>5.00 *</td>
<td>0.03</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSE Total</td>
<td>1.51</td>
<td>2.47</td>
<td>0.75</td>
<td>0.10</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DigitsF Total</td>
<td>1.47</td>
<td>0.33</td>
<td>0.07</td>
<td>4.04 *</td>
<td>0.43</td>
<td>3.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DigitsB Total</td>
<td>1.51</td>
<td>0.30</td>
<td>0.00</td>
<td>0.55</td>
<td>1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOT Total</td>
<td>1.47</td>
<td>0.91</td>
<td>0.81</td>
<td>4.51 *</td>
<td>0.34</td>
<td>3.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSP Span</td>
<td>1.47</td>
<td>4.85 *</td>
<td>0.24</td>
<td>6.88 **</td>
<td>0.66</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWM BetweenErrors</td>
<td>1.45</td>
<td>4.42 *</td>
<td>0.21</td>
<td>10.89 **</td>
<td>0.07</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COWA Total</td>
<td>1.51</td>
<td>0.05</td>
<td>0.71</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COWA Verrors</td>
<td>1.51</td>
<td>0.78</td>
<td>1.11</td>
<td>0.19</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COWA Accuracies</td>
<td>1.51</td>
<td>0.26</td>
<td>1.64</td>
<td>1.18</td>
<td>2.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOSP Silhouettes</td>
<td>1.47</td>
<td>0.01</td>
<td>2.79</td>
<td>10.63 **</td>
<td>0.73</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRM Accuracy</td>
<td>1.47</td>
<td>1.71</td>
<td>0.67</td>
<td>5.12 *</td>
<td>0.38</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRM Reaction</td>
<td>1.51</td>
<td>0.08</td>
<td>0.57</td>
<td>0.15</td>
<td>1.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMTS Accuracy</td>
<td>1.51</td>
<td>0.02</td>
<td>0.51</td>
<td>0.02</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS Accuracy</td>
<td>1.51</td>
<td>0.50</td>
<td>6.01 *</td>
<td>0.91</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS Reaction</td>
<td>1.51</td>
<td>0.91</td>
<td>0.69</td>
<td>4.97 *</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DWR SI</td>
<td>1.51</td>
<td>1.79</td>
<td>5.21 *</td>
<td>2.33</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DWR SI</td>
<td>1.51</td>
<td>1.80</td>
<td>0.15</td>
<td>0.00</td>
<td>2.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRT Accuracy</td>
<td>1.43</td>
<td>0.30</td>
<td>0.48</td>
<td>0.00</td>
<td>1.50</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CRT Reaction</td>
<td>1.43</td>
<td>0.00</td>
<td>3.48</td>
<td>0.22</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRT Reaction</td>
<td>1.51</td>
<td>1.40</td>
<td>0.75</td>
<td>0.10</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DV Accuracy</td>
<td>1.51</td>
<td>0.22</td>
<td>0.22</td>
<td>1.81</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DV Reaction</td>
<td>1.51</td>
<td>1.83</td>
<td>2.91</td>
<td>0.04</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS Nausea</td>
<td>1.49</td>
<td>1.47</td>
<td>0.02</td>
<td>4.37 *</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: COWA = Controlled Oral Word Association test; CRT = Choice Reaction Time; DigitsB = Digit Span Backward; DigitsF = Digit Span Forward; DMS = Delayed Matching to Sample; DWR = Delayed Word Recognition; DOT = Digit Ordering Test; DV = Digit Vigilance; IWR = Immediate Word Recognition; MMSE = Modified Mini Mental State examination; MADRS = Montgomery-Asperg Depression Rating Scale; MOT = Motor Screening; POMS = Profile of Mood States; PRM = Pattern Recognition Memory; Rerrors = repetition errors; SMTS = Simultaneous Matching to Sample; SRM = Spatial Recognition Memory; SRT = Simple Reaction Time; SSP = Spatial Span; SWM = Spatial Working Memory; TRP = tryptophan; UPDRS = United Parkinson’s Disease Rating Scale; VAS = Visual Analogue Scale; Verrors = violation errors; VOSP = Visual Object and Space Perception test. **p ≤ .05. ***p < .01. ***p < .001.
8.6.2.3  Mood

There was a significant treatment by time effect on the MADRS ($F_{1,51} = 4.04, p = .02$), as shown in Table 8-5 and Figure 8-3. The mood of participants was worse after ATD at 6.5 hrs (ATD: $M = 1.92$, $SE = 0.26$; placebo: $M = 1.38$, $SE = 0.24$) than at baseline (ATD: $M = 0.54$, $SE = 0.14$; placebo: $M = 0.88$, $SE = 0.16$) or 4.5 hrs (ATD: $M = 0.58$, $SE = 0.14$; placebo: $M = 0.53$, $SE = 0.13$).

![Figure 8-3 Effect of treatment and time on MADRS.](image)

There were five participants (4 patients and 1 control) whose score increased by 5 points between baseline and 6.5 hrs on the MADRS during ATD. These participants were not statistical outliers. When this group was removed from the ANOVA, the interaction between ATD and time was not significant ($F_{1,49} = 0.79, p = .46$). The characteristics for these participants are shown in Table 8-6.

Table 8-6 Study B. Participants with a Score Increase of 5 Points at 6.5 hrs

<table>
<thead>
<tr>
<th>Participant</th>
<th>Diagnosis</th>
<th>H &amp; Y</th>
<th>Age at PD onset</th>
<th>Gender</th>
<th>Age</th>
<th>FH</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>*</td>
<td>*</td>
<td>F</td>
<td>87</td>
<td>no</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>PD</td>
<td>2</td>
<td>50</td>
<td>M</td>
<td>59</td>
<td>no</td>
<td>Amantadine, Benzatropine, Domperidone, Pergolide, Selegeline, Sinemet, Cilazapril (TD2 only), Inhibace (TD1 only)</td>
</tr>
<tr>
<td>3</td>
<td>PD</td>
<td>3</td>
<td>58</td>
<td>M</td>
<td>64</td>
<td>no</td>
<td>Amantadine, Pergolide, Selegeline, Sinemet, Viagra</td>
</tr>
<tr>
<td>4</td>
<td>PD</td>
<td>3.5</td>
<td>58</td>
<td>F</td>
<td>65</td>
<td>no</td>
<td>Rotigotine, Sinemet, Frumil</td>
</tr>
<tr>
<td>5</td>
<td>PD</td>
<td>1.5</td>
<td>57</td>
<td>F</td>
<td>60</td>
<td>no</td>
<td>*</td>
</tr>
</tbody>
</table>

8.6.2.4  Movement

There were no effects of treatment on UPDRS as shown in Table 8-5. There were no effects of treatment on the UPDRS composite tremor scores ($F_{1,19} = 1.01, p = .33$); only scores for patients were analysed.
There was a significant interaction of treatment by diagnosis on MOT ($F_{1,51} = 15.38, p = .000$). As shown in Figure 8-4, patient response was faster during ATD ($M = 1188.43, SE = 71.12$) compared to placebo ($M = 1380.67, SE = 81.19$), but control response was slower during ATD ($M = 1299.70, SE = 53.76$) compared to placebo ($M = 1212.39, SE = 61.37$). An independent samples $t$-test revealed there was no difference between patients and controls on the placebo scores for MOT ($t_{53} = 0.39, p = .83$).

![Figure 8-4](image)

**Figure 8-4 Effect of treatment and group on MOT Reaction Time (ms).**

8.6.2.5 Neuropsychological

There were no effects of treatment, treatment and diagnosis, or treatment and gender on CANTAB (PRM, SRM, and SMTS), CDR (SRT, CRT, DV, and DWR), Digit Span Forward and Backward, DOT, VOSP, COWA, and ODFAS. Only statistically significant results for individual assessments are presented and referred to in the text.

Global Cognitive Status

There was a significant interaction of treatment by diagnosis on 3MS ($F_{1,47} = 0.78, p = .005$). As shown in Figure 8-5, patient performance worsened during ATD ($M = 94.83, SE = 1.07$) compared to placebo ($M = 97.33, SE = 0.92$), whereas control performance improved during ATD ($M = 98.09, SE = 0.79$) compared to placebo ($M = 96.75, SE = 0.68$).
Memory

There was no significant interaction of treatment by delay on DMS so accuracy and reaction time were analysed without delay interval, as per the a priori decision outlined in section 8.5. There was a significant interaction of treatment by diagnosis in DMS Accuracy ($F_{1,51} = 6.02, p = .02$). The performance of patients was enhanced on DMS Accuracy during ATD ($M = 80.83, SE = 3.19$) compared to placebo ($M = 77.08, SE = 2.48$), as shown in Figure 8-6, whereas the performance of controls worsened during ATD ($M = 79.01, SE = 2.14$) compared to placebo ($M = 85.79, SE = 1.88$).

Although not having a significant effect, the means and standard errors of each delay interval on DMS are displayed in Figure 8-7 as a graphical example of the a priori decision to analyse DMS (all delays) only (see section 8.5).
There was a significant interaction of treatment by diagnosis on DMS %Corr ($F_{1,51} = 6.62, p = .01$). The performance of patients was enhanced on DMS %Corr during ATD ($M = 84.69, SE = 2.46$) compared to placebo ($M = 81.35, SE = 2.09$), whereas the performance of controls worsened during ATD ($M = 83.01, SE = 1.86$) compared to placebo ($M = 88.24, SE = 1.58$), as shown in Figure 8-8. As explained in section 8.5, DMS %Corr is a different variable to DMS Accuracy as it reports the percentage of times a correct response is made on a participant’s first response rather than merely a percentage of the correct responses in total, as occurs in DMS Accuracy.
There was a significant interaction of treatment by diagnosis on IWR ($F_{1,51} = 5.21$, $p = .03$). The performance of patients was worse during ATD ($M = 0.76$, $SE = 0.04$) compared to placebo ($M = 0.84$, $SE = 0.03$), whereas the performance of controls during ATD ($M = 0.88$, $SE = 0.03$) was better compared to placebo ($M = 0.86$, $SE = 0.02$) but to a lesser extent than patient performance, as shown in Figure 8-10.

Working Memory

There was a main effect of treatment on SSP ($F_{1,47} = 4.85$, $p = .03$). Performance on SSP was worse during ATD ($M = 4.67$, $SE = 0.13$) than during placebo ($M = 4.94$, $SE = 0.13$), as shown in Figure 8-11.
There was no significant interaction of treatment by level on SWM so accuracy was analysed without level as a factor, as per the a priori decision outlined in section 8.5. There was a main effect of treatment on SWM Between errors ($F_{1,45} = 4.42, p = .04$), as shown in Figure 8-12. Errors were measured in this task and, thus, the means show performance worsened during ATD ($M = 43.47, SE = 2.62$) compared to performance during placebo ($M = 39.45, SE = 2.24$).

Although not having a significant effect, the means and standard errors of each level for SWM Between errors are displayed in Figure 8-13 as a graphical example of the a priori decision to analyse SWM Between errors (all levels) only (see section 8.5).
20.6.2.6 **Treatment Order**

Twenty six participants received the placebo drink first and ATD second (placebo/ATD) and 29 received the ATD drink first and placebo second (ATD/placebo). The ANOVA showed nine significant interactions of treatment by order, as shown in Table 8-5, and one 3-way interaction of treatment by diagnosis by order that was not tabulated. All are represented in Figure 8-14. There was no consistent difference between the groups; sometimes ATD/placebo was better, sometimes not. The nature of these effects is explained in section 8.7.5.
Figure 8-14 Interactions of treatment by order.

Note: ■ = ATD; ■ = placebo; TD1 = first test day; TD2 = second test day
8.7 Discussion

The present study investigated the effects of ATD on movement, mood and cognition in 20 patients with PD and 35 healthy age and gender matched control participants.

The principal findings for the present study were:

a. A significant reduction in free TRP such that the increase during placebo was higher for patients than for controls.

b. Differential effects on global cognition, visual memory, and verbal memory, such that performance by patients was improved in delayed visual recognition memory and psychomotor speed but was impaired in global cognition and immediate verbal recognition memory after ATD.

c. Impaired performances in spatial working memory by all participants after ATD.

d. Females were faster at responding in a delayed visual memory task after ATD.

e. ATD was associated with a mood lowering effect at 6.5 hrs post treatment in all participants.

ATD has been used previously in one PD patient (McCance-Katz et al., 1992) and, after commencement of the present study, in a Dutch study with a group of 15 patients and 15 matched control participants (Leentjens et al., 2006; Scholtissen et al., 2006b). Whereas the earlier study by McCantz-Katz et al. found ATD was associated with bradykinesia, depression, and bradyphrenia in the depressed patient being treated with an SSRI, the later study found only one effect of treatment by diagnosis – when the patients did not show an effect of ATD on the long interval of the SRT task – but not on any of the other movement, mood or cognitive assessments they used. These included the POMS, UPDRS, Visual Verbal Learning Task, Concept Shifting Task, Simple Reaction Time Task, and Finger Precuing Task.

8.7.1 Biochemical

Free TRP level was reduced during ATD and increased after placebo, as expected. The percentage of reduction after ATD was not as great as that discussed by Hood et al. (2005), however, it is in line with the level of reduction found in ATD studies with older participant groups (Porter et al., 2000; Porter et al., 2005; Scholtissen et al., 2006b). It should be noted, also, that the lowest level the assay apparatus could detect was 500 ng/ml; this may have excluded much lower levels being included in the analysis. The increase in level during placebo is consistent with the literature (see section 3.5.3).

There was an interaction between group and treatment, which appear to have been secondary to differences in increase following the placebo. PD patients and controls had equivalent free TRP levels during ATD, but during placebo – and particularly at 4 hrs – the levels of patients increased by a greater margin. The reason for this is not clear because there is no indication that PD patients have problems with protein synthesis or slowed liver function (Professor Tim Anderson, Professor of
Parkinson’s disease, personal communication, December 7, 2007). It is possible the decarboxylase inhibiting medication (e.g. carbidopa and benserazide) taken by a majority of PD patients may have led to the higher plasma levels of TRP in this group since this medication blocks the peripheral catabolism of levodopa into DA and 5-HTP into 5-HT. One study has demonstrated carbidopa co-administered with 5-HTP results in a 14-fold increase in plasma 5-HTP levels (Gijsman et al., 2002). It is possible such an increase may slow the conversion of TRP into 5-HTP and thus maintain elevated levels of plasma TRP after placebo amino acids load. The TRP/LNAA ratio would have provided more information about the central effects of this treatment by group differential, but this ratio was not available.

8.7.2 Movement

No effects of treatment were observed in movement as assessed by the UPDRS, corroborating the result found by Scholtissen et al. (2006b) that 5-HT does not significantly influence scores on this measure. However, ATD appears to have motoric effects because patients were faster in the MOT task during ATD than they were during placebo.

One explanation for an effect on the MOT may be that this task comprises rudimentary psychomotor elements in addition to motoric ones. Bradyphrenia is a symptom of PD – particularly as reported from clinical observation – although this has not been reliably demonstrated empirically (Davidson & Knight, 1995). It is often noted that slowed psychomotor speed is as likely to arise with (a) comorbid depression, (b) slowed physical rather than psychomotor speed, or (c) the cognitive load accompanying the test used for assessment (for references refer section 5.11.1.4). To the extent the MOT is a passive task – in that it does not have high cognitive demand – and because it requires the participant to search as well as respond, it may well be measuring reaction time as well as movement time; albeit reaction time with little requirement for processing time due to this low cognitive demand.

Scholtissen and colleagues found all participants – irrespective of diagnosis – were faster during ATD in their not dissimilar SRT, but for PD patients, this occurred on the long interval only (i.e., longer interval between interval and response therefore, possibly, higher cognitive load). The SRT used in their study assessed both reaction time and movement time; the participant had to let go the response button on cue (i.e., reaction time) and touch a square shape on a computer screen as quickly as possible (i.e., movement time). They found no treatment by diagnosis effect on movement time in this simple task. Nor did they find an effect in a more complicated reaction time task to the SRT – the Finger Precuing Task – which requires the participant to select one of four fingers in response to a precue. The present research also did not find an effect on the more complicated CRT. Together this suggests ATD has an enhancing effect on reaction time during less demanding tasks and that this effect may also include movement time; particularly since MOT requires a full arm movement to effect a response.
Reaction time and movement time have previously been correlated in PD. Zimmermann et al. (1992) examined reaction time with tasks of differing cognitive load. They found no differences in reaction time with cognitive load, however, they did find that late- and, especially, early-in-the-course patients were impaired in movement times when the task had a heavier cognitive load; both compared to controls and compared to their own performance in a passive simple reaction time task. These authors conclude there is an overlap of motor processes and cognitive load in PD.

Supporting evidence for this was observed in an imaging study when cognitive impairment in PD became apparent when faster speeds were required (Sawamoto et al., 2002). The authors of this study reiterated the conclusion of the previous authors that cognitive slowing and motor slowing are significantly correlated. Although the aforementioned research concluded this overlap occurred during high demand tasks, the present results suggest the link is apparent also in low demand tasks when 5-HT is appreciably reduced. A definitive answer, however, requires further investigation with several measures of pure motor speed because it may be the present findings on MOT are due to chance. Certainly, this is possible given that ATD – and also combined serotonin-catecholamine depletion – has not previously been shown to affect reaction time in a number of different cognitive tasks (see section 4.3.7.2). In the meantime, two factors need to be considered when interpreting the present results:

a. The medication status of the patients in the present study compared to the Dutch study. Scholtissen et al. (2007) report their patients were not taking lisuride (i.e., DA agonist), selegeline, or levodopa. Patients in the present study were taking levodopa and DA agonists and this may have affected the results. However, all participants acted as their own control and were taking the same medication on both treatment arms of the study. Also, although not taking lisuride, selegeline, or levodopa, Scholtissen et al. reported eighty percent of their patients were still taking dopaminergic enhancing medication (albeit unspecified).

b. Therapeutic efficacy of the medication. Dopaminergic medication could mean patients were operating at near normal levels of movement and at this level there may be no ability to detect changes in movement. The MOT may be a more sensitive test to the effects of reduced 5-HT and its inhibition of DA activity, whereas the UPDRS may have a ceiling effect in PD patients taking dopaminergic medication.

c. The stage of disease progression. It may be that the patients in the Dutch study were less handicapped by the physical nature of their disease. They were not taking levodopa implying their disease was less severe and very early-in-the-course. Because the publications for this study (Leentjens et al., 2006; Scholtissen et al., 2006b) did not include years from diagnosis, the only way to compare this is to use the Hoehn
and Yahr score to determine where patients were in the course of their disease course; this stage was the same for both studies.

The non-significant effect of treatment on composite tremor scores does not support a role of 5-HT in the generation of tremor. Scholtissen et al. did not differentiate between types of motor complaint on the UPDRS and the present study did not investigate akinetic items. To the extent reduced 5-HT is associated with the akinetic variant of PD (Liu et al., 1999) and that three quarters of the UPDRS items concern bradykinesia, hypokinesia, or rigidity, it would be interesting to investigate this further with ATD and assessment techniques other than clinical observation, for example,

a. A computerised system that measures moment-to-moment fluctuations in the position signal of the output from a digitising tablet (as described by, Aly et al., 2007).

b. A lightweight, wireless, finger-worn motion sensor, which transmits information to a wrist-worn transceiver and then on to a computer (as described by, Giuffrida, 2007).

8.7.3 Mood

There was a significant ATD by time effect suggesting ATD affects mood after 6 hours. It is possible this effect arose as a result of four patients and one control having a 5 point increase on their MADRS scores during ATD because this was absent when this subgroup was removed from the ANOVA. In general, participants in the present study were not vulnerable to mood effects during ATD and, thus, the second hypothesis for this study is not supported. The results endorse those reported after the present study was commissioned by a group of authors investigating the same group of PD patients from the Netherlands as Scholtissen et al (Leentjens et al., 2006).

It is interesting to note the absence of a significant main effect of treatment on mood given the reasonable proportion of participants having a family history for major depression – which is in contrast to the study by Leentjens et al (2006), that excluded participants with this family history – and the evidence that predisposes such people to the adverse consequences of serotoninergic imbalance (Benkelfat et al., 1994; Klaassen et al., 1999b; Ruhe et al., 2007; Sobczak et al., 2002b). ATD has been used extensively to study the role of 5-HT in mood in both healthy adult and psychiatric populations. The results of these studies have been examined in literature reviews (Bell et al., 2001; Booij et al., 2003; Fusar-Poli et al., 2006; Young & Leyton, 2002) and re-analyses (Booij et al., 2002; Ruhe et al., 2007) with a consensus that a number of people are vulnerable to adverse mood changes during this challenge. It was hypothesised – given the abnormalities in the serotoninergic system discussed in section 5.12 – that people with PD would form a similar cohort. The present finding suggests that there may be other explanations for the development of depression in PD.

A dopaminergic hypothesis has been proposed for depression in PD (for proposal and review see, Brown & Gershon, 1993) and it is possible the current findings reflect this alternative hypothesis. All patients were taking dopaminergic enhancing compounds which, if the dopaminergic hypothesis was operating, would mean adequate supplies of DA with which to resist serotoninergic challenge. However,
patients taking dopaminergic enhancing medications still get depressed and there is some evidence that the frequency of depression in PD is the same after levodopa therapy as before (Cummings, 1992; Veazey et al., 2005). Thus, a number of other explanations for the present non-significant findings are offered:

a. The inclusion criteria regarding current medications may have been too encompassing. Five patients were taking selegeline and seven were taking amantadine. Both these drugs have antidepressant effects and although amantadine is effectively dopaminergic and selegeline, at the dose prescribed to these patients – is not predicted to influence mood (Tom & Cummings, 1998) – their inclusion may have confounded the ‘purity’ of the challenge.

b. The depressive relapse induced by ATD in the sole PD patient represents an anomaly. The 55 year old man studied during the first ATD and PD study (McCance-Katz et al., 1992) had been in remission from depression for only four weeks and was still being treated with a SSRI. Compared to placebo, ATD induced a temporary return of symptoms but not so great as to be above the cut-off score for major depression on the HAM-D. People recovered from depression – irrespective of remission duration – are vulnerable to a return of symptoms during ATD (Booij et al., 2002; Van der Does, 2001a), but this finding has not been upheld in older remitted depressed patients (Porter et al., 2005), suggesting the vulnerability does not extend to older persons.

c. It is possible the present study included only a group of patients with resilience to depression. Early-in-the-course depression appears before or shortly after the onset of PD (see section 5.10) and early-in-the-course volunteers with depression were excluded from the study.

d. ATD is suitable for demonstrating presynaptic integrity but may be less sensitive to assessing post-synaptic dysfunction (Leentjens et al., 2006). There are a number of studies showing markers for pre-synaptic 5-HT activity are altered in PD (Kish, 2003), unfortunately few studies have investigated post-synaptic integrity in this condition. Although participants in the present study were telephoned the day after each testing day to check they were okay, no valid mood recordings were taken, thus, there is too little data with which to speculate on adaptive responses (see sections 3.5.5 and 4.2).

e. Depression in PD may arise as a result of a number of different mechanisms. Noradrenergic dysfunction may have an important role given the pathological involvement of NA in both PD and major depression. Post-mortem studies have reported extensive cell loss in the locus coeruleus of PD patients (Chan-Palay & Asan, 1989), while TCAs and SRNIs are both used successfully to treat depression in PD.
(Ghazi-Noori et al., 2006; Takahashi et al., 2005; Veazey et al., 2005; Zesiewicz & Hauser, 2000). Although studies comparing and combining 5-HT and NA precursors in healthy participants have not demonstrated mood effects unless accompanied by a psychological challenge (Harrison et al., 2002b; Hughes et al., 2004; Leyton et al., 1999; Matrenza et al., 2004; Nathan et al., 2002), this needs further investigation in PD.

f. Because there was a significant interaction of treatment by time on the MADRS (see Figure 8-3), it may be that older persons in general are vulnerable to the mood effects of ATD. However, due to the slower rates of precursor absorption or uptake into the brain, this means mood lowering may be experienced only at the outer extreme of the observation period. This requires further examination at observation points later than 6.5 hours. Although there was no effect of treatment by time for older remitted depressed patients when assessed with the MADRS 27 hours after initial ATD administration (Porter et al., 2005), it is still possible older persons experience mood effects from ATD during the interim 20 hours.

g. A further explanation for the treatment by time effect is that a number of people with a propensity for depression were inadvertently included in the study. That is, a number of participants unknowingly having a family history of depression.

8.7.4 Cognition
The most significant finding in support of the cognitive hypothesis was:

a. A treatment by diagnosis effect in global cognitive status, which reflected an enhanced performance by controls and impaired performance by PD patients during ATD.

However, there were other significant findings in this study:

b. A treatment by diagnosis effect in visual memory, which was due to improved performance by PD patients and impaired performance by controls during ATD.

c. A treatment by diagnosis effect in verbal memory, which was due to enhanced performance by controls and the impaired performance by patients during ATD.

d. A significant impaired performance by all participants in working memory during ATD.

Serotonin has a role in learning and memory (McEntee & Crook, 1991) and studies using ATD to investigate cognition have found diverse effects across different cognitive domains (see section 4.3). Because 5-HT inhibits the activity of ACh and DA, it could be expected ATD would facilitate enhanced performance by a group having a shortfall in these neurochemicals. However, this theory did not bear up when patients with severe cholinergic deficits experienced a worsening of global cognitive...
status during ATD (Porter et al., 2000). Possibly because of this finding in SDAT patients, the recent study by Scholtissen et al. (2007) expected to find negative effects of ATD in PD patients in their cognitive tasks. Instead they found virtually no differential effect of diagnosis and treatment. There are a number of factors that distinguish the present study from that of Scholtissen et al., not least the significant effects on global cognitive status and memory, which are discussed below.

8.7.4.1 Global Cognitive status

The Scholtissen et al. study did not include an assessment of global cognitive status. This is surprising given the findings on the 3MS in the two studies with older patients and controls (Porter et al., 2000; Porter et al., 2005) and the modulating role 5-HT has on the cholinergic and dopaminergic systems. The pattern emerging from the present study and those of Porter and colleagues, is that the 3MS is sensitive to serotonergic change in some older persons; those with SDAT, remitted depression, and now PD, but not in healthy older persons, in whom if anything, there is an improvement.

It should be noted that in both the present and the recovered depressed studies, the change in 3MS score is not a clinically significant reduction in patient score, rather, the result is indicative of a group differential in the effects of ATD on global function. It should be noted also that the 3MS may be picking up subtle changes in performance in these older patients groups purely because its psychometric properties mean it is pitched at a more appropriate level of difficulty than other more specific tests.

8.7.4.2 Verbal Memory

IWR is a recognition task with a visual-word rather than visual-pattern component. Although the controls improved their performance on this task, by comparison the performance by patients were markedly impaired. The performance of PD patients is congruent with the effects seen in healthy younger adults in episodic memory (see section 4.3.2.2). Recognition memory tasks assess the ability to acquire new information and, as such, are measures of encoding and consolidation.

Two ATD studies have demonstrated the specificity of ATD for influencing memory at the encoding and consolidation stages. The first study used event-related potentials to demonstrate that impaired delayed recall was not mirrored by brain activity in areas underscoring the retrieval process (McAllister-Williams et al., 2002). The second study used fMRI scanning to demonstrate ATD attenuated activation in the hippocampus during encoding – and possibly consolidation – but did not affect brain activity during retrieval (Van der Veen et al., 2006). In view of this neuroimaging data – and that from animal studies showing ATD reduced TRP and 5-HT levels in the hippocampus and impaired recognition memory (Lieben et al., 2004a; Lieben et al., 2004b) – it is germane to suggest hippocampal 5-HT projections are more sensitive to ATD.

The relevance of this proposition to the rationale for the present study is that patients with PD have a severe cholinergic deficit, hypothesised to differentiate performances of patients and controls.
Moreover, PD patients, with cognitive impairment, have smaller hippocampi than healthy age-matched control subjects and hippocampal volumes similar to patients with mild SDAT (Camicioli et al., 2003). Reductions in acetylcholine have been shown to disrupt encoding in both CA3 and CA1 subregions of the hippocampus (Rogers & Kesner, 2003) and combined reductions of 5-HT and ACh in this structure, to impair spatial working memory in rats. Thus, it is possible combined hippocampal, cholinergic, and serotonergic pathology accounts for the deficits in verbal memory demonstrated in the present study. In fact a scenario such as this was proposed by Richter-Levin and Segal (1993) who reviewed the animal data on 5-HT and ACh and concluded reduced serotonergic modulation of hippocampal interneuron activity and impaired modulation of cholinergic effects in the hippocampus both contribute to cognitive deficits.

This is not to say this occurred in the present study or that 5-HT is the prime modulator in this cognitive deficit. The cholinergic system is critical and other neurotransmitters systems have a role in the hippocampus and elsewhere in the brain, however, the present results are consistent with this proposal.

An alternative and more parsimonious hypothesis for the performance differential of the groups in this study is that IWR is a task requiring little cognitive demand. PD patients have been shown, under normal conditions, to improve verbal memory performance when external aids are used (Ivory, 1994; Lezak, 1995), as occurs in IWR.

The improved performance by the healthy older controls is interesting given ATD is associated with verbal memory impairment in younger adults (see section 4.3.2.2). To the extent the cholinergic system is altered with ageing, it could be hypothesised that including the RAVLT into the neuropsychological battery may have resulted in a performance that corroborated those found in studies with younger participants. However, the effect of ATD on the RAVLT across the adult lifespan was investigated by Sambeth et al. (2007) and no effects of age on verbal memory were demonstrated.

Sambeth et al. (2007) did, however, find that compared to males, female performance on the RAVLT was worse after ATD. This result suggested the differential in female and male brains after depletion noted in Nishizawa et al. (1997) manifested greater effects in females in verbal memory. The gender effect in Sambeth et al. and in Study A – the data of which was included in the meta-analysis – was not replicated in the present study. The explanation for this is that without a Bonferroni correction, the result from Study A needs to be interpreted with caution; despite a post hoc analysis showing the effect was still present in the older females, it is possible the result from the meta-analysis was still driven by the performance of the pre-menopausal women.

**8.7.4.3 Visual Memory**

There was a significant interaction between treatment and diagnosis in visual memory as assessed by the DMS task from CANTAB. Contrary to the performance in IWR, the patients’ improvement in
accuracy on DMS was accompanied by their improved ability to make a correct response on the very first touch; by comparison, control scores on this task were reduced during ATD. ATD studies have not generally demonstrated an effect in visual recognition memory. One study found an impaired performance in this task when change scores (i.e., mean difference between scores at the end of presentation and those after a 25 min delay) for individual participants were analysed, however, for the immediate trial there was no effect of treatment (Rubinsztein et al., 2001). The format of that study’s task was similar to the one used in the present study and, thus, the performance of older controls in the present research may fit with this finding. However, the significant improvement by PD patients represents a considerable deviation in both domain specificity and type of memory – visual not verbal, recognition not recall. Although not statistically significant in a repeated measures analysis of variance omnibus, enhanced performance in pattern recognition was observed post hoc in female SDAT patients (Porter et al., 2003b), which is interesting given PRM assesses encoding and that memory encoding is particularly disrupted in SDAT (Greene et al., 1996). No effects were found for PRM in the present study; however, the discrepancy between results on PRM and DMS may be due simply to differing sensitivity of the tasks in different patient groups. PRM may be sensitive to picking up performance differences for groups having a mean score of 18 on the MMSE, but have ceiling effects for those with a mean score of 29; whereas, the DMS may be more sensitive, irrespective of cognitive ability. Or, one task may be assessing memory with its recognition delay of 20 s (PRM); but the other, working memory as well as memory, with its recognition delay of 12 s and its more complicated imagery (DMS).

It has been suggested that 5-HT has different effects depending on the memory task used (Sambeth et al., 2007). Recognition tasks generally assess encoding and consolidation and have traditionally been used alongside recall tasks to differentiate the retrieval aspects of memory. Recall tasks have consistently been impaired during ATD and support provided by at least one neuroimaging study has this impairment associated with reduced hippocampal activity during encoding (Van der Veen et al., 2006). Although encoding, consolidation and retrieval do not necessarily equate to a sequential process of memory, there are different brain regions involved in memories.

Recent studies have tied both memory encoding and consolidation to the DLPFC and medial PFC regions (vmPFC studied only, Akirav & Maroun, 2006; Blumenfeld & Ranganath, 2006; review, Blumenfeld & Ranganath, 2007; Lee et al., 2000a; Rolls, 2000) and Izquierdoa et al. (2007) have speculated that the selective, time-dependent and specific receptor-mediated roles these two areas have in modulating this process can be mapped to the same role they have in immediate memory for congruent information.

There are strong anatomical connections between the DLPFC and areas involved with memory, like the hippocampus (Hyman et al., 1990; Izquierdo et al., 2007). The accruement of work by Raymond Kesner and colleagues (Kesner, 1998; Kesner et al., 1993; Kesner & Hopkins, 2001, 2006) suggests the hippocampus may not be as important as the cerebral cortex for maintaining visual object
information in short-term memory; as demonstrated by the unchanging performance of patients with bilateral hypoxia-induced damage to the hippocampus during a DMS task with delay intervals from 1-20 s (Kesner & Hopkins, 2001). The implication is that the cerebral cortex is important for the initial stage of memory formation but that the hippocampus is essential for the consolidation of new information into memory (Squire & Knowlton, 2000). It is speculative, but what may have been occurring for the PD patients in the present study, is an effect of ATD on frontal as well as hippocampal function and the improvement in aspects of the DMS underscored by this region; but in SDAT patients, the effect of ATD was in a task (i.e., PRM) underscored by hippocampal activity.

It is possible the differential performances of PD and SDAT in DMS and PRM may be due to the characteristics of the tasks, as discussed above. However, recent imaging and computational modelling studies have demonstrated the involvement of the hippocampus in both visual and verbal recognition memory (Stark & Squire, 2001) and – at least in simulated pattern recognition memory – this involvement occurs at the 5-HT_{1A} receptor site (Meeter et al., 2006). It is possible given 5-HT_{1A} receptors have an inhibitory function in the hippocampal formation (Yasuno et al., 2003) – presynaptically on glutamate interneurons and post-synaptically on GABA interneurons (Aghajanian & Sanders-Bush, 2002) to modulate ACh release (Barnes & Sharp, 1999) – that ATD manipulated the ability of the SDAT patients in the Porter (2003b) study and the PD patients in the present study to recognise patterns by reducing activity at these sites.

Note: the gender by treatment interaction on response time in DMS is an isolated result and is likely to have occurred by chance. The a priori decision to look for patterns in the results meant the Bonferroni correction was not used; if it had then this result may not have arisen with the altered significance level.

8.7.4.4 Working Memory

There were significant main effects of treatment on SWM and SSP with both showing decrements during ATD. There were no effects in other tasks having a working memory component (e.g., DigitsF, DigitsB, DOT), which is interesting given performance on DigitsF was significantly affected by ATD in Study A. The reason this was not replicated in the present study is because the significant effect in Study A was the interaction between treatment, dose and gender. Participants in the present study had a high dose of treatment.

Despite population studies showing impaired performances by PD patients in spatial working memory and executive function tasks (see section 5.11.1.1), there was no diagnosis by treatment differential in tasks of this type in the present study.

Working memory impairments have been found in two other ATD studies with older persons (Porter et al., 2003b; Porter et al., 2005) but generally not in studies with younger adults. A possible explanation for this is that working memory is affected during ATD but that some tasks are not sensitive to picking this up in some groups. Tentative support for this specificity and sensitivity comes
from a recent ATD study showing altered activation in the DLPFC and medial PFC during a 2-back verbal working memory task, despite the absence of significant score differences (Allen et al., 2006). Also, from ATD studies demonstrating increased activation in the medial PFC, ACC and areas of the DLPFC in the absence of effects on two executive function tasks (Evers et al., 2005; Horácek et al., 2005). Working memory tasks that monitor and manipulate – as well as maintain information in working memory – like SWM and SSP (D'Esposito et al., 1999; Klingberg et al., 1997; Manoach et al., 1997; Owen et al., 1996b; Salmon et al., 1996; Tsukiura et al., 2001) – are known to activate areas in the DLPFC (Smith & Jonides, 1998; Smith et al., 1998a), thus it may be possible that ATD is facilitating a change in brain activity which is not always reflected in test scores.

It has previously been reported that supranormal levels of 5-HT may be deleterious in spatial working memory and from this, it could be argued, a severe reduction would facilitate an enhancement (Luciana et al., 1998). However, the opposite occurred in two spatial working tasks during ATD in the present study. It is worth noting the possibility that older persons are more sensitive to the effects of ATD when performing working memory tasks; this has certainly been demonstrated before (Porter et al., 2003b; Porter et al., 2005).

8.7.4.5 Attention and Consciousness

There were no effects on the four measures used to assess fluctuating consciousness or attention: SRT, CRT, DV, and ODFAS. This included a comparison of the three observation times for the CRT that were staged across the afternoon testing period. ATD has previously been shown to improve performance during focused attention tasks but not during sustained attention tasks (see section 4.3.6.). Sustained attention is impaired in patients with carcinoid syndrome after ATD. This is a condition associated with disturbed 5-HT metabolism and high levels of excreted 5-HIAA (Russo et al., 2003b) so it is interesting that the performance of PD patients – who likewise experience aberrations in the serotonergic system – on sustained attention tasks, was not affected during ATD. The lack of scoring on the ODFAS in this group of non-demented PD patients, on the other hand, is not surprising given the items in this task are designed to assess extreme forms of altered consciousness, as occurs in DLB.

8.7.5 Order Effects

Given the crossover design of this study, there was always the possibility of practice and novelty effects, despite the fact that, in most cases, some tasks were familiar at time of testing on the first visit and reliable repeat versions were used. Practice effects generally result in scores being higher on the second visit. Essentially, if there are practice effects, order by treatment interactions indicate there is a significant departure from an equal increase from first to second visit between the groups – in this case referring to the group who received placebo first (placebo/ATD) versus the group who received placebo second (ATD/placebo). Novelty effects generally result because unfamiliarity with the test or the testing environment prompts a better response; treatment by order interactions indicate a significant departure from this pattern. The departures can be explained by the action of the
manipulation combining with the characteristics of the groups, despite their being matched for age, gender ratio and PVIQ. The nature of the task is also important since it may have different properties depending on when it is presented; it may be more sensitive when a participant is unfamiliar with it, but reach ceiling when a participant is familiar. Interpreting these interactions is complex, especially when there are a number of them and they don’t conform to a congruent pattern of cognitive function. In the present study, the treatment order interactions in section 8.6.1.10 are interpreted as follows:

a. There were practice effects with 3MS, VOSP Silhouettes, and SRM Accuracy which were magnified by ATD. Performance the second visit on the 3MS, VOSP, and SRM was enhanced when ATD was given on the first visit, suggesting a carry-over effect of learning.

b. There were novelty effects on DigitsF, SSP, SWM, and POMS (note: higher scores on SWM reflect worsened performance and on POMS poorer mood). These effects were enhanced on SSP, SWM and POMS, but lessoned on DigitsF, when ATD was given on the first visit, that is, when the task was unfamiliar.

c. Irrespective of practice or novelty effects, ATD enhanced performance on DOT and PRM when the task was familiar.

d. There was a practice effect on VOSP Silhouettes shown by the two diagnostic groups having placebo second (ATD/placebo) and the control group having placebo first. However, when ATD was given to the group of PD patients having placebo second, it negated any practice effect and impaired performance.

8.8 Conclusion
The present research corroborates previous studies by demonstrating impairment in verbal memory, but also a specific sensitivity of older patient groups, but not in healthy controls, to impairment in global cognitive status during ATD. Moreover, working memory is impaired in older persons, irrespective of disease or health. Conversely, visual memory and psychomotor performance by PD patients is enhanced compared with the effects in healthy controls.

The present study – and those of Porter and colleagues (Porter et al., 2003b; Porter et al., 2005) – has demonstrated a particular sensitivity of the 3MS to the effects of ATD.

The finding in verbal memory is consistent with that found in other ATD studies and – although this has not been observed in older groups – when data from older patients were included in a meta-analysis of studies (Sambeth et al., 2007), a significant impairment was found.

Performance on working memory tasks is impaired in older – but not younger – adults during ATD. Although an independent effect of age was not found in the pooled analysis of Sambeth et al. (2007), the combination of an effect observed in three studies (this one plus, Porter et al., 2003b; and Porter et al., 2005) is suggestive of impairment. Neuroimaging studies have demonstrated ATD does have an
effect on neural activity, even when this is not evidenced in the scores of cognitive tasks (Allen et al., 2006; Evers et al., 2005; Horácek et al., 2005).

The participants demonstrated resilience on all other neuropsychological tasks; likewise, to ratings on some of the movement and mood assessments. The effects of ATD are subtle and were not found in many areas during the present study. This suggests that, even in PD and ageing, the 5-HT system is still relatively resilient in the face of such a profound challenge.
9 STUDY C: THE EFFECTS OF ACUTE TRYPTOPHAN DEPLETION ON MOVEMENT, MOOD AND COGNITION IN HEALTHY OLDER PERSONS

9.1 Introduction
The serotonergic system is altered during ageing but how this is manifested in behavioural terms is not clear. Older age can be associated with depression and limited mobility, and decreased cognitive function may be considered a consequence of advancing age for some people (Jolles et al., 1995). Much of the research into the biochemical basis of behaviour has tended to be around developing pharmaceutical strategies for pathological conditions and little is known about psychoneuropharmacology in healthy older persons. ATD is a technique that has been successfully utilised to examine the role of serotonin in behaviour in healthy young adults and in pathological conditions. It is perfectly suited to investigate these same factors in healthy older persons. It is also suitable for examining gender differences, as has been demonstrated in the past (Moreno et al., 2006; Sambeth et al., 2007; Schmeck et al., 2002).

As noted, there are age-related changes in the dopaminergic, noradrenergic, cholinergic and serotonergic neurotransmitter systems. These changes are accompanied in normal ageing also by lowered volumes of grey matter which occur as a consequence of reduced synaptic density (Hedden & Gabrieli, 2004). The pattern of this reduced volume is not uniform across the brain, with the PFC, striatum, and medial temporal lobe being areas most susceptible to change. Specifically, as per Hedden and Gabrieli (2004),

a. Frontostriatal The PFC has the greatest volumetric change in adulthood, with an average decline of 5% per decade after age 20. There are also reliable smaller declines per decade in the striatum of around 3%. These reductions are accompanied by reductions in DA and 5-HT concentrations, transporters, receptor densities, and markers; all of which affect the integrity of frontostriatal circuitry and, ultimately, the ability of older persons to optimise performance in cognitive tasks. An example of this has been demonstrated with the correlation between D$_2$ receptor availability and performance on tasks of attention, response inhibition, also processing speed and episodic memory. Poorer performances on executive function and memory tasks, along with slower information processing speed, are associated with abnormalities in white matter, which in older persons, have been shown to be reduced in anterior brain regions. It could be argued that these changes contribute to cognitive deficits by interfering with the connections between the PFC and the striatum, or the hippocampus, or both.

b. Hippocampus and Medial Temporal Lobes In contrast to the PFC, anatomical studies have produced little degeneration in these structures in the normal ageing brain. More recently, imaging studies have demonstrated 2-3% decline in hippocampal and
parahippocampal gyrus volume, with the possibility of 1% reductions per annum after age 70. The degree of volumetric reductions is hypothesised to predict explicit memory performance after age 60. As activity in these structures is accompanied by changes in PFC activity relative to healthy younger adults, it is also hypothesised older people engage frontal regions to compensate for reduced hippocampal function.

The present study was instigated to investigate the effect of ATD in older persons with the understanding that age-related alterations observed in the serotonergic system, even if small, may combine with alterations in other neurotransmitter systems. For example, 5-HT₂ receptor numbers are reduced sharply in middle age in the frontal cortex and has been correlated with declines in striatal DA receptors (Wang et al., 1995). To the extent these 5-HT receptors activate cells in the SNpr, it may be this reflects the interaction between the serotonergic, noradrenergic, and dopaminergic systems in the frontostriatal circuit. That is, mirrored action by either system to compensate for reductions in the other. If this were the case, then it is likely functions mediated by these anatomical regions and these neurotransmitter systems would be affected. For example, mood, some aspects of movement, executive function, working memory, attention, and processing speed.

It is known that the above cognitive functions, along with encoding information into episodic memory, are the most likely to decline in older persons, and they do so in a consistent and continuous manner (Craik et al., 1995; Hedden & Gabrieli, 2004; Nilsson, 2003). It is also known that persons who live to the eighth decade (the old-old) will experience a sharp decline in short-term memory around this time (Hedden & Gabrieli, 2004). Moreover, the progression of age exposes a greater risk for the cognitive deficits associated with pathological changes in the CNS; cognitive deficits that may, or may not, be attributable to diseases such as SDAT and PD. It is essential to study the brain over the life span, not just to determine the presence of vulnerability in the CNS, but to establish age-equivalent norms with which cognitive data from neurodegenerative conditions can be compared. What is not known is the extent to which the serotonergic system accentuates or attenuates the changes where they occur.

As noted in the previous chapter, there have been several studies investigating 5-HT function in a range of cognitive domains, but none, including Study A, in a comprehensive and systematic manner over a wide range of cognitive tasks in older persons. This raises a number of issues:

a. There is only one study that examined the effect of age (Sambeth et al., 2007). However, analysis and inferences were limited to one task of verbal memory.

b. The majority of cognitive ageing research compares the performances of older with younger persons (mostly students). Middle age is a time when neuronal loss occurs and to some extent this manifests in cognitive decrements that can affect work and relationships (Jolles et al., 1995). Some aspects of the serotonergic system decline more in mid than late life (Sheline et al., 2002) and for these reasons research needs to include people aged in their 50s and 60s.
c. Studies investigating ATD in older adults (Leentjens et al., 2006; Porter et al., 2003b; Porter et al., 2000; Porter et al., 2005; Scholtissen et al., 2006b) and Study A, have not compared mood and cognitive effects between the younger-old and the older-old.

d. There is growing consensus that the role of the serotonergic system is in maintaining healthy episodic memories at the encoding and consolidation phases. There is also the possibility that this system modulates attention and frontally directed cognitions (see sections 4.3.3.2 and 4.3.6). To date, there are no data that robustly confirms these effects in older persons or across gender.

e. A pattern is emerging that ATD impairs global cognitive status in older patient groups. In contrast, ATD has been shown to not alter performance significantly in healthy older persons (Porter, 2005).

It has previously been reported that recovered depressed patients are at risk for mood lowering during ATD, yet this has not been replicated in older patients (Porter et al., 2005). There is a possibility that the serotonergic system is more resilient to challenge in older persons – as well as healthy younger adults – since ATD had no effect, likewise, on cortisol levels (Porter et al., 2007b). Interestingly, older persons are treated with SSRIs and – notwithstanding the beneficial effects for many patients and, therefore, the implication that the serotonergic system is involved in depression – there is still relatively little evidence-based data for efficacious use in older persons without comorbidity (Taylor & Doraiswamy, 2004). Moreover, in older persons, these drugs have been linked to the development of extrapyramidal side effects (Gormley et al., 1997) and falls (Hartikainen et al., 2007), which suggests the serotonergic system may be implicated in some aspects of movement.

9.2 Aim

The aim of the present study was to use the technique of ATD to investigate the effect a reduction in central serotonin would have in some aspects of behaviour in healthy adults aged over 50 years and to investigate both younger-old and older-old non-patient groups. A large number of assessments were utilised covering a wide range of cognitive domains. Study A in elderly participants demonstrated an association between ATD and poorer performance on tasks of verbal memory, executive function and working memory in females having a 100 g dose. The pattern of memory impairment at the encoding and consolidation stage has been well established in younger persons. The effect on working memory is inconclusive in both younger and older cohorts. The present study sought to clarify this uncertainty with a number of working memory and attentional tasks, as a complement to DigitsF. It also sought to examine the differential effect of gender during ATD, given younger females were more likely to experience mood lowering and older females more likely to demonstrate cognitive impairment. Assessments of movement and mood were included also in the battery.

Hypothesis 1 ATD will be associated with impaired working memory with increasing age.
The rationale for this hypothesis is that DigitsF and DigitsB were impaired in older persons during two ATD studies (Porter et al., 2003b; Porter et al., 2005) and in Study A. These results raised the potential of a differential effect of ATD in working memory in older compared to younger adults, which required further investigation.

9.3 Method
The study comprised healthy adults aged over 50 years divided into two groups based on age; young-old: 50-69 yrs; old-old: 70-89 yrs. The participants comprised a pool of people from which the control group in Study B was formed.

The design, procedure and assessment of this study followed that outlined for Study B.

9.3.1 Analysis
The analysis was the same general plan as that outlined in Study B (see section 8.5) except diagnosis was not entered as a variable and age was entered as a covariate. If there was a significant interaction of treatment by age, the ANOVA was rerun as a post hoc analysis with age converted from a covariate to a between-subject variable, with young-old and old-old as the two participant groups.

Study B also outlined the analysis protocol for the ANOVA which was run with order as a variable and level or time as factors. That is, if there were no interactions of treatment by order, level, or time then the analysis was re-run without them. This meant a dependent variable could have 3 (treatment, age, gender), 4 (treatment, age, gender, order or level or time), or 5 (treatment, age, gender, order and level or time) factors entered into the analysis.

In all respects the analysis for Study C was the same as that outlined in Study B, except Composite Tremor score was not assessed.

9.3.2 Statistical
The procedure for statistical analysis is outlined in section 6.4. Free TRP level at baseline on both test days was compared with a paired samples \(t\)-test to see if there was a significant difference in the levels at this time. An independent samples \(t\)-test was done for the UPDRS screening score to see if there was a significant difference between groups.

9.3.2.1 Power Calculation
Based on a previous study in older persons (Porter et al., 2003b), the primary outcome of this research was score on DigitsB. The study was powered to have 80% power to show a 1 point difference in the means \(SD_{\text{diff}} = 1.4\) of ATD and placebo, when \(n = 40\), at a two-tailed significance level of .05.

9.4 Results
There were 43 participants accepted for the study, 35 of whom formed the control group for Study B. The demographic data are shown in Table 9-1. All women in the present study were menopausal and not on hormone replacement therapy. All participants completed both test days.
9.4.1.1 **Demographic**

There were no differences between the groups on all demographic variables and baseline scores.

**Table 9-1 Study C. Means and Standard Deviations for Demographic Variables**

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Young-Old</th>
<th>Old-Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age</td>
<td>69.23</td>
<td>11.22</td>
<td>50-88</td>
</tr>
<tr>
<td>PVIQ</td>
<td>110.95</td>
<td>10.34</td>
<td>90-128</td>
</tr>
<tr>
<td>Educ Stage</td>
<td>3.44</td>
<td>1.39</td>
<td>2-5</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.37</td>
<td>1</td>
<td>27-30</td>
</tr>
<tr>
<td>MADRS</td>
<td>0.21</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>POMS</td>
<td>9.4</td>
<td>9.96</td>
<td></td>
</tr>
<tr>
<td>UPDRS</td>
<td>1.34</td>
<td>2.04</td>
<td></td>
</tr>
</tbody>
</table>

n (m/f) | 43(22/21) | 21(10/11) | 22(12/10) |

Note: M = mean; SD = standard deviation; n = number; m = male; f = female; MADRS = Montgomery-Asberg Depression Rating Scale; MMSE = Mini Mental State examination; POMS = Profile of Mood States; PVIQ = predicted verbal IQ (from the NART: National Adult Reading Test); UPDRS = United Parkinson’s Disease Rating Scale.

9.4.1.2 **Family History of Depression**

Of the 43 participants 27.9% had a family history of depression.

9.4.1.3 **Somatic Effects**

The amino acid drinks were well tolerated with no serious side effects. No participant vomited but irrespective of treatment, 37% of participants experienced nausea during the morning of both test days and 19% experienced diarrhea on the day following both test days. Table 9-2 records the somatic effects for each gender for each treatment arm.

**Table 9-2 Study C. Number of Participants Experiencing Somatic Complaints**

<table>
<thead>
<tr>
<th></th>
<th>Nausea</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo t(m/f)</td>
<td>Depletion t(m/f)</td>
</tr>
<tr>
<td>Participants</td>
<td>7 (5/2)</td>
<td>8 (4/4)</td>
</tr>
</tbody>
</table>

Note: t = total; m = male; f = female.

9.4.2 **All Measures**

The significant main effects of treatment and interactions between treatment and other variables are shown in Table 9-3. The means and standard errors for all main effects and interactions are presented in Appendix I.

9.4.2.1 **Missing Data**

Participants were excluded from the analysis on particular dependent variables if their data were incomplete and this is represented in the degrees of freedom for each variable. These are listed following as Variable (n = m/f): Biochemical (2/8); POMS (2/1); PRM Accuracy and Reaction time (0/1); CRT Accuracy and Reaction time (2/1); SWM Between errors (0/1).
Table 9-3 Study C. Effects of Treatment (ATD vs Placebo) on All Variables
Repeated Measures ANOVA

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>Treatment (F)</th>
<th>Treatment x Age (F)</th>
<th>Treatment x Order (F)</th>
<th>Treatment x Gender (F)</th>
<th>Treatment x Time (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>free TRP 4 hrs</td>
<td>1,30</td>
<td>5.23 *</td>
<td>22.03 ***</td>
<td>2.86 ***</td>
<td>4.75 *</td>
<td></td>
</tr>
<tr>
<td>MADRS Total</td>
<td>1,40</td>
<td>1.26</td>
<td>1.10</td>
<td></td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>POMS Total</td>
<td>1,35</td>
<td>0.09</td>
<td>0.19</td>
<td></td>
<td>15.26 *</td>
<td>2.14</td>
</tr>
<tr>
<td>UPDRS Total</td>
<td>1,40</td>
<td>4.61 *</td>
<td>5.65 *</td>
<td></td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>MOT Accuracy</td>
<td>1,40</td>
<td>0.08</td>
<td>0.05</td>
<td></td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>MOT Reaction time</td>
<td>1,40</td>
<td>0.17</td>
<td>0.62</td>
<td></td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>3MS Total</td>
<td>1,40</td>
<td>0.38</td>
<td>0.12</td>
<td></td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>MMSE Total</td>
<td>1,40</td>
<td>1.06</td>
<td>1.32</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>DigitsF Total</td>
<td>1,38</td>
<td>1.09</td>
<td>0.97</td>
<td></td>
<td>7.77 **</td>
<td>1.43</td>
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<tr>
<td>DigitsB Total</td>
<td>1,40</td>
<td>0.06</td>
<td>0.01</td>
<td></td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>DOT Total</td>
<td>1,38</td>
<td>1.86</td>
<td>1.36</td>
<td></td>
<td>4.15 *</td>
<td>1.66</td>
</tr>
<tr>
<td>SSP Span</td>
<td>1,40</td>
<td>0.28</td>
<td>0.01</td>
<td></td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>SWM BetweenErrors</td>
<td>1,37</td>
<td>2.01</td>
<td>2.55</td>
<td></td>
<td>5.99 *</td>
<td>0.64</td>
</tr>
<tr>
<td>COWA Total</td>
<td>1,40</td>
<td>0.67</td>
<td>0.95</td>
<td></td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>COWA V errors</td>
<td>1,40</td>
<td>0.58</td>
<td>0.70</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>COWA R errors</td>
<td>1,40</td>
<td>4.01</td>
<td>3.89</td>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>VOSP IncomLetters</td>
<td>1,40</td>
<td>1.40</td>
<td>1.25</td>
<td></td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td>VOSP Silhouettes</td>
<td>1,38</td>
<td>1.28</td>
<td>0.96</td>
<td></td>
<td>28.74 ***</td>
<td>0.32</td>
</tr>
<tr>
<td>PRM Accuracy</td>
<td>1,39</td>
<td>0.01</td>
<td>0.19</td>
<td></td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>PRM Reaction time</td>
<td>1,39</td>
<td>0.52</td>
<td>0.64</td>
<td></td>
<td>0.45</td>
<td></td>
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<tr>
<td>SRM Accuracy</td>
<td>1,38</td>
<td>0.81</td>
<td>0.40</td>
<td></td>
<td>27.25 ***</td>
<td>0.38</td>
</tr>
<tr>
<td>SRM Reaction time</td>
<td>1,40</td>
<td>3.24</td>
<td>3.96</td>
<td></td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td>SMTS Accuracy</td>
<td>1,40</td>
<td>0.00</td>
<td>0.02</td>
<td></td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>SMTS Reaction time</td>
<td>1,40</td>
<td>2.39</td>
<td>3.09</td>
<td></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>DMS Accuracy</td>
<td>1,38</td>
<td>0.09</td>
<td>0.48</td>
<td></td>
<td>10.23 **</td>
<td>4.11 *</td>
</tr>
<tr>
<td>DMS Reaction time</td>
<td>1,40</td>
<td>0.00</td>
<td>0.01</td>
<td></td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>DMS %Corr</td>
<td>1,38</td>
<td>0.06</td>
<td>0.46</td>
<td></td>
<td>12.57 **</td>
<td>4.51 *</td>
</tr>
<tr>
<td>IWR SI</td>
<td>1,40</td>
<td>0.00</td>
<td>0.03</td>
<td></td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>DWR SI</td>
<td>1,40</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>CRT Accuracy</td>
<td>1,35</td>
<td>2.83</td>
<td>2.27</td>
<td></td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>CRT Reaction time</td>
<td>1,37</td>
<td>2.57</td>
<td>4.09 *</td>
<td></td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>SRT Reaction time</td>
<td>1,40</td>
<td>3.62</td>
<td>3.86</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DV Accuracy</td>
<td>1,40</td>
<td>2.16</td>
<td>2.20</td>
<td></td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>DV Reaction time</td>
<td>1,40</td>
<td>5.05 *</td>
<td>4.57 *</td>
<td></td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>VAS Nausea</td>
<td>1,38</td>
<td>0.02</td>
<td>0.06</td>
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<td>2.96</td>
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Note: COWA = Controlled Oral Word Association test; CRT = Choice Reaction Time; DigitsB = Digit Span Backward; DigitsF = Digit Span Forward; DMS = Delayed Matching to Sample; DWR = Delayed Word Recognition; DOT = Digit Ordering Test; DV = Digit Vigilance; IWR = Immediate Word Recognition; MADRS = Montgomery-Asperg Depression Rating Scale; MOT = Motor Screening; MMSE = Mini Mental State examination; MADRS = Montgomery-Asperg Depression Rating Scale; MOT = Motor Screening; MMSE = Mini Mental State examination; POMS = Profile of Mood States; PRM = Pattern Recognition Memory; Rerrors = repetition errors; SMTS = Simultaneous Matching to Sample; SRM = Spatial Recognition Memory; SWM = Spatial Working Memory; TRP = tryptophan; UPDRS = United Parkinson’s Disease Rating Scale; VAS = Visual Analogue Scale; Verrors = violation errors; VOSP = Visual Object and Space Perception test.

*p ≤ .05. **p < .01. *** p < .001.

No participant scored on this assessment in either treatment.
9.4.2.2 Biochemical

Measurements are expressed in nanograms per millitre (ng/ml). To reiterate, the minimum measurement of TRP is 500 ng/ml, thus reductions in TRP levels may be lower than the data suggest.

At baseline, there was no significant difference in free TRP between ATD ($M = 1670.64$, $SD = 126.56$) and placebo ($M = 1700.92$, $SD = 119.87$; $t_{35} = 1.72$, $p = .86$). There was a main effect of treatment with free TRP levels being lower during ATD ($M = 899.09$, $SE = 42.03$) than during placebo ($M = 3080.38$; $SE = 175.48$; $F_{1,30} = 5.23$, $p = .029$). There was a significant interaction of treatment by time ($F_{1,30} = 4.76$, $p = .012$); during ATD this represented a 69% reduction in free TRP at 4 hrs and 69% at 6.5 hrs; also a 177% increase in free TRP at 4 hrs and 67% at 6.5 hrs. The means and standard errors for these are displayed in Figure 9-1.

![Figure 9-1 Effect of treatment on free TRP level at different observation times.](noimage)

9.4.2.3 Mood

There were no significant effects of treatment on mood.

9.4.2.4 Movement

There was a main effect of treatment on UPDRS score ($F_{1,40} = 4.61$, $p = 0.03$). Participants’ scores were worse during ATD ($M = 1.40$, $SE = 0.33$) compared to placebo ($M = 1.12$, $SE = 0.22$). There was a significant interaction of treatment by age ($F_{1,40} = 5.65$, $p = 0.022$). Movement ability of the old-old group was more disabled during ATD ($M = 2.34$, $SE = 0.51$) compared to placebo ($M = 1.76$, $SE = 0.34$), while there was little difference in the scores of the young-old during ATD ($M = 0.39$, $SE = 0.52$) compared to placebo ($M = 0.46$, $SE = 0.34$), as shown in Figure 9-2.
9.4.2.5 Neuropsychological

Memory

There was a significant interaction of treatment by gender on DMS Accuracy \((F_{1,38} = 4.11, p = .05)\). The performance by males was worse during ATD \((M = 76.71, SE = 2.66)\) compared to placebo \((M = 86.00, SE = 1.95)\), while there was little difference in the performance of females during ATD \((M = 83.68, SE = 2.71)\) compared to placebo \((M = 84.62, SE = 1.99)\), as shown in Figure 9-3.

There was a significant interaction of treatment by gender on DMS %Corr \((F_{1,38} = 4.41, p = .04)\). Male performance was worse during ATD \((M = 80.85, SE = 2.10)\) compared to placebo \((M = 88.20, SE = 1.57)\), while there was little difference in the performance of females during ATD \((M = 86.77, SE = 2.14)\) compared to placebo \((M = 87.61, SE = 1.60)\), as shown in Figure 9-4.
Psychomotor

There was a main effect of treatment on Digit Vigilance Reaction time ($F_{1,40} = 5.05, p = .03$). Participants had a faster response time during ATD ($M = 429.27, SE = 6.36$) compared to placebo ($M = 432.81, SE = 5.64$), as shown in Figure 9-5. There was a significant interaction of treatment by age on Digit Vigilance Reaction time ($F_{1,40} = 4.57, p = .04$). The young-old group performed faster during ATD ($M = 420.30, SE = 9.42$) compared to placebo ($M = 429.391, SE = 8.22$), whereas there was little difference in the performance of the old-old group during ATD ($M = 436.86, SE = 9.23$) compared to placebo ($M = 435.91, SE = 8.06$), as shown in Figure 9-6.
There was a significant interaction of treatment by age on CRT Reaction time ($F_{1,37} = 4.09, p = 0.05$). The reaction time of the old-old group was slower during ATD ($M = 494.08, SE = 11.12$) compared to placebo ($M = 479.22, SE = 9.95$), whereas there was little difference in the reaction time of the young-old group during ATD ($M = 452.90, SE = 11.69$) compared to placebo ($M = 450.63, SE = 10.47$), as shown in Figure 9-7.

9.4.2.6 Treatment Order

Twenty one (21) participants received the placebo drink first and ATD second (placebo/ATD) and 22 received the ATD drink first and placebo second (ATD/placebo). The ANOVA showed eight significant interactions of treatment by order, which are presented in Table 9-3 and graphed in Figure 9-8. There was no consistent pattern to the treatment by order effects because sometimes the performance of the ATD/placebo group was influenced by the interaction while at other times; it was the placebo/ATD group.
9.5 Discussion

The present study is the first to study the influence of age and gender specifically in healthy older persons during ATD. A wide range of assessments were administered to 43 healthy older persons, of whom 22 were male and 21 were female.

The principal findings for the present study were:

a. ATD was associated with a significant reduction in free TRP.
b. Motor function in the old-old group was worse after ATD.

c. No effects of ATD on mood.

d. ATD was associated with a slowed reaction time in the oldest group.

e. A gender differential in visual recognition memory, such males recognised fewer patterns on their first attempt.

Previous results from older participants have come from studies investigating patient populations with the healthy control group supplying the data (i.e., Leentjens et al., 2006; Porter et al., 2003b; Porter et al., 2005; Scholtissen et al., 2006b). These studies report ATD was not associated with mood changes. With regards to cognitive function, ATD has been associated with either non-significant effects on tasks assessing a range of cognitive domains or with impaired encoding and consolidation. This impairment has been demonstrated in a recent study with combined group of patients and healthy controls when ATD impaired delayed recall and recognition memory (Scholtissen et al., 2006b) or in females only (Study A). Impairment in working memory has also been demonstrated in a combined group of patients and healthy controls combined (Porter et al., 2005) or in females only (Study A).

9.5.1 Biochemical
The reduction in free TRP after ATD, although smaller than the range reviewed by Hood et al. (2005), was as expected; as was the increase after administration of the placebo treatment. The smaller percentage reduction in the present study may be attributable to the assay artifact, discussed in section 8.7.1, or the slower absorption of older persons. A comparison of the raw data from this study with those of Porter and colleagues (2000, 2005), however, revealed a particularly consistent, across-the-board reduction by the present participants. This, plus the larger sample size, suggests the slightly lower percentage increase is more likely to be a result of the artifact.

9.5.2 Movement
ATD was associated with a change in scores on the main measure of movement. However there are three factors which place doubt on the suitability of the UPDRS for assessing movement in this study.

a. The UPDRS is an assessment of motor function in PD and is not generally used to assess motoric function in healthy people (Scholtissen et al., 2006b); it was included in the present study because data was required on this assessment for Study B and it was important all participants were administered the same type, number and order of assessments.

b. There were no significant effects on the other movement assessment, MOT.

9.5.3 Mood
In accord with most ATD studies in healthy people, there were no effects on mood in this cohort of older persons. The non-significant finding occurred in the presence of approximately one third of the
participants having a family history of depression and a hypothesised predisposition to a mood lowering response to ATD.

9.5.4 Neuropsychological

The most significant finding for the neuropsychological hypothesis of the present study was that no measure of working memory was significantly affected by ATD. The hypothesis was based on the ATD associated impairment on DigitsF from Porter et al (2005) and Study A. Although not a prerequisite to the hypothesis, the main effect on spatial working memory in Study B suggested older persons may be more challenged in this domain during ATD. This was examined in Study C with a larger sample size.

9.5.4.1 Global Cognitive Status

The lack of significant effects in the global cognition tasks is consistent with effects found in other ATD studies in healthy older persons (Study A, Study B and, Porter et al., 2000).

9.5.4.2 Memory

The significant gender by treatment interaction on DMS did not fit the pattern observed in the present study or the pattern of results in other ATD studies. The a priori decision not to apply the Bonferroni correction and to look for patterns among the results, meant this was more likely to be an isolated occurrence due to chance.

It is interesting – given the strong indication in the literature that ATD affects verbal memory – that this was not found in the present study which comprises a large group of participants. It may be the IWR task is not sensitive to the effects of ATD and a more responsive task, like the RAVLT, could have shown a result. Unfortunately, this task was not included in the battery (see section 10.2.3).

9.5.4.3 Working Memory

It is interesting also that there were no significant working memory effects in the present study, given the suggestion of an effect on the Digit Span task in the literature (Porter et al., 2003b; Porter et al., 2005) and in Study A; also in SWM and SSP in Study B and, to a lesser extent, the working memory component of the RAVLT in the Sambeth et al (2007) study. This had not been observed in younger adults but the emerging pattern was suggesting something else going on for older persons. The present study had the advantage of comprising a larger group of healthy persons and it may be that the effect is observed only in studies comprising mixed patient and healthy groups, e.g. patients with SDAT, recovered depression, and PD. That is, patient groups may be more susceptible to ATD effects in working memory and this may confuse interpretations of the effect.

9.5.4.4 Psychomotor Speed

The most significant finding with respect to age is the effect on psychomotor speed. The young-old group speeded up their responses on DV, but on DV and CRT the old-old group slowed down. There
were no accompanying effects on accuracy in either of these tasks suggesting a true effect of psychomotor speed rather than a response tendency.

It is possible, because the means for the old-old group were only milliseconds apart and the standard deviations overlapped, that the overall effect of ATD on DV was driven by the response of the young-old group. The impaired performance by the old-old in two aspects of psychomotor function is very interesting, especially since ATD improved aspects of this in the PD patients in Study B (See section 8.6.2.4), and in PD patients and controls from the Scholtissen et al. study (2007). It may be that very old persons have fewer resources in terms of increasing psychomotor speed. Whether this comes from limited neurotransmitter reservoirs or neuronal disruptions as a consequence of other pathology is uncertain. Unquestionably, increasing age is associated with lower concentrations of 5-HT, ACh, DA, and NA. It is also associated with neuronal disruption. For example, disruptions to frontostriatal-thalamic circuitry as a consequence of hyperintense lesions have been correlated with cognitive slowing in normal ageing. It has been shown that most persons aged over 60 have underlying cortical and subcortical hyperintensities (de Leeuw et al., 2001) and – at least in persons aged over 70 – that a significant association exists between hyperintensities in these particular regions and psychomotor speed (O’Brien et al., 2002). It may be argued that during ATD, this pathology constrains the degree of adaptation shown in the young-old group to this challenge.

To the extent the effect on DV, at least, was not explained by the old-old performance, it is possible ATD affected vigilance and psychomotor speed secondary to activity in other neurotransmitter systems. In the young-old group this may have meant one or a number of factors attributable to other neurotransmitter systems. For example, heightened arousal and alertness to external stimuli via the noradrenergic system, increased stimulus processing in cortical regions by the cholinergic system, or increased activation in cognition and motor function through the dopaminergic system (Robbins, 1997). Several studies have examined reaction time in vigilance or specific reaction time tasks during different challenges to biogenic neurotransmitter systems and, while there is demonstrable modulation in reaction time by all systems, the only consistent pattern came from precursor studies providing non-significant results. For example:

a. DA augmentation with acute L-dopa had no effect in PD patients (SRT and CRT, Molloy et al., 2006), while antagonism with haloperidol impaired reaction time in healthy older persons (DV, Beuzen et al., 1999). NA augmentation both improved reaction time and had no effect (Coull, 1998); while NA antagonism impaired it (Coull, 1998) or had no effect (CRT, Nathan et al., 2000). Acute tyrosine/phenylalanine depletion (ATPD) had no effect on reaction time in both young adults (Stroop, Scholes et al., 2007) and female participants studied (DV, SRT, and CRT, Harrison et al., 2004).
b. 5-HT augmentation with some SSRIs has been associated with improved psychomotor speed (CRT, Nathan et al., 2000; Schmitt et al., 2002), while ATD had no effect in the young adults (TOL, Booij et al., 2005b; Stroop, Evers et al., 2006a; DV, SRT, and CRT, Harrison et al., 2004; SRT and CRT, Riedel et al., 1999; Stroop, Scholes et al., 2007).

c. Combined monoamine depletion had no effect in the young adults (DV, SRT, and CRT, Matrenza et al., 2004; Stroop, Scholes et al., 2007).

9.6 Order Effects

In reference to the explanation offered for treatment by order effects in section 8.7.5, most of the graphs in the Results section 9.4.1.7 can be interpreted as follows:

a. There were practice effects for DOT, VOSP Silhouettes, DMS %Corr and Accuracy, and SWM (note: higher scores on SWM reflect worsened performance). These effects were enhanced on DOT and VOSP when ATD was given in the second visit, that is, at the retrieval stage since the task was familiar. Performance on the second visit on DMS and SWM, however, was enhanced when ATD was given on the first visit, suggesting a carry-over effect of learning.

b. There were novelty effects on SRM Accuracy and DigitsF. These effects were enhanced on DigitsF, but lessoned on SRM, when ATD was given on the first visit, that is, when the task was unfamiliar.

c. Irrespective of practice or novelty effects, ATD worsened score on POMS when the task was unfamiliar (note: higher scores on POMS report poorer mood).

9.7 Conclusion

This was the first study to investigate ATD in a large sample of healthy older men and women. Congruent with studies in younger adults there were no effects on mood, despite the inclusion of a group having vulnerability to mood lowering during ATD.

There was a decrease in the motor function of the old-old group, however, in real terms this was minimal and the reliability of the UPDRS for assessing this cohort is questionable.

In contrast to other ATD studies, older persons were not susceptible to encoding and consolidation impairments in verbal memory. Further, the larger participant group of present study demonstrated that working memory in older persons is not affected by ATD.

The most interesting finding is the slowed reaction time of the old-old group during ATD.
10 CONCLUDING REMARKS

The present research is the first to investigate the functional role of serotonin using the technique of acute tryptophan depletion in a large group of healthy older persons. It is also the most comprehensive examination of this role in Parkinson’s disease (PD).

A broad background of the serotonergic system and a literature review of 5-HT challenge studies in movement, mood, and cognition combine with three studies to offer insights into the integrity of the serotonergic system in older persons and patients with PD.

10.1 Principal Findings

When the present research is considered along with findings for studies with SDAT and recovered depressed patients, there is an emergent picture of how older persons respond to a challenge to the 5-HT system during ATD. Specifically there is:

a. Less evidence of mood effects, even in groups with hypothesised vulnerability (e.g., recovered depression, SDAT, and PD) to the mood effects of ATD. Despite age-related changes to the serotonergic system which suggested that there might be an increased vulnerability in older persons towards a mood lowering response during the ATD challenge, the present research demonstrated the resilience of older persons instead. This was evident despite the number of participants with concomitant risk factors, for example, female gender and family history of depression.

b. A significant effect on movement. ATD was associated with improvement in the response time of the PD group on a motor task and an increase in the response time of the old-old group on two psychomotor tasks.

c. The combined results of Study A and C suggest very little in the way of ATD effects on executive function and working memory; combined with the Sambeth et al. study (2007), they suggest a subtle effect which may be detectable if the group is large enough (e.g., Sambeth, 2007) or if patients are included in the overall group.

d. Few consistent effects were found across studies in the healthy aged controls, although delayed visual memory may be impaired, perhaps especially in males.

e. A significant impairment in global cognitive status during ATD in conditions having a severe cholinergic deficit compared with the healthy elderly.

Cross-sectional studies have reported psychomotor speed and working memory – along with episodic memory – show linear life-long declines (Park et al., 2002; Park et al., 1996). The present research suggests that the decline in psychomotor speed may be related to age-related declines in serotonergic function such that the older the person the greater the serotonergic dysfunction and, thus, the greater the impairment in this cognitive function.
When the integrity of 5-HT’s role in episodic memory is considered, there appears to be gender and domain differences in older persons that are not apparent in younger adults. In visual memory, this occurs as a deficit for males but a reaction time improvement for females. In verbal memory, there is a deficit for females; however, because there was no replication in other verbal memory tasks, the effect in females is not as unambiguous as that seen in studies with younger females. Generally, males outperform females on visuospatial tasks and females outperform males on verbal memory tasks (Herlitz et al., 1997). The results of the present research could be suggesting age- and gender-related differences in the serotonergic system may be accounting for this differential effect on memory. Certainly, PET data has previously suggested that gender-specific effects of age on central serotonergic function may relate to differences between men and women in behaviour (Meltzer et al., 2001). However, a more circumspect explanation may be that these were chance findings occurring because there were a large number of variables.

It is possible the profile of ATD responses in older persons is associated with this group having cholinergic deficits. This profile consistently includes impairment in global cognitive status. An enhancing effect on visual memory has not been a feature in the cognition of healthy young adults so the improvement in the performance of older patient groups is unique. There are innate and intimate interconnections between the cholinergic and serotonergic systems, such that a severe reduction in 5-HT levels may differentially influence cognitive function when the cholinergic system is already challenged, as it is in persons having advanced age, SDAT, and PD.

10.2 Critique of the Research

There are several limitations to the studies presented in this thesis and it is important these are disseminated along with the results.

10.2.1 Matching

The control participants in Study B were not matched to the patients on a one-to-one basis but were matched on the gender ratio, then PVIQ and age. There is the possibility that this technique of post data collection could lead to the results being manipulated through the selective elimination of particular participants for some arbitrary reason. An alternative is to use all collected data and conduct an analysis of co-variance, including PVIQ and age as covariates.

10.2.2 Treatment

Some of the limitations regarding ATD in general have been outlined elsewhere (see section 3.8) but are supplemented by the following limitations from the present research.

a. It is not clear that the placebo drink is neutral in its effects on the central serotonergic system. This could mean an under- or over-estimation of cognitive effects in ATD, since ATD may be being compared to an enhancement of TRP availability (Evers et al., 2004). The TRP/LNAA ratio was not measured in the present research so there is no data with which to discount this as a limiting factor.
b. The technique of ATD is a global challenge and, as such, it is difficult to interpret to what the behavioural outcomes were specifically attributable. If there was a no significant change in any outcome measure, did this mean the brain was resistant to the acute reduction or did it compensate and adapt? For example, do the somatodendritic autoreceptors intervene and facilitate continuing post-synaptic activity? Is there up- or down-regulation at pre- or post-synaptic receptor sites and if so, at what point does this occur within the testing schedule? Such adaptive effects presumably underlie the lack of profound effects in most ATD studies despite an apparently massive manipulation of the serotonergic system.

c. The differential sensitivity of tasks may give the impression of specificity in different cognitive domains; however, the results may be reflecting the differential psychometric properties of the tasks instead.

10.2.3 Biochemical Assessment

One of the strongest criticisms that could be made against the present research is that the ratio of tryptophan (TRP) to other large neutral amino acids (LNAAs) in plasma was not assayed, thus raising the question of how the reduction in 5-HT synthesis was established. Plasma sampling is an important part of pharmacological challenge studies because outcome effects need to be mapped back to a robust change in biological parameters. It is particularly important in ATD studies to have some measure of these parameters because serotonin levels in the CNS are determined by (a) the amount of TRP remaining in circulating plasma after protein synthesis, (b) how much TRP is unbound and thus available for transport into the brain, and (c) the TRP/LNAA ratio because – as the best indicator of brain serotonin level (Fernstrom et al., 1979) – this ratio confirms the manipulation is having the desired effect. The minimum biological information required in an ATD study is the total plasma TRP level, but it is preferable to have the free plasma TRP (from now on labeled free TRP) level and the TRP/LNAA ratio. A further reason for measuring and taking account of this ratio would be to assess the neutrality of the placebo drink.

The absence of this assessment was due purely to a lack of funding for what are extremely difficult and expensive assays. It should be noted, however, as demonstrated in a previous study (Golightly et al., 2001), that the active 100 g drink significantly reduces the TRP/LNAA ratio and that the 100 g placebo drink, despite an increase in free TRP levels, does not alter this ratio. Moreover, that this technique has been shown to be specific to 5-HT synthesis and not dopamine, since the tyrosine/LNAA ratio was not altered significantly in the same study (Golightly et al., 2001).

10.2.4 Cognitive Assessment

When the present study commenced there were no published studies recording the effect of ATD on cognition in PD and inconclusive findings on the effect of ATD on recall and recognition tasks. There were no ATD studies of PDD and DLB and very little information available on the state or function of
the serotonergic system in the latter condition. The battery of tests in Studies B and C was based on
the effects of ATD in SDAT and on what was expected to happen in conditions having greater
cholinergic deficits – and thus a greater 5-HT/ACh imbalance – than SDAT. It was also designed to
accommodate the possible fatigability, limited and fluctuating attention, and floor effects of the DLB
patients. Past experience with a group of fasting older persons undergoing two long and tiring
experimental days was taken into consideration in conjunction with task characteristics – including
task length – when deciding which tasks to include into the neuropsychological battery. There are
clearly areas that, in hindsight, could have been assessed in more detail. For example, the assessment
of immediate and delayed verbal memory recall with the RAVLT.

10.2.5 Movement Assessment
The ATD effects on movement observed on the MOT in Study B and the UPDRS in Study C are
inconsistent with the literature and may be due to chance findings. It may have been preferable to
include more tests of motor function, such as, measures that differentiate different types of movement
or that assess movement in different limbs. The positive findings on UPDRS and measures of
movement and reaction time suggest movement needs to be considered in future ATD studies.

10.2.6 Analysis
Given the large number of variables in the ANOVAs it may have been preferable to use a Bonferroni
to limit the occurrence of chance findings. However, the a priori decision to look for patterns in the
results meant this correction was omitted. This produced a number of isolated findings in the three
studies which did not fit into an overall pattern and could be chance findings. For example, order
effects did not really fit into a pattern and were mentioned only briefly in the interpretation. The fact
they did appear, however, deserves an explanation.

10.2.7 Order Effects
There were a number of interaction effects of treatment by order in Studies B and C. Order effects
have not been consistently reported in the ATD literature because, either order effects were absent or
the wording constraints of published articles meant they were simply not reported.

The crossover design meant a potential confounding by practice effects when the effect of ATD was
greatest on the second visit. The only way around this is to omit the within-subject design and have a
parallel study or perform an analysis of the first session only, both of which were feasible only with a
much larger participant sample.

Conversely, a greater effect of ATD on the first visit could be potentially explained by novelty effects.
The way to avoid this is to have a mock-up of the test day and all testing procedures. This was
impractical given each experimental day was 8 hours long and the two protracted experimental days
came on top of a screening session. There was a limit to how much time busy participants could be
expected to donate.
The large number of interaction effects in Studies B and C is problematic because it implies something going on that could confound other significant results. Also, it was difficult to interpret the interactions because there is no pattern of replication across congruent cognitive tasks. More critical, however, is that this information has limited importance to the primary hypothesis of these studies; that there would be a differential effect of ATD between two diagnostic groups, not two diagnostic groups blended for order.

10.3 Strengths of the Research
There are a number of factors that contribute to the strength of the present research and which inform researchers and clinicians using 5-HT manipulations in PD and in healthy older persons.

10.3.1 Integral Part of a Larger Research Plan
The three studies that form this thesis are part of an ongoing series of studies using the same procedure and assessments. It was intended a fourth study would be included with which PD patients could be compared to patients with PDD and DLB. Recruitment drives failed to supply the prerequisite number of comparative participants, but this latter study is ongoing and the data from the present studies will be used at a later date. The systematic procedure and battery of assessments designed as part of the present research are invaluable for discerning similarities and differences in these related patient groups. For example, to observe whether serotonin functions differently in each condition or whether function is in fact the same across conditions, but differs in degree.

It was foreseen that a fifth study would be initiated comprising dementia patients medicated with cholinesterase inhibitors. An undercurrent of the present research was that cognitive impairments from a cholinergic deficit in PD patients would be attenuated during ATD. Including patients on cholinesterase inhibitors will mean this hypothesis will be tested more extensively.

10.3.2 Large Sample Size
Studies B and C comprised 55 and 43 participants respectively. These are large sample sizes with which to observe behavioural differences between treatments and between groups. The only other study to use ATD (Scholtissen et al., 2006b) in PD patients used a 15:15 patient to control ratio compared to the present 20:35 ratio. The larger samples for Studies B and C meant less chance of sampling variability and less chance of type II error.

The large sample sizes meant gender could be examined more robustly than has been possible in previous ATD studies. Because there were gender effects in the Porter et al study (Porter et al., 2003b), a decision was made for the present research to specifically include gender as a factor in the ANOVA. A larger sample size meant 33 and 22 males plus 22 and 21 females, respectively, could be studied for Studies B and C. The decision to examine gender was validated when Sambeth et al (2007) found gender effects in their pooled analysis.
10.3.3 Wide Age Range

The age ranges for Studies A and C were 60-81 and 50-89, respectively. This meant a wide age range – particularly in Study C – with which to examine the effects of age. It meant also enough people across the ample age distribution to split the larger group into a young-old and an old-old group each having 20 participants, when an effect of age was found.

By including people from the fifth decade, the research included middle age persons. Research has shown cognitive changes occur in this decade (Hedden & Gabrieli, 2004) and also that the serotonergic system may have already begun to change in some brain regions (Sheline et al., 2002). Although including a cohort of 20-49 year olds would have been optimal, the present results are still able to be compared with other studies using ATD in younger people because the procedure is generally standardised across studies.

10.3.4 Experimental Design

The three studies used a double-blind, placebo-controlled, counterbalanced, randomised, and crossover design. Double-blind meant research outcomes would not be influenced by the placebo effect or observer bias; placebo-controlled meant two virtually identical experiments were conducted differing only in the treatment administered; counterbalanced, randomised, and crossover meant an equivalent number of participants received either the active or placebo treatments on their first visit and the other treatment on their second visit, also participants were randomly allocated to either group for the treatment order. In study B, the two study groups were matched for gender, IQ and age.

Another benefit of the research design was that participants were randomised to receive the two computerised batteries – CANTAB and CDR – either first or second. This meant any effects of ATD on the tasks in these batteries would be an effect of treatment and not an artifact of the experimental apparatus. For example, CANTAB used a touchscreen and the participant was required to touch the screen in front of them with their forefinger in response to a command or stimulus; whereas CDR used a response box and the participant was required to make a response by using either thumb to press buttons on the box resting in their lap.

10.3.5 Standardised Procedure

The research comprised a standardised technique to compare results against those from other groups. ATD has been used in a large number of studies and the same composition has been used in most of these studies. There are recent studies experimenting with encapsulated amino acids and different doses, but mounting evidence – including that from Study A – indicates the optimal dose for ATD effects is the same as that used in Studies B and C.

10.3.6 Safety of the Technique for Older Persons

Studies in younger adults have already shown ATD is a safe and effective method for reducing 5-HT. The present research demonstrated this is also the case in older adults, even very old persons. No participant vomited and only a third of participants reported an initial mild nausea as a result of the
drinks’ taste. Although 3 participants withdrew from Study A, at least one as a consequence of adverse side effects, no person withdrew from Studies B and C. This may have been because the drink was mixed differently in the latter studies, making it less frothy and, thus, more palatable; there is an advantage of using this mixing technique in future ATD studies. The advantage of using ATD as a method for reducing central 5-HT levels is that it can be used efficaciously and safely with other older patient groups.

10.3.7 Advantage of the High Dose
There were a number of significant effects in studies B and C where a high dose of amino acids was used. The treatment was associated with minimal side effects. These encourage the use of a high dose in future ATD studies in older persons.

10.3.8 Guide for Treatment Strategies
In clinical practice, depression in PD is generally treated with SSRIs or SNRIs like venlafaxine (Leentjens et al., 2006). These first choice treatments are used primarily because they have fewer side effects than other antidepressants. It is not based on efficacy, pathophysiological, or empirical arguments. The latest Cochrane review (Ghazi-Noori et al., 2006) found no evidence for the superior efficacy of any antidepressant medication over placebo. Moreover, recent research is suggesting that regular use of SSRIs is associated with a dangerous side effect profile that includes increased risk of fractures and falls – in a manner not dissimilar to the TCAs – and cardiovascular effects, such as bradycardia, dysrhythmia, and syncope (Pacher & Ungvari, 2001). Although ATD involves an acute manipulation of serotonin and is unlikely to result in the receptor changes that probably occur during chronic SSRI treatment, the results from the present ATD studies, plus those of Leentjens et al. (2006), add to the knowledge of serotonin’s role in behaviour in PD. To this extent they may be used to guide future treatment strategies.

10.3.9 Older Persons
Two studies have now been conducted with the express purpose of examining ATD in healthy older persons. This has not been done previously. There is an emerging profile of ATD effects that differentiate older from younger adults, and very old from middle aged adults. There are differences between younger and older people in 5-HT levels and the present research suggests that this difference extends to serotonergic functionality.

It is expected the results of the present research will guide pharmacological strategies for enhancing the cognitive impairment experienced by some older persons.

The present results show that a gross and significant reduction in 5-HT levels does not interfere with mood, or with many cognitive functions. This means older persons are as surprisingly resilient as their younger cohorts to this serotonergic challenge. It may be the reduction induces adaptation in 5-HT receptors, in which case, an ‘aged’ system has a similar integrity to a ‘young’ system.
10.4 Future Research

Scientific investigations like the present research often generate more questions to be answered along with the answers to questions they have already posed. In like manner, the present research suggests the following interesting avenues for future research.

a. The changes that occurred in motor function and psychomotor speed during ATD in PD and old-old persons indicate the role of serotonin in movement needs to be examined further in these two groups. Studies could include an assessment of specific types of movement, movement in cognitive tasks having different levels of difficulty, and tasks that assess different limbs in movement.

b. The present research found ATD associated with a significant worsening of mood at 6.5 hrs but no other statistically significant effects of ATD on mood in older persons. This information may be useful for research investigating strategies for treating depression in older persons. The resilience of this group towards a drastic reduction in 5-HT means strategies targeting other neurotransmitters or therapies may also be more effective in treating depression.

c. ATD is a global challenge and thus it is unclear what is happening to the serotonergic system at receptor level. It may be that different receptors perform different functions and that the integrity of these functions is different in older persons, and in PD. The results of the present research thus inform investigations into pharmacological strategies, e.g., novel 5-HT$_{1A}$ antagonists (see, Schechter et al., 2002) for cognitive decline in older persons, whether this is related to pathological or non-demented impairment. Moreover, for the nootropic (i.e., cognitive enhancers) compounds which could be developed to supplement or counteract the side effects of psychoactive drugs used to treat affective, sleep disorder or gastrointestinal disorders (Jolles et al., 1995).

ATD is a robust experimental manipulation which has been used widely to research the role of the serotonergic system in a range of neuropsychiatric conditions. While there may be variability in the neutrality of the placebo treatment, there is no doubt that the depletion treatment induces a very significant but transient reduction in 5-HT function for a time period during which various neuropsychiatric functions can be investigated. The studies in this thesis used this technique to investigate the role of 5-HT in neuropsychiatric function in patients with Parkinson’s disease and in healthy older persons. The studies are part of a series in healthy older persons and in SDAT, recovered depression and PD and can be interpreted as such. No previous studies have used this technique in such large group of older persons.

Various interpretations of the results have been discussed in the sections above. Overall, the following important conclusions can be drawn.
a. In impaired elderly groups (e.g., SDAT, recovered depression, PD), ATD reduces global cognition, as measured by a commonly used clinical measure.

b. Despite this, in groups with concomitant cholinergic deficit there is an improvement in aspects of memory.

c. There are effects on movement induced by ATD. This research suggests that ATD induces a differential improvement in PD but impairment in the very elderly.

d. All groups were surprisingly resistant to the often reported effects of ATD on mood.

Various treatments for depression, cognitive impairment and motor disorder currently used and being developed in the elderly involve 5-HT manipulation. The data presented here on the effects of a global reduction in 5-HT synthesis should at least be taken into account when considering the likely effects of these treatments. The data adds to knowledge of the role of 5-HT in mood, motor function and cognitive function, particularly in the elderly.
11 APPENDICES
Dear Sir or Madam,

Dr Richard Porter and I are carrying out research on Parkinson’s disease at the Christchurch School of Medicine and Health Sciences. We hope that the research will contribute to significant improvements in the quality of life for older people suffering from Parkinson’s disease.

We need to carry out the same research on people with Parkinson’s disease or dementia with Lewy bodies, and on a control group of healthy people from a comparable age group. We have started recruiting participants with Parkinson’s disease and we wish to recruit volunteers for the control group.

I am writing to request your help in recruiting people aged 60 years and over. Enclosed with this letter is a brief outline of the study which could be added to your usual newsletter. Our contact details are on the brief for your members to contact us directly. I have also included a brochure about the study and I would be grateful if you would distribute it to anyone you think would be willing to take part.

Both Dr Porter and I would be happy to discuss the study with you or your members.

Yours faithfully,

Janet Mace
11.2 Appendix B

Brief overview of the ATD and PD/DLB Study for newsletters

Janet Mace and Dr Richard Porter are researching the role of serotonin in Parkinson’s disease (PD) and the related dementia with Lewy bodies (DLB). They are looking for people aged 60 years and older to volunteer as control participants for their study. Little is known about serotonin in PD and DLB so your participation would contribute to furthering knowledge and may be used to guide future treatments to help people with these conditions. Participation will involve coming to the Christchurch School of Medicine for two separate occasions, ingesting a protein drink, answering some questions and performing some simple memory tests. In appreciation of your participation in two test sessions you will receive $100 in petrol or book vouchers. We have approval for the study from the Canterbury Ethics Committee. For more information, Janet or Richard can be contacted by phone: 03 372 0400, by email: janet.mace@chmeds.ac.nz and richard.porter@chmeds.ac.nz or by letter: c/- Janet Mace, ATD and PD/DLB Study, Dept. of Psychological Medicine, Christchurch School of Medicine and Health Sciences, P. O. Box 4345, Christchurch.
Information Sheet

Dietary challenge, memory, mood and movement in Parkinson’s disease and in Lewy body dementia.

Introduction
You are invited to take part in a research study being conducted by Janet-Lee Mace, Richard Porter, John Dalrymple-Alford, Tim Anderson and Chris Collins to try to understand the role of chemicals in Parkinson’s disease (PD) and Lewy body dementia (DLB).

Tryptophan is a substance which occurs naturally in foods and is made into serotonin, a chemical in the brain which may be involved in mood and in helping us to learn and remember things. The major research question which we are interested in is the relationship between brain serotonin levels and learning and memory in PD and DLB. We are also interested in the role of the serotonin system in depression and movement in these two conditions.

By using a special drink containing a mixture of naturally occurring substances (amino acids) which are found in everyday food, brain serotonin may be lowered. This is completely reversible and the effect lasts for only a few hours. In order to understand more about the relationship between brain serotonin and memory, movement and depression, we wish to study the effects of serotonin reduction.

Your participation in this study is completely voluntary. If you agree to take part you may withdraw at any time, for any reason and this will in no way affect your future health care.

More about this study
What are the aims of this study? We hope to find out more about the role of serotonin in memory, mood and movement in Parkinson’s disease and Lewy body dementia. The information we obtain will be used to investigate new treatments for memory, mood and movement problems in these conditions.
Who can participate in this study?
Anyone with Parkinson’s disease and Lewy body dementia who is not suffering from depression and who is otherwise currently medically well. If you have had certain other illnesses in the past or are taking certain medications this may also mean that you cannot take part.

Healthy people without Parkinson’s disease or Lewy body dementia and who don’t have depression, memory problems or any other serious illness. Once again if you have had certain other illnesses in the past or are taking certain medications this may also mean that you cannot take part.

How many participants will be involved? Approximately 100 people.

Where will the study be held? Van der Veer Institute, near Christchurch Hospital.

What will happen during the study? If you consent to the study we will ask you to attend, at your convenience, for two sessions at least a week apart. You would be asked to fast from midnight the night before each session but if you are on medication you should take this at about 7.00am on the day you come for the tests.

The timetable for the testing day will be as follows:

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.30 am</td>
<td>Arrive by taxi at the Van der Veer Institute</td>
</tr>
<tr>
<td></td>
<td>Complete questionnaires and interviews to rate movement and symptoms of depression</td>
</tr>
<tr>
<td></td>
<td>Give blood sample (10mls – two teaspoons)</td>
</tr>
<tr>
<td>9.00 am</td>
<td>Drink test drink</td>
</tr>
<tr>
<td></td>
<td>Relax, read or listen to the radio</td>
</tr>
<tr>
<td>1.00 pm</td>
<td>Give blood sample (10mls)</td>
</tr>
<tr>
<td></td>
<td>Complete questionnaires and interviews to rate movement and symptoms of depression</td>
</tr>
<tr>
<td>1.30 pm</td>
<td>Take tests of learning and memory</td>
</tr>
<tr>
<td></td>
<td>Complete questionnaires and interviews to rate movement and symptoms of depression</td>
</tr>
<tr>
<td></td>
<td>Give blood sample (10mls)</td>
</tr>
<tr>
<td></td>
<td>Have a cup of tea/coffee/juice and a sandwich which we will provide for you</td>
</tr>
<tr>
<td>4.00 pm</td>
<td>Return home by taxi</td>
</tr>
</tbody>
</table>

The drink you will be asked to consume will be either a tryptophan depleting drink or a placebo (a placebo is a “dummy drink” which looks and tastes exactly like a tryptophan depleting drink). The drink has an unpleasant taste but we will give you mints to help take this away. The placebo is necessary to ensure any changes are real effects due to changes in serotonin, and not due to something else. Some people may feel slightly nauseated (a little sick) following the drink but this will wear off.
What are the tests of memory? They involve trying to remember things such as words or patterns on a computer screen, and they involve repeating information back to the interviewer. These tests will take approximately 1½ hours.

Why blood tests? The blood tests are for research purposes and are taken to measure tryptophan. The tests are for assessing the amount of tryptophan in your blood. No DNA (genetic material) will be stored or analysed. We will ask for your permission to send a portion of your blood to Brisbane, Australia, for analysis. If you don’t want this to happen, this will not affect your taking part.

Risks and Benefits

What are the risks of participation? You may find that the drink tastes unpleasant and it may make you nauseated or sick for a short time. It is also possible that during one of the tests you may experience symptoms of depression. This will be temporary and will rapidly resolve. There may be some bruising and discomfort in your arm similar to that which occurs after you have a blood test at your doctors.

What are the benefits of participation? It is possible some of your memory may improve temporarily. There is no long term treatment for memory impairment in PD and DLB, but your participation would be contributing to knowledge which will help to find new treatments (medication and dietary) for memory problems, as well as movement problems and depression in PD and DLB.

Will my GP know I am in the study? We prefer to advise your GP that you are taking part in this study, however, this is your decision.

Will I be reimbursed for my time and expenses? We will pay for taxis to and from your home to the department on the days of the testing. In view of the length of time which you will be giving up for the study, we will also offer a payment of $50 petrol or book vouchers for each of the two testing days.

Participation

Your participation in this study is entirely voluntary (your choice)

If you agree to take part, you are free to withdraw from this study at any time, for any reason.

You do not have to answer any questions you do not wish to answer

If you have any queries or concerns about your rights as a participant in this study you are free to contact a Health and Disability Services Consumer Advocate, ph. (03) 377 7501 or 0800 377 766.

Confidentiality

We will take all precautions to maintain confidentiality. All data will be stored in secure areas. The data will be available only to the study investigators. All forms and computer files will be marked with numbers only, not names. No names will be used when the results of this study are published.
Results
How can I get results of this research? When this study is over you may have a summary of the key results. Detailed results will be published in international scientific journals.

Compensation
There may be compensation available to you in the unlikely event that you are injured taking part in this research. If you suffer physical injury as a result of your participation in this clinical trial, you may be covered by ACC. You should note, however, that eligibility for cover is not automatic and you would be assessed by ACC according to the provisions of the Act. You would be in the same position as a claimant who has suffered physical injury as a result of medical error or negligence, or as a result of medical mishap, i.e. an adverse consequence of treatment which is both rare and severe.

If your claim for cover is accepted by ACC, your entitlement to compensation would depend on a number of factors, such as whether you are an earner or non-earner. You should note that in most cases ACC provides only partial reimbursement of costs and expenses and there is no lump sum compensation payable under current ACC legislation. You should also be aware that if you have cover under the ACC legislation, your right to sue the researcher(s) or anyone else involved in the clinical trial is extremely limited. If you have any questions about cover or entitlements under the ACC scheme, you should contact your nearest ACC branch office for further information before you consent to participate in this trial.

This study has received ethical approval from the Canterbury Ethics Committee.

Where can I get more information about the study?

Richard Porter may be contacted by, telephone: 03 372 0400, by email: richard.porter@chmeds.ac.nz or by letter: Assoc Professor Richard Porter, ATDPD Study, Department of Psychological Medicine, Christchurch School Medicine and Health Sciences, P.O. Box 4345, Christchurch.
Consent Form

Dietary challenge, memory, mood and movement in Parkinson’s disease and in Lewy body dementia.

I have been invited to take part in a study of the role of the serotonin system in Parkinson’s disease and Lewy body dementia being conducted by Janet-Lee Mace, Richard Porter, John Dalrymple-Alford, Tim Anderson, Chris Collins, Roger Mulder and Caroline Bell.

I have heard and understand an explanation of this study and/or I have read the information sheet. I have been given an opportunity to discuss the study and ask questions about it. I am satisfied with the answers I have been given.

I have had enough time to consider whether to take part and to discuss my decision with a person of my choice and the researcher. I know who to contact if I have any questions about the study.

I understand that:

1. My taking part in this study is voluntary (my choice).
2. I am free to withdraw from the study at any time and for any reason and this will not affect my future healthcare.
3. The study involves drinking a drink on 2 test days, which contains amino acids.
4. I will undertake tests of memory and learning.
5. I will be interviewed, complete questionnaires and have blood tests in order to obtain detailed assessments of depression and movement.
6. I am free to stop these interviews and tests at any time and to refuse to answer any questions I don’t want to answer.
7. My participation in this study is confidential and no information that could identify me will be used in any reports generated on this study.
8. The compensation provisions for this study are covered by accident compensation legislation within its limitations.
9. I understand that this study has received ethical approval from the Canterbury Ethics Committee.
Consent Form

I consent to take part in this study, entitled “Dietary challenge, memory, mood and movement in Parkinson’s disease and in Lewy body dementia”.

Participant’s signature: ______________________________  (print name) __________________
Date: __________________________

I am willing to have my General Practitioner contacted regarding my participation in this study

YES     NO     (please circle your choice)

Participant’s signature: ______________________________
Date: __________________________

I wish to receive a copy of the results of this study:

YES     NO     (please circle your choice)

Investigators signature: ______________________________
Investigators name: ______________________________
Date: __________________________

Contact phone numbers:

Janet Mace (03 372 0400 Ext.85418)
janet.mace@chmeds.ac.nz

Richard Porter (03 338 5059 Ext. 85426, 021 2623615, or 03 338 5059 anytime)
richard.porter@chmeds.ac.nz

John Dalrymple-Alford (“John D-A” 364 2998 or 366 7002 Ext. 6382)
john.dalrymple-alford@canterbury.ac.nz
11.5 Appendix E
Reminder Handout for the Test Days

CHRISTCHURCH SCHOOL OF MEDICINE & HEALTH SCIENCES
DEPARTMENT OF PSYCHOLOGICAL MEDICINE

Serotonin, memory, mood and movement in Parkinson’s disease and in Lewy body dementia.

Information for the Research day

On the research day, we ask you to please:

1. Not eat from midnight before the research day. Drink only water.
2. Take your usual medications with water at 7am.
3. Bring along a book, CDs and audiotapes if you wish. From 9.00am you will be sitting quietly for about 4 hours and you may prefer to read your own literature and listen to your own music.
4. Bring your reading glasses and/or hearing aid; and the medications you take during the day.
5. Be ready for the taxi to pick you up to arrive at the clinic at 8.30 am.
6. Arrange to have someone stay with you the night after the research day. The research day will finish around 4.00 pm and we will provide a taxi to take you home.

Taxi
As we don’t want you to drive yourself home at the end of the day, we will pay for a taxi to pick you up on the morning of the Research Day and return you home again at around 4.00pm. The morning taxi is arranged by either the researcher or the participant (this is you). Please let the researcher know if you would like him/her to arrange the taxi.

If you are arranging the taxi, please phone Blue Star Taxis on 379 9799 and ask to be picked up on (date) ______________________________ and ______________________________, at (time) ________________,


to go to the Clinic on the ground floor of the Van der Veer Institute, 16 St Asaph Street, Christchurch.

Please ask your taxi driver to come into the clinic with you to sign a taxi chit for the taxi ride. The Department of Psychological Medicine will then pay the taxi company for you journey.
The researchers’ names are Janet Mace and Richard Porter.

If you have any queries before the Test Day you can phone either Richard Porter or Janet Mace at the School of Medicine on ph 372 0400. Alternatively, you can email richard.porter@chmeds.ac.nz; janet.mace@chmeds.ac.nz

If you have any queries on the Test Day you can phone Janet Mace at the Van der Veer Institute on 372 8609. If you have any queries after the Test Day you can phone Dr Richard Porter on the hospital phone 337 7969 and the telephone operator will ask him to phone you back.

**A reminder of what will happen during the study**

We invite you to come to the clinic at the Van der Veer Institute for two sessions, at least a week apart. We ask you to fast from midnight the night before each session but if you are on medication you should take this at about 7.00am on the day you come for the tests. The timetable for the testing day will be as follows.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
</table>
| 8.30 am| Arrive by taxi to the Van de Veer Institute  
  Complete questionnaires and interviews to rate movement and symptoms of depression  
  Give blood sample (10mls – two teaspoons) |
| 9.00 am| Drink test drink  
  Relax, read or listen to the radio |
| 1.00 pm| Give blood sample (10mls)  
  Complete questionnaires and interviews to rate movement and symptoms of depression |
| 1.30 pm| Take tests of learning and memory  
  Complete questionnaires and interviews to rate movement and symptoms of depression  
  Give blood sample (10mls)  
  Have a cup of tea/coffee/ juice and a sandwich which we will provide for you |
| 4.00 pm| Return home by taxi |

What is the drink you will be asked to consume? The drink will be either a tryptophan depleting drink or a placebo (a placebo is a “dummy drink” which looks and tastes exactly like a tryptophan depleting drink). The drink has an unpleasant taste but we will give you mints to help take this away. The placebo is necessary to ensure any changes are real effects due to changes in serotonin, and not due to something else. Some people may feel slightly nauseated (a little sick) following the drink but this will wear off.

What are the tests of memory? They involve trying to remember things such as words or patterns on a computer screen, and they involve repeating information back to the interviewer. These tests will take approximately 1½ hours.

**Thank you very much for your participation**
11.6 Appendix F
Assessments (in Alphabetical Order)

11.6.1 Mood Assessment
11.6.1.1 Montgomery - Asperg Depression Rating Scale (MADRS)

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent sadness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Reported sadness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Inner tension</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Reduced sleep</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Reduced appetite</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Concentration difficulties</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Lassitude</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Inability to feel</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Pessimistic thoughts</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Suicidal thoughts</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
### 11.6.1.2 Profile of Mood States (POMS)

<table>
<thead>
<tr>
<th>Mood State</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friendly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tense</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worn out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unhappy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear-headed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lively</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confused</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorry for things done</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shaky</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listless</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Peeved</td>
<td></td>
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</tr>
<tr>
<td>Considerate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On edge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grouchy</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panicky</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hopeless</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unworthy</td>
<td></td>
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</tr>
</tbody>
</table>

1 = Not at all  
2 = A little  
3 = Moderately  
4 = Extremely
### 11.6.2 Motor Assessment

#### 11.6.2.1 United Parkinson’s Disease Rating Scale (UPDRS)

<table>
<thead>
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11.6.3 Neuropsychological Assessment

11.6.3.1 Controlled Oral Word Association (COWA) task

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11.6.3.2 Digit Span

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<tr>
<td>Knuckle</td>
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<tr>
<td><strong>4- Legged Animals</strong> (30 sec)</td>
<td>1 pt each</td>
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<tr>
<td>Arm-Leg</td>
<td>Body part: limb etc</td>
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<td>Lesser correct</td>
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<td>Laughing-Crying</td>
<td>Feeling: emotion</td>
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<tr>
<td>Other correct answer</td>
<td>0</td>
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<tr>
<td>Eating-Sleeping</td>
<td>Essential for life</td>
<td>2</td>
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<td></td>
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<tr>
<td>Other correct answer</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Repetition</strong></td>
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<td></td>
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</tr>
<tr>
<td>‘I would like to go home/out’</td>
<td>2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 or 2 missed/wrong words</td>
<td>0</td>
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<tr>
<td><strong>Read and Obey</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Close your eyes’</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Obey without prompting</td>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>Obey after prompting</td>
<td>2</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reads aloud (spont. or by</td>
<td>0</td>
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<tr>
<td><strong>Writing</strong> (1 min.)</td>
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<tr>
<td>I would like to go home/out</td>
<td></td>
<td></td>
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<td></td>
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<td>MMS: Spontaneous sentence:</td>
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<td><strong>Copying 2 Pentagons</strong> (1 min)</td>
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<tr>
<td>Each Pentagon:</td>
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</tr>
<tr>
<td>5 approx. equal sides</td>
<td>4</td>
<td></td>
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<td></td>
<td>4</td>
</tr>
<tr>
<td>5 but unequal (&gt;2:1) sides</td>
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<td></td>
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</tr>
<tr>
<td>Other enclosed figure</td>
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<td></td>
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</tr>
<tr>
<td>2 or more lines</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Intersection:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 corners</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Not 4 corner enclosure</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Three-Stage Command</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>___ Take this paper with your L/R hand</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>___ Fold it in hand, and</td>
<td></td>
<td></td>
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<tr>
<td>___ Hand it back to me</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Second Recall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Something to wear</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Colour</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Good person’s quality</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>
11.6.3.5 One Day Fluctuation Assessment Scale

1. Falls
Has the patient fallen today?
Yes ______ (1) No ______ (0)
If yes, how many times? ______

Have the patient had any ‘near falls’ today? (a near fall is when the patient almost fell but was saved by somebody else or objects such as furniture or a walking aid)
Yes ______ (1) No ______ (0)

2. Fluctuation
a) Has the patient had a period or periods today when he/she seemed to be confused and muddled and then a period or periods when he/she seemed to be improved and functioning better?
Yes ______ (1) No ______ (0)
If yes, how much of the day was he/she confused?
(i) 25% (¼) of the day (1)
(ii) 25-75% (¼ -¾) of the day (2)
(iii) 75% (¾) or more of the day (3)

b) How great was the difference today between the worst period of function and the best period of function?
(i) A slight degree of variation (0)
(ii) A moderate degree of variation which had a large effect on his/her ability to function at the same level throughout the day (1)
(iii) A marked degree of variation which had a large effect on his/her ability to function at the same level throughout the day (2)
Give examples of worst period and best period of functioning:
Worst: ____________________________
Best: ______________________________

3. Drowsiness
Has the patient been excessively drowsy today?
Yes ______ (1) No ______ (0)
If yes, for how much of the day was he/she

(i) 25% (¼) of the day (1)
(ii) 25-75% (¼ -¾) of the day (2)
(iii) 75% (¾) or more of the day (3)
Were there any periods when he/she was
Yes ______ (1) No ______ (0)

4. Attention
Did the patient have difficulty focusing attention (for example, Was he/she easily distractible, or did he/she have difficulty keeping track of what was being said) throughout the day?
Yes ______ (1) No ______ (0)
Was the patient’s thinking disorganised or incoherent (for example, was he/she easily distractible, or did he/she have difficulty following conversations, unclear or illogical flow of ideas, or unpredictable switching from subject to subject) throughout the day?
Yes ______ (1) No ______ (0)

6. Altered level of consciousness
Overall, how would you rate this patient’s level of consciousness today?
(i) Alert (normal) (0)
(ii) Lethargic (drowsy, easily aroused) (1)
(iii) Stuporous (difficult to arouse) (2)

7. Communication
a) How well does the patient understand what you communicate to him/her (you may use speaking, writing or gesturing)?
(i) Understands almost everything you communicate (0)
(ii) Understands some of what you communicate (1)
(iii) Understands almost nothing of what you communicate (2)

b) How well does the patient communicate (by writing, speaking or gesturing)?
(i) Well enough to make him/her easily understood at all times throughout the day (0)
(ii) Can be understood sometimes or with some difficulty (1)
(iii) Can rarely or never be understood for whatever reason (2)
### Rey Auditory Verbal Learning Test (RAVLT)

<table>
<thead>
<tr>
<th>List A</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>List B</th>
<th>Recall List B</th>
<th>List A</th>
<th>Immediate Recall List A</th>
<th>Delayed Recall List A</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Curtain</td>
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</tr>
<tr>
<td>Bell</td>
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<td></td>
<td>Bird</td>
<td>Bell</td>
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<td>Garden</td>
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<td>Boat</td>
<td>Farmer</td>
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<td>Church</td>
<td>House</td>
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<td></td>
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<td></td>
<td>Fish</td>
<td>River</td>
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<td></td>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>Total</strong></td>
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11.6.3.7 Visual Object in Space Perception (VOSP) task

Shape Detection Screening Test

<table>
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<tr>
<th>Practice</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>-</td>
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</table>

Incomplete Letters

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<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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</thead>
<tbody>
<tr>
<td>Practice</td>
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<td>B</td>
<td>D</td>
<td>M</td>
<td>S</td>
<td>K</td>
<td>X</td>
<td>Y</td>
<td>H</td>
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</table>

<table>
<thead>
<tr>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>camel</td>
<td>elephant</td>
<td>penguin</td>
<td>pig</td>
<td>cow</td>
<td>rabbit</td>
<td>snail</td>
<td>crocodile</td>
<td>frog</td>
<td>bear</td>
</tr>
<tr>
<td>cup</td>
<td>corkscrew</td>
<td>dustpan</td>
<td>bike</td>
<td>shoe</td>
<td>ladder</td>
<td>spanner</td>
<td>tractor</td>
<td>key</td>
<td>deckchair</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>kanga</td>
<td>rhino</td>
<td>sheep</td>
<td>seal</td>
<td>duck</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>scissors</td>
<td>axe</td>
<td>watch</td>
<td>binoculars</td>
<td>glasses</td>
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</table>
11.6.4  Physical State

11.6.4.1  Visual Analogue Scale

Please circle the words that best describe how you feel right now.

<table>
<thead>
<tr>
<th></th>
<th>0 = Not at all</th>
<th>1 = A little</th>
<th>2 = Moderately</th>
<th>4 = Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>How hungry are you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How sleepy are you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How nauseous are you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How happy are you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>How irritable are you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
### 11.6.5 Screening Assessment

#### 11.6.5.1 The Mini International Neuropsychiatric Interview (M.I.N.I.)

*If YES to any question, go to the corresponding MINI module →*

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you been <strong>consistently</strong> depressed or down, <strong>most of the day, nearly every day, for the past two weeks?</strong></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?</td>
<td>YES</td>
<td>NO</td>
<td>A</td>
</tr>
<tr>
<td>Have you felt sad, low or depressed <strong>most of the time</strong> for the last two years?</td>
<td>YES</td>
<td>NO</td>
<td>B</td>
</tr>
<tr>
<td>In the past month did you think that you would be better off dead or wish you were dead?</td>
<td>YES</td>
<td>NO</td>
<td>C</td>
</tr>
<tr>
<td>Have you <strong>ever</strong> had a period of time when you were feeling ‘up’ or ‘high’ or so full of energy or full of yourself that you got into trouble or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.)</td>
<td>YES</td>
<td>NO</td>
<td>D</td>
</tr>
<tr>
<td>Have you <strong>ever</strong> been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family? Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified?</td>
<td>YES</td>
<td>NO</td>
<td>D</td>
</tr>
<tr>
<td>Have you <strong>ever</strong> on more than one occasion, had spells or attacks when you suddenly felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way? Did the spells peak within 10 minutes?</td>
<td></td>
<td></td>
<td>E</td>
</tr>
<tr>
<td>CODE YES ONLY IF THE SPELLS PEAK WITHIN 10 MINUTES.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you feel anxious or uneasy in places or situations where you might have a panic attack or panic-like symptoms, or where help might not be available or escape might be difficult; like being in a crowd, standing in a line (queue), when you are away from home or alone at home, or when crossing a bridge, travelling in a bus, train or car?</td>
<td>YES</td>
<td>NO</td>
<td>F</td>
</tr>
<tr>
<td>In the past <strong>month</strong> were you fearful or embarrassed being watched, being the focus of attention, or fearful of being humiliated? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.</td>
<td>YES</td>
<td>NO</td>
<td>G</td>
</tr>
<tr>
<td>In the past <strong>month</strong> have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (e.g., the idea that you were dirty, contaminated or had germs, or fear of contaminating others, or fear of harming someone even though you didn’t want to, or fearing you would act on some impulse, or fear or superstitions that you would be responsible for things going wrong, or obsessions with sexual thoughts, images or impulses, or hoarding, collecting, or religious obsessions).</td>
<td>YES</td>
<td>NO</td>
<td>H</td>
</tr>
<tr>
<td>Question</td>
<td>YES</td>
<td>NO</td>
<td>H</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
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</tr>
<tr>
<td>In the past <strong>month</strong>, did you do something repeatedly without being able to resist doing it, like washing or cleaning excessively, counting or checking things over and over, or repeating, collecting, or arranging things, or other superstitious rituals?</td>
<td></td>
<td></td>
<td>H</td>
</tr>
<tr>
<td>Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury to you or someone else?</td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>EXAMPLES OF TRAUMATIC EVENTS INCLUDE SERIOUS ACCIDENTS, SEXUAL OR PHYSICAL ASSAULT, A TERRORIST ATTACK, BEING HELD HOSTAGE, KIDNAPPING, FIRE, DISCOVERING A BODY, SUDDEN DEATH OF SOMEONE CLOSE TO YOU, WAR, OR NATURAL DISASTER.</td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>During the past month, have you re-experienced the event in a distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?</td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>In the past 12 months, have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?</td>
<td></td>
<td></td>
<td>J</td>
</tr>
<tr>
<td>Have you worried <strong>excessively</strong> or been anxious about several things over the past 6 months?</td>
<td></td>
<td></td>
<td>O</td>
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### 11.6.5.2 National Adult Reading Test (NART)

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11.7 Appendix G

Means and Standard Errors for Study A

Table G-1 Study A. Means and Standard Errors (SE) for ATD and Placebo Treatment Effects

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<td>97.00</td>
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Table G-2 Study A. Means and Standard Errors (SE) for Treatment by Dose Interactions

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Table G-4 Study A. Means and Standard Errors (SE) for Treatment by Gender by Dose Interactions

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High dose study
### Appendix H

Means and Standard Errors for Study B

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Table H-1 Study B. Means and Standard Errors (SE) for ATD and Placebo Treatment Effects
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Table II-3 Study B. Means and Standard Errors (SE) for Treatment by Gender Interactions

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### Table H-5 Study B. Means and Standard Errors (SE) for Treatment by Diagnosis by Gender interactions

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### 11.9 Appendix I

Means and Standard Errors for Study C

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## Table I-3 Study C. Means and Standard Errors (SE) for Treatment by Gender Interactions

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### Table I-4 Study C. Means and Standard Errors (SE) for Treatment by Time Interactions

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<td>457.20 7.84</td>
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<td>469.42 7.47</td>
</tr>
<tr>
<td>VAS Nausea</td>
<td>0.07 0.04</td>
<td>0.05 0.03</td>
<td>0.25 0.08</td>
<td>0.28 0.09</td>
<td>0.34 0.22</td>
<td>0.11 0.08</td>
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</tbody>
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### Table I-2 Study C. Means and Standard Errors (SE) for Treatment by Order Interactions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment x Order</th>
<th>Placebo first</th>
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<th>Placebo second</th>
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<tr>
<td></td>
<td>ATD</td>
<td>Placebo</td>
<td>ATD</td>
<td>Placebo</td>
</tr>
<tr>
<td>POMS Total</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>DigitsF Total</td>
<td>7.30 1.78</td>
<td>9.15 1.73</td>
<td>12.32 1.77</td>
<td>9.44 1.73</td>
</tr>
<tr>
<td>DOT Total</td>
<td>9.38 0.50</td>
<td>9.93 0.49</td>
<td>9.35 0.49</td>
<td>8.59 0.48</td>
</tr>
<tr>
<td>SSP BetweenErrors</td>
<td>8.13 0.60</td>
<td>7.01 0.50</td>
<td>7.25 0.58</td>
<td>7.49 0.49</td>
</tr>
<tr>
<td>VOSP Silhouettes</td>
<td>34.89 4.07</td>
<td>38.20 3.28</td>
<td>47.98 3.88</td>
<td>39.88 3.14</td>
</tr>
<tr>
<td>PRM Accuracy</td>
<td>11.27 0.40</td>
<td>8.61 0.50</td>
<td>9.01 0.39</td>
<td>10.81 0.54</td>
</tr>
<tr>
<td>SMTS Accuracy</td>
<td>68.87 2.45</td>
<td>82.95 2.49</td>
<td>78.97 2.40</td>
<td>71.84 2.44</td>
</tr>
<tr>
<td>DMS %Corr</td>
<td>85.54 2.73</td>
<td>84.00 2.00</td>
<td>74.85 2.67</td>
<td>86.61 1.95</td>
</tr>
<tr>
<td></td>
<td>88.08 2.15</td>
<td>86.69 1.61</td>
<td>79.53 2.11</td>
<td>89.13 1.58</td>
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Appendix J

Preliminary data of the four participants who completed two tests days for Study D are presented.

Table J-1 Study D. Demographic and Baseline data

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Age</th>
<th>Ed level</th>
<th>PVIQ</th>
<th>MMSE</th>
<th>H &amp; Y</th>
<th>MADRS</th>
<th>UPDRS</th>
<th>Handedness</th>
<th>Side of PD onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>401</td>
<td>DLB</td>
<td>M</td>
<td>72</td>
<td>2</td>
<td>100</td>
<td>29</td>
<td>2.5</td>
<td>10</td>
<td>*</td>
<td>*</td>
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<tr>
<td>402</td>
<td>DLB</td>
<td>F</td>
<td>71</td>
<td>2</td>
<td>102</td>
<td>20</td>
<td>2.5</td>
<td>0</td>
<td>R</td>
<td>*</td>
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<tr>
<td>403</td>
<td>PDD</td>
<td>F</td>
<td>71</td>
<td>3</td>
<td>101</td>
<td>26</td>
<td>3</td>
<td>10</td>
<td>*</td>
<td>R</td>
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</tr>
<tr>
<td>405</td>
<td>DLB</td>
<td>M</td>
<td>79</td>
<td>2</td>
<td>102</td>
<td>18</td>
<td>0</td>
<td>6</td>
<td>L</td>
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</tr>
</tbody>
</table>

Note: DLB = dementia with Lewy bodies; Ed level = educational level; PD = Parkinson’s disease; PDD = Parkinson’s disease with dementia; H & Y = Hoehn and Yahr; MADRS = Montgomery-Asberg Depression Rating Scale; MMSE = Modified Mental State examination; PVIQ = predicted IQ (from the NART: National Adult Reading Test); UPDRS = United Parkinson’s Disease Rating Scale.

Table J-2 Study D. Means for Choice Reaction Time

<table>
<thead>
<tr>
<th>ID</th>
<th>ATD</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>CRT Accuracy (%)</th>
<th>Placebo</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>CRT Reaction Time (ms)</th>
<th>Placebo</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
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<td>90</td>
<td>95</td>
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<td>90</td>
<td>90</td>
<td>90</td>
<td>530.74</td>
<td>471.56</td>
<td>620.72</td>
<td>507.84</td>
<td>444.67</td>
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<tr>
<td>402</td>
<td>90</td>
<td>85</td>
<td>^</td>
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<td></td>
<td>65</td>
<td>55</td>
<td>60</td>
<td></td>
<td>3174.13</td>
<td>3506.82</td>
<td>^</td>
<td>9532.15</td>
<td>4213.27</td>
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<tr>
<td>403</td>
<td>95</td>
<td>85</td>
<td>95</td>
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<td>90</td>
<td>100</td>
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<td>738.5</td>
<td>709</td>
<td>689.76</td>
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<td>55</td>
<td>85</td>
<td>^</td>
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<td>60</td>
<td>^</td>
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<td>1024.24</td>
<td>^</td>
<td>2845.5</td>
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Note: ^ = no data available.
### Table J-2: Study D. Means for Assessments

Note: ^ = no data available

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<th>Patient ID #403</th>
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<td>ATD Mean</td>
<td>Placebo Mean</td>
<td>ATD Mean</td>
<td>Placebo Mean</td>
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<td>freeTRP 4 hrs</td>
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<td>8.9,5</td>
<td>0.0,^</td>
<td>3.3,^</td>
<td>12.1,4</td>
<td>3.3,7</td>
<td>9.2,16</td>
<td>20.13,15</td>
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<tr>
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<td>60.54,79</td>
<td>71.68,^</td>
<td>47.53,^</td>
<td>22.42,^</td>
<td>84.101,59</td>
<td>59.58,^</td>
<td>39.75,^</td>
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<tr>
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<td>7.84</td>
<td>9.81</td>
<td>12.51</td>
<td>6.81</td>
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<tr>
<td>MOT Reaction time (ms)</td>
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<td>1304.20</td>
<td>1208.40</td>
<td>1754.56</td>
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<td>DigitsB Total</td>
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<td>7</td>
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<td>PRM Accuracy (%)</td>
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<td>70.83</td>
<td>75.00</td>
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<td>PRM Reaction time (ms)</td>
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<td>60.00</td>
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<td>46.67</td>
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<td>DMS Reaction time (ms)</td>
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<td>4464.50</td>
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<td>3982.00</td>
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<tr>
<td>DMS %Corr</td>
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<td>45.00</td>
<td>55.00</td>
<td>50.00</td>
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<td>0.444</td>
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<td>100</td>
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<td>86.67</td>
<td>100</td>
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<td>2.3,7</td>
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