The dopaminergic system and human spatial working memory: A behavioural, electrophysiological and cerebral blood flow investigation.

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Abstract

Dopamine appears to play a critical role in regulating spatial working memory (SWM) in non-human primates, and SWM deficits are observed in patients with Parkinson’s disease and schizophrenia. Unfortunately, the current experimental literature in humans is marred by inconsistent behavioural findings, and there is a void in neuroimaging studies examining dopaminergic manipulation of SWM-related brain activity. The present thesis used a combination of behavioural neurocognitive testing and brain imaging to further examine dopaminergic manipulation of SWM in healthy humans, using two pharmacological challenges: 1) acute tyrosine depletion (TPD) (to acutely deplete tonic dopamine), and 2) D₁/D₂ receptor activation using the dopamine receptor agonist pergolide (to stimulate dopamine neurotransmission) under conditions of TPD.

The effects of TPD on behavioural performance were examined using three SWM tasks: 1) a delayed-recognition task previously impaired by TPD (Experiment 1) and 2) two delayed-response tasks designed to vary only in response requirements (Experiment 2). The findings demonstrated an apparent failure of TPD to impair performance on any of the tasks. Further, the effects of TPD on SWM-related brain activity during a SWM n-back task were examined using regional Cerebral Blood Flow (rCBF) measured by H₂¹⁵O Positron Emission Tomography (Experiment 2), and Steady State Visually Evoked Potentials (SSVEP) measured by Steady State Probe Topography (Experiment 4). TPD failed to produce discernable effects on either neural networks (task-related rCBF) or temporal electrophysiological activity (SSVEP) associated with the SWM n-back task. In contrast, D₁/D₂ receptor stimulation under dopamine depleted conditions impaired performance on both a SWM delayed-response task (Experiment 1) and SWM n-back task (Experiment 2), and resulted in task-related increases in fronto-temporal SSVEP latency (suggestive of increased inhibition) and decreases in parieto-occipital SSVEP amplitude (suggestive of increased activation) during the early delay period of the SWM n-back task (Experiment 4). These changes are consistent with the undisputed role of frontal and parietal regions in n-back task performance, and with previous evidence of dopaminergic modulation of these regions in animals and humans.
In summary, TPD did not modulate SWM behavioural performance on four different SWM tasks with differing task demands, and failed to produce measurable changes to either SWM-related neural networks (task-related rCBF) or cortical electrophysiological activity (SSVEP) associated with the SWM n-back task. The implication of these findings, when taken together with previous studies, is that the degree of dopaminergic depletion achieved with TPD may be insufficient to consistently and robustly modulate SWM networks in healthy humans, questioning the utility of TPD as a probe of dopaminergic function. In addition, these findings demonstrate the complexity of stimulating D₁/D₂ receptors under dopamine depleted conditions, and highlight the critical importance of baseline dopamine levels in influencing the effects of acute dopamine challenge on SWM performance.
Acknowledgments

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of this thesis. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made.

Kathryn Anne Ellis
October, 2005
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<tbody>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AMPT</td>
<td>$\alpha$-methyl-paratyrosine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AQC</td>
<td>6-aminoquinolyl-N-hydroxysuccinimidyl Carbamate</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann’s Area</td>
</tr>
<tr>
<td>BAL</td>
<td>Balanced Control/Placebo</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral Blood Flow</td>
</tr>
<tr>
<td>CDR</td>
<td>Cognitive Drug Research</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CPT</td>
<td>Continuous Performance Task</td>
</tr>
<tr>
<td>CPT-AX</td>
<td>Continuous Performance Task, AX version</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic Acid</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>EOG</td>
<td>Electro-Oculography</td>
</tr>
<tr>
<td>ERP</td>
<td>Event-Related Potential</td>
</tr>
<tr>
<td>FC</td>
<td>Fourier Coefficients</td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<tr>
<td>FWHM</td>
<td>Full Width Half Maximum</td>
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<tr>
<td>GABA</td>
<td>Gamma-Aminobutyric Acid</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>HVA</td>
<td>Homovanillic Acid</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz (cycles per second)</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>kΩ</td>
<td>Kilo-Ohms</td>
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<td>L-DOPA</td>
<td>L-dihydroxyphenylalanine</td>
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<tr>
<td>LNAA</td>
<td>Large Neutral Amino Acids</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
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<tr>
<td>LTM</td>
<td>Long Term Memory</td>
</tr>
<tr>
<td>M</td>
<td>Mean/average</td>
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<tr>
<td>MAO</td>
<td>Monoamine Oxidase</td>
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<td>MET</td>
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<td>Milligrams</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NMADA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>N-SWM</td>
<td>Non-Spatial Working Memory</td>
</tr>
<tr>
<td>NV</td>
<td>Neurovascular</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PFC</td>
<td>Prefrontal Cortex</td>
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<tr>
<td>PLA</td>
<td>Placebo</td>
</tr>
<tr>
<td>rCBF</td>
<td>Regional Cerebral Blood Flow</td>
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<tr>
<td>RT</td>
<td>Reaction Time</td>
</tr>
<tr>
<td>sec</td>
<td>Seconds</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SED</td>
<td>Standard Error of the Difference of the Means</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SSPT</td>
<td>Steady State Probe Topography</td>
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<td>SSVEP</td>
<td>Steady State Visually Evoked Potential</td>
</tr>
<tr>
<td>SWM</td>
<td>Spatial Working Memory</td>
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<tr>
<td>TPD</td>
<td>Acute Tyrosine/Phenylalanine Depletion</td>
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<td>TPD+P</td>
<td>Acute Tyrosine/Phenylalanine Depletion + Pergolide</td>
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<td>VAL</td>
<td>Valine</td>
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<td>VLPFC</td>
<td>Ventrolateral Prefrontal Cortex</td>
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<td>3-MT</td>
<td>3-methoxytyramine</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-HydroxyTryptamine (Serotonin)</td>
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Chapter One

1 Introduction

1.1 OVERVIEW

Working memory is arguably one of the most studied neuropsychological constructs of the last 20 years, and can be broadly construed as the process of retaining information which is no longer present in the environment, internally manipulating and/or transforming this information, and using this information to guide behaviour. By its nature, working memory is the foundation of most, if not all, higher cognitive functions (Smith and Jonides, 1998, Wager and Smith, 2003), and has been described “as the hub of cognition” (Haberlandt, 1997). It is essential for many everyday tasks, such as reading a sentence, understanding spoken language, conducting mental arithmetic, reasoning, decision making and problem solving.

Dopamine levels within the prefrontal cortex (PFC) appear critical in modulating spatial working memory (SWM) in non-human primate (Goldman-Rakic et al., 1996). Further, deficits in working memory are observed in many psychiatric disorders in which abnormality of the dopaminergic system is implicated, including schizophrenia (e.g. Park and Holzman, 1992, Weickert et al., 2000, Meyer-Lindenberg et al., 2001, Abi-Dargham et al., 2002, Callicott et al., 2003) and Parkinson’s disease (e.g. Lange et al., 1992, Kulisevsky et al., 1996, Postle et al., 1997a, Bublak et al., 2002). However, despite the publication of a number of important studies over the last 13 years, the relationship between dopamine and SWM in healthy humans remains unclear. The current experimental literature in humans is marred by inconsistent behavioural findings, and there is a void in neuroimaging studies examining the effects of dopaminergic manipulation of SWM-related brain activity.

Therefore, the general aim of this thesis was to extend upon the understanding of the effects of dopamine in modulating SWM in healthy humans by conducting a series of behavioural and neuroimaging studies.
The first two chapters of this thesis, through reviews of the literature, provide an introduction to working memory, the human dopaminergic system, and dopaminergic manipulation of working memory. The current chapter is separated into two main sections. In the first section, the concept of working memory will be introduced and an overview of working memory neuroimaging studies in both non-human and human primates will be presented. The latter section of this chapter will provide an overview of the human dopaminergic system. In Chapter 2, a review of the literature examining the pharmacology of working memory is presented, with a specific focus on acute drug challenge studies of the dopaminergic system. Based on the reviews presented in Chapters 1 and 2, the second chapter will culminate in the specific aims and research questions to be addressed in this thesis.

Chapter 3 of this thesis will present issues of general methodology. Chapters’ 4 to 7 will detail the experimental studies, and each experimental chapter will discuss the findings and possible implications of the individual experiment. The final chapter (Chapter 8) will discuss the conclusions and implications of the thesis findings, and address the contribution of this research to the literature.

1.2 HISTORY AND DEFINITIONS

Although visual working memory is arguably one of the most studied neuropsychological constructs, working memory is notoriously difficult to define (Postle et al., 2003, Owen et al., 2005). A number of cognitive theories of working memory have been proposed, which have been previously reviewed in detail (Miyake and Shah, 1999). Arguably the most influential working memory theory in human cognitive psychology is the multi-component model of Baddeley and Hitch (1974). This model assumes that three separable components are involved in working memory: a controlling attentional system known as the central executive, and two subsidiary slave systems - the phonological loop which is responsible for holding and manipulating verbal information, and the visuo-spatial sketch-pad which performs a similar function for visual, spatial and possibly kinesthetic information (Baddeley and Hitch, 1974, Baddeley, 1992, Baddeley, 2003). However, within neuroscience, Jacobsen (1935, 1936) has been credited as the pioneer of working memory research based on findings observed over 30 years before Baddeley and Hitch’s important
model was proposed. In this research, Jacobsen demonstrated that primates with bilateral frontal lobe lesions showed a deficit in a simple working memory task (the delayed-response task; see Section 1.3 below for task descriptions).

Working memory has been examined in a number of species including rodents, non-human primates and humans, and differences in definitions often reflect differences between species and resulting research interests. The discussion of working memory within this thesis will largely be restricted to human and non-human primate literature (for reviews of working memory rodent literature, see Castner et al., 2004, Dalley et al., 2004, Dudchenko, 2004). There are two definitions which arguably form the basis of most characterizations of working memory within the human literature. The first is Baddeley’s conceptualization of working memory: “as a cognitive system for the temporary storage and manipulation of remembered information” (Baddeley, 1981, Baddeley, 1992, Baddeley, 1998b, Baddeley, 1998a, Baddeley, 2001, Baddeley, 2003). The second is Goldman-Rakic’s definition of working memory (based on non-human primate literature): “as the process by which a remembered stimulus is held “on-line” to guide behaviour in the absence of external cues or prompts” (Goldman-Rakic, 1992, Goldman-Rakic, 1996). Like most definitions of working memory, these interpretations are similar in that they highlight the temporary storage of information which is no longer present in the environment, and the manipulation of this information within working memory (i.e. Baddeley) and/or the use of this information in guiding behaviour (i.e. Goldman-Rakic).

1.3 WORKING MEMORY SUB-PROCESSES AND TASKS

While working memory is characterised by maintenance of information during a delay period, a number of additional sub-processes are required for the working memory process to be successfully completed. At its simplest, working memory may take the form of a phone number remembered for just long enough to dial, before the content of working memory passively decays (Goldman-Rakic, 1996). This form of working memory more closely resembles the conceptualisation as it pertains to the non-human primate literature, and can be considered to encompass three main sub-processes: 1) initial sensory and perceptual processes involved in stimulus encoding and motor preparation, 2) information maintenance during a delay period, and 3)
execution processes involved in making a response (Goldman-Rakic, 1996). However, within the human literature, working memory is often conceptualised as a more complex construct which serves as a workspace for holding items of information in mind as they are manipulated, modified, and used in higher cognitive functions. Many definitions of working memory in the human literature more closely resemble this later conceptualization, with working memory involving not only storage and response, but a degree of manipulation of information during the delay period (D'Esposito et al., 2000). In this latter conceptualization, additional sub-processes such as “manipulation” of information are suggested.

While there are a vast number of working memory tasks that have been used in the human and non-human working memory literature, working memory tasks can generally be considered as either variants of the delayed-response task, or as SWM tasks which include additional “executive” components. These paradigms are outlined below.

1.3.1 Delayed-response tasks
The delayed-response task takes two common forms – the delayed-recall task and delayed-recognition task, which will be discussed separately.

Delayed-recall tasks
The delayed-recall task has been used in both non-human primate (for a review, see Goldman-Rakic, 1987) and human research (Luciana et al., 1992, Mehta et al., 2004, Mehta et al., 2005a). This task originated in the non-human primate literature, with arguably the most well known version being the oculomotor (spatial) delayed-response task, in which monkeys were trained to fixate on a central location during a brief (typically 0.5 second) presentation of a peripheral cue, and during a subsequent delay period (typically ranging between 1 – 6 seconds) (Fuster and Alexander, 1971, Funahashi et al., 1989). The monkey was required to remember the location of the cue during the delay, and at the completion of the delay a probe stimulus (fixation target) prompted a response (a saccadic eye movement to the location of the original cue). In humans, the delayed-recall task has often been modified, with the “recall” component being altered from an oculomotor movement to a motor process such as touching a screen to indicate the location of the original stimulus (Luciana et al., 1992, Mehta et
al., 2004, Mehta et al., 2005a). While there are variations of the delayed-recall task, all versions of this paradigm include initial information encoding, maintenance during a delay period, and a response that is not dichotomous or constrained and involves motor preparation and output.

**Delayed-recognition tasks**
The delayed-recognition (or delayed-matched-to-sample) task is a measure of working memory recognition with minimal motor preparation and output and is a modification of the delayed-recall tasks used in human research (Muller et al., 1998, Harmer et al., 2001, Kimberg and D'Esposito, 2003, Postle et al., 2003, Muller et al., 2004, Muller et al., 2005). Specifically, these tasks differ in response requirements, involving a forced choice response, in which at the completion of the delay the participant is presented with one or more possible response options to be compared to the stimulus. Delayed-recognition tasks involve less response preparation and generally less motor output than delayed-recall tasks, but involve some decision making in the response process.

**1.3.2 SWM tasks with “executive components”**
In addition to delayed-response paradigms, there are a number of tasks which test working memory but also include additional executive demands. Two commonly used examples are outlined below.

**N-back task**
In recent years the n-back task has become one of the most common tasks used in neuroimaging studies of working memory (see meta-analysis conducted on 24 studies Owen et al., 2005). The n-back is a continuous task in which participants must monitor a series of stimuli (spatial locations, letters, objects etc.) and identify whether each stimulus is the same as the one presented \( n \) trials earlier (commonly \( n = 1, 2 \) and/or 3) (Gevins and Cutillo, 1993, Cohen et al., 1997). This task requires trial specific sub-processes such as on-line monitoring, updating and manipulation of remembered information and response selections. In addition the n-back task requires task general processes such as sustained attention and maintaining task goals (and instructions) for successful completion. An advantage of the n-back task is that the
load on working memory can be parametrically increased without modifying other task demands.

**Self-ordered strategic search task**

Taken from the CANTAB computerised assessment system (CeNeS Ltd), this test assesses both working memory and strategy. Participants must search for blue tokens which were hidden behind coloured boxes on the screen, by touching the boxes to open them up. Once a token had been found behind a particular box, then that box would never be used again to hide a token, and that spatial location had to therefore be remembered. This task measures errors and reaction time, in addition to a strategy score. The strategic search task has previously been used in studies examining dopaminergic modulation of SWM (e.g. Harmer et al., 2001, Mehta et al., 2001, McLean et al., 2004, Roiser et al., 2004) (see Chapter 2 for discussion).

**Summary**

In its simplest form (as assessed by basic delayed-response tasks) working memory processes are relatively easy to dissociate into separate “stages” – i.e. encoding, maintenance (sometimes known as “holding”), and response. However, more complex working memory tasks, such as the n-back task, are less easy to segregate into stages. Neuroimaging studies have suggested segregation of the n-back task into “perceptual” and “mnemonic/delay” stages (e.g. see Cohen et al., 1997); however it is important to note the likely overlap of a number of different sub-processes during the mnemonic/delay stage of tasks such as the n-back, including manipulation (and/or additional executive processes) and maintenance.

## 1.4 NEUROANATOMY OF WORKING MEMORY

### 1.4.1 Prefrontal Cortex (PFC)

The PFC is a large expanse of cortex that is connected through cortico-cortical projections to all areas of the neocortex, and has rich connections to limbic and subcortical structures (Rezai et al., 1993). It is also established that the PFC provides both excitatory and inhibitory input to distributed neural circuits that are required to support performance in a range of diverse tasks (Knight et al., 1999). The PFC may be divided into a number of major regions, as presented in Figure 1.1
The effects of experimental lesions of the non-human primate PFC have played a predominant role in the current conceptualisations of prefrontal function in the human, and most notably working memory (Curtis and D'Esposito, 2004). Following Jacobsen’s seminal work (1935, 1936) which demonstrated that primates with bilateral frontal lobe lesions showed a deficit in simple working memory task a number of studies have demonstrated that lesions of the PFC were related to impaired performance on the delayed-response task (Mishkin, 1957, Gross and Weiskrantz, 1962, Butters and Pandya, 1969, Goldman and Rosvold, 1970, Butters et al., 1971, Goldman et al., 1971, Passingham, 1975, Mishkin and Manning, 1978, Petrides, 1991, Funahashi et al., 1993, Petrides, 1995). These observations of a critical role for the PFC in working memory have now long been accepted in modern neuroscience, and have been the focus of a large body of neuroimaging work to elucidate the nature of the PFC/working memory relationship.

1.4.2 Delay related activity with the PFC
Perhaps the greatest advance in our understanding of the role of the PFC in working memory began in the 1970’s, with single cell electrophysiological recordings of neurons in the PFC of awake monkeys, performing various delayed-response tasks (Fuster and Alexander, 1971, Kubota and Niki, 1971, Fuster, 1973, Niki, 1974b, Niki, 1974a, Kojima and Goldman-Rakic, 1982, Miller et al., 1996). Arguably the most
influential of these studies employed the oculomotor delayed-response task (discussed above) in which monkeys were trained to fixate on a central location during a brief (generally 0.5 second) presentation of a peripheral cue, and during a subsequent delay period (generally ranging between 1-6 seconds) (Fuster and Alexander, 1971, Funahashi et al., 1989). The monkey was required to remember the location of the cue during the delay, and at the completion of the delay a probe stimulus (fixation target) prompted a response (a saccadic eye movement to the location of the original cue).

These electrophysiological studies demonstrated that during the delay period of the task (i.e. when the visual stimulus was not present), selective neurons within the lateral PFC showed elevated and persistent activity lasting throughout the delay period, before the initiation of a response (Fuster and Alexander, 1971, Kubota and Niki, 1971, Fuster, 1973, Niki, 1974b, Niki, 1974a, Kojima and Goldman-Rakic, 1982, Miller et al., 1996). This sustained firing has been interpreted as a possible cellular correlate of the mnemonic event, and it has been suggested that this neuronal discharge is a reflection of information being held “online” (Goldman-Rakic, 1996). Such neuronal discharge in the absence of stimuli or responses has been recorded for as long as 12-15 seconds during the delay period of a task (Kojima and Goldman-Rakic, 1982, Funahashi et al., 1989). It has further been inferred that neurons within the PFC form so called “memory fields”. This concept is based on the findings that a given neuron within the PFC only increases its firing rate during the delay period and only for specific spatial locations, and this preference for specific locations remains constant across time with the neuron not firing when the stimulus location is different. Further, when sustained firing of the neuron is not maintained throughout the delay, errors are more likely to occur (Funahashi et al., 1989, Goldman-Rakic, 1996).

Wilson et al. (1994) have also suggested that memory field formation relies on interactions between pyramidal and non-pyramidal cells (similar to the importance of pyramidal-non-pyramidal interactions in establishing the orientation specificity of primary visual neurons (for a review, see Sillito and Murphy, 1986)), and have highlighted the importance of inhibition in memory field generation. Using single cell recordings, Wilson et al. (1994) demonstrated that similar to pyramidal cells, non-pyramidal neurons reveal a preference for specific locations. In addition, an apparent synergistic, inverse relationship between pyramidal and non-pyramidal neurons was
observed during the delay component of an oculomotor delayed-response task (i.e. as a non-pyramidal neuron increases its rate of discharge, a nearby pyramidal neuron decreases its rate). Findings of shorter latency for non-pyramidal cells further suggested that feed forward inhibition from the non-pyramidal neurons may possibly play a role in establishing the prefrontal memory fields (Wilson et al., 1994).

Electrophysiological recordings in humans have supported these findings of an important role for the PFC during working memory (for a review, see Ruchkin et al., 2003). Working memory performance has been associated with changes in ongoing electroencephalography (EEG), in components of the averaged event-related potential (ERP) elicited by a discontinuous stimulus, and in steady state visually evoked potentials (SSVEP) elicited by a continuous stimulus. For example, there is a considerable literature showing changes in theta (4-7Hz), alpha (9-13Hz) and gamma (20-80Hz) activity in frontal regions during working memory (Klimesch et al., 1993, Gevins et al., 1996, Gevins et al., 1997, McEvoy et al., 1998, Sarnthein et al., 1998, Tallon-Baudry et al., 1998, Smith et al., 1999, Tallon-Baudry et al., 1999, Gevins and Smith, 2000, Tallon-Baudry et al., 2001, Halgren et al., 2002, Jensen et al., 2002, Jensen and Tesche, 2002, Schack et al., 2002). In addition, there is evidence that ongoing EEG and stimulus registered ERPs are sensitive to variations in the working memory load (Gevins et al., 1996, Gevins et al., 1997, Krause et al., 2000). Recent studies have demonstrated changes in SSVEP during the delay period of working memory tasks. Silberstein et al. (2001) observed that the delay of a SWM delayed-response working memory task was associated with increases in amplitude of the SSVEP signal within the frontal cortex. Consistent with these findings, Perlstein et al. (2003) demonstrated increases in SSVEP amplitude in the frontal cortex during a non-spatial working memory (N-SWM) delayed-response task.

The advent of haemodynamic neuroimaging in the early 1990’s precipitated a number of studies in humans which further supported the role of the PFC in working memory. A large number of studies have demonstrated sustained working memory related activity in the lateral PFC that can be distinguished from transient activity associated with the perceptual and motor events that precede and follow the delay (Cohen et al., 1997, Courtney et al., 1998, Petit et al., 1998). There are a number of informative reviews detailing the involvement of the lateral PFC during the delay of working
memory tasks in humans (Smith and Jonides, 1997, Courtney et al., 1998, D'Esposito et al., 1998, Postle and D'Esposito, 1999, Cabeza and Nyberg, 2000, D'Esposito et al., 2000, Owen, 2000, Postle et al., 2000, Owen et al., 2005), and it is now largely undisputed that the lateral PFC is critical in working memory function in both the human and non-human primate.

However, one of the most contentious issues in working memory neuroscience research is the functional organization of the lateral PFC, which has been the focus of much of the human working memory neuroimaging literature. An important division of the lateral PFC exists between the dorsal and ventral PFC. The dorsolateral PFC (DLPFC) occupies the gross morphological features of the superior and middle frontal gyri in the human brain. In both the human and the monkey brain, the DLPFC occupies several architectonic areas. Arguably the most commonly used reference map was outlined by Brodmann, who devised 47 cortical areas on the basis of cytoarchitectural differences (see Figure 1.2 below), and this map will be referenced throughout this thesis. The most anterior section of the DLPFC is part of the frontopolar/rostral region, corresponding to Brodmann Area (BA) 10, the posterior DLPFC corresponds to BA 8 and the rostral part corresponds to BA 6. Within these regions is the critical mid-sector of the DLPFC cortex, which is occupied by BA 9 and 46 and is most often implied when using the term “dorsolateral PFC”. Within this thesis the terminology DLPFC will be specifically directed towards this mid-sector (BA 9/46), with discussion of BA 8, 6 or 10 stated specifically and separately for clarity. The VLPFC is situated inferior to the mid-DLPFC. This area encompasses BA 45 and BA 47. As will be discussed below, both the DLPFC and VLPFC are thought to be involved in working memory.
There are two main theories as to the role of the DLPFC and VLPFC in working memory: the domain specific model (suggesting a division based on modality of information; i.e. SWM versus N-SWM), and the process specific model (suggesting a division based on the type of process; i.e. maintenance versus manipulation), which have been well reviewed previously (Smith and Jonides, 1997, Courtney et al., 1998, D'Esposito et al., 1998, Postle and D'Esposito, 1999, D'Esposito et al., 2000, Owen, 2000, Postle et al., 2000, Owen et al., 2005). A brief discussion of the two main divergent theories is presented below.

1.4.3 Domain Specific Model
The domain specific model was first proposed by Goldman-Rakic and colleagues (Goldman-Rakic, 1994, Goldman-Rakic, 1995a). This theory proposes a stimulus (or modality) related dissociation of the lateral PFC in temporary storage of information, in which the DLPFC subserves spatial information and the VLPFC is recruited for non-spatial information. The origins of this theory are based on three main lines of evidence. First, lesions of the DLPFC of non-human primates have been observed to impair spatial memory (for a review, see Levy and Goldman-Rakic, 2000), and lesions of the VLPFC interfere with processing of non-spatial information, including form and colour (Passingham, 1975, Mishkin and Manning, 1978). Second, there is evidence that, more posterior, visual information may be segregated into anatomically distinct pathways, with the occipito-parietal pathway (or “dorsal stream”) functionally
specialised for identifying spatial locations and the occipito-temporal pathway (or “ventral stream”) subserving object features (Ungerleider and Mishkin, 1982).

Third, electrophysiological evidence in the non-human primate indicates that SWM and N-SWM delayed-response tasks activate different populations of neurons within the PFC, with a domain-differential distribution in DLPFC and VLPFC regions, respectively (Wilson et al., 1993). As outline above, early electrophysiological studies primarily employed a SWM task (the oculomotor delayed-response task), and demonstrated considerable evidence of sustained firing in the DLPFC. In 1993, Wilson et al. (1993) published an important electrophysiological study which demonstrated a physiological dissociation between the DLPFC and VLPFC in terms of stimulus, specifically showing that electrodes within the VLPFC (BA 12 and 45, just below the principal sulcus on the inferior convexity of the PFC) exhibited delay related responses to faces and objects, while in the same animals the DLPFC (BA 46) revealed sustained activity to spatial location. Similar findings have subsequently been reported (O'Scalaidhe et al., 1997, O'Scalaidhe et al., 1999). It has further been suggested that reciprocal cortico-cortical connections between the parietal and PFC (Selemon and Goldman-Rakic, 1988, Cavada and Goldman-Rakic, 1989) and between the inferior temporal lobes and PFC (Barbas and Mesulam, 1981, Barbas and Pandya, 1987) may form the respective anatomical bases for spatial and object working memory networks.

1.4.4 Process Specific Model
Petrides and colleagues (e.g. Petrides, 1989, Petrides, 1994, Owen et al., 1996, Owen et al., 1999), supported more recently by others (D'Esposito et al., 1998, D'Esposito et al., 2000) have proposed a contrasting model of the segregation of DLPFC/VLPFC function. According to this model, the VLPFC is the site where information is initially retrieved from posterior association areas, and held active to guide behaviour. In contrast, DLPFC is recruited only when “monitoring” and “manipulation” of information held in working memory is required. A central requirement in the process specific model of the lateral PFC is that within a given domain (i.e. SWM or N-SWM), both the dorsal lateral and ventral lateral cortical regions must be observed to show distinct functional roles during the working memory task (Owen et al., 1999). As injury to PFC in humans is rarely restricted to a single area within the lateral PFC,
testing this divisional hypothesis is problematic (Pierrot-Deseilligny et al., 1991, Ptito et al., 1995). The process specific theory therefore initiated a range of studies examining the difference between both SWM and N-SWM, and maintenance and manipulation processes within each modality during working memory tasks. For example, Owen et al. (1996) used Positron Emission Tomography (PET) imaging (combined with MRI co-registration of images) to examine this issue by comparing and contrasting PFC activation during 5 SWM tasks with varying manipulation and maintenance demands (Owen et al., 1996). The findings of this study revealed that when the task required the organization and execution of a sequence of spatial moves retained in working memory, VLPFC (BA 47) blood flow changes were observed bilaterally, while when tasks involved additional active monitoring and manipulation of spatial information within working memory, additional activation foci were observed in mid-DLPFC (BA 46 and 9). These findings were consistent with the process specific model and have been replicated in an additional PET study (Owen et al., 1999). Further, using functional magnetic resonance imaging (fMRI) Owen et al. (1998) demonstrated that the same regions of the lateral PFC were activated during performance of visual spatial and visual N-SWM tasks when all factors unrelated to the type of stimulus material were appropriately controlled.

1.4.5 Current status of models

There are studies in the primate literature which question the domain specific model, since lesions and/or cooling of the DLPFC have been previously observed to cause impairments in both SWM and N-SWM (Fuster and Bauer, 1974, Bauer and Fuster, 1976, Quintana and Fuster, 1993, Petrides, 1995). Similarly, single-unit recording studies have revealed activation of both DLPFC and VLPFC regions during SWM and N-SWM delayed-response tasks (Rosenkilde et al., 1981, Fuster et al., 1982, Quintana et al., 1988, Rao et al., 1997). Within the human imaging literature (fMRI and PET), there is evidence both for and against the domain specific hypothesis. For example, D’Esposito et al. (1998) and Owen et al. (1997) critically examined SWM and N-SWM studies, and revealed no evidence for a dorsal/ventral dissociation based on modality. However, Courtney et al. (1998) argues that this may be due to differences in interpretation. First, activation within the superior frontal sulcus have been observed in a number of SWM tasks (Jonides et al., 1993, Baker et al., 1996, Courtney et al., 1996, Mellet et al., 1996, Owen et al., 1996, Smith et al., 1996,
Courtney et al., 1998, Petit et al., 1998), and has often been disregarded as it was within the premotor cortex (and may be related to hand or eye movement) (Courtney et al., 1998). However, these activations may potentially overlap with dorsolateral regions as defined in non-human primate studies, specifically in light of the spatial resolution of PET imaging (which is in the order of millimetres). Second, when comparing literature between human and monkey studies, assumptions on which cortical areas overlap is influenced by differences between the two species (i.e. the VLPFC and DLPFC are separated by the principal sulcus in the non-human primate, but not within the human brain) and may result in misattribution of activations. A study by Rao et al. (1997) in non-human primates’ may best reflect the domain specific model. In this study, a task that required the maintenance of both spatial and object information resulted in half of the PFC neurons (with delay activity) showing both object and spatial tuning. These findings suggested that while different parts of the lateral frontal cortex may emphasise processing by different information types (or modalities), this segregation may not be absolute.

While D’Esposito et al. (1998) observed no evidence of a segregation of spatial and non-spatial information into dorsal/ventral lateral PFC, they presented evidence of a lateralisation of spatial information. When the VLPFC is activated, there tends to be greater activation in the right hemisphere during spatial tasks and greater left hemisphere activation during non-spatial tasks (D’Esposito et al. 1998). In terms of the process specific model, D’Esposito et al. (1998) concluded that tasks that activated DLPFC were more likely to engage processes requiring computation or transformation of information (i.e. executive processes) in addition to maintenance in working memory. This has been generally supported in a recent meta-analysis of working memory studies in humans, which reviewed 60 neuroimaging studies (Wager and Smith, 2003) Therefore, as will be discussed further below, there is evidence for division within the brain between spatial and non-spatial information; potentially in terms of the PFC, but more likely this is reflected as hemispheric lateralisation (or dominance) specifically in more posterior regions (Wager and Smith, 2003, Owen et al., 2005).
1.4.6 Working memory neuroanatomy: A distributed network

In addition to the PFC, working memory involves cooperative activity of multiple distributed cortical regions. Within the non-human primate, sustained activation has been observed in the posterior parietal cortex during the delay of a SWM task similar to that observed in the PFC (Constantinidis and Steinmetz, 1996; Chafee and Goldman-Rakic, 1998). An important role for the posterior parietal cortex, and also the inferior temporal cortex is also suggested based on their position as end stages of the ventral and dorsal visual pathways, respectively (Ungerleider et al., 1998).

To date, over 60 neuroimaging studies of working memory have been conducted, and have established that working memory activates a distributed network. This data is discussed in a number of informative reviews and meta analyses (i.e. Courtney et al., 1996; D’Esposito et al., 1998; Smith and Jonides, 1999; Wager and Smith, 2003). Regions most commonly activated during working memory tasks include the PFC, posterior parietal cortex and supplementary motor area regardless of the type of information retained (Wager and Smith, 2003).

1.4.7 Spatial vs. Non-spatial information

Within the literature, there has been some suggestion that working memory for spatial information may be right hemisphere lateralised, while non-spatial information activates a left hemisphere dominant network (Wager and Smith, 2003). For example Smith et al. (1996) demonstrated that verbal and object working memory were associated with a left hemisphere network, in contrast to spatial information which was associated with a right hemisphere network. Such lateralisation is consistent with general neuropsychological findings, as it has been consistently demonstrated that lesions of the right hemisphere can result in visuospatial disorders such as spatial agnosia, while lesions of the left hemisphere are more likely to be associated with language (speech, reading, and writing) and praxic disorders (for reviews, see Heilman et al., 1986; Joseph, 1988; Kandel, 1991; Hodgson and Kennard, 2000).

At least one meta-analysis has supported the lateralisation of spatial and non-spatial information to right and left hemispheres, respectively (D’Esposito et al., 1998). However, a more recent meta-analysis failed to find clear indication of dissociation between right and left hemisphere. In this analysis, Wager and Smith (2003) did
observe some right hemisphere lateralisation in the PFC for spatial tasks with greater demands, suggesting that the hypothesised hemispheric lateralisation may be more likely to occur in difficult working memory tasks, rather than simple delayed-response paradigms.

A pattern of dissociation between spatial and non-spatial storage in the posterior cortices has been recently demonstrated. Specifically, spatial information was most frequently found to activate the superior parietal cortex, while object information appeared to activate the inferior temporal cortex. It must be noted, however, that non-spatial information did activate the parietal cortex – just to a lesser degree than spatial information (Wager and Smith, 2003).

1.4.8 N-back task neuroanatomy

There have now been at least 24 studies of the n-back task with fMRI or PET imaging, based on searches within Pubmed and Current Contents databases. Early haemodynamic imaging studies of the n-back task employed a sequential letter version of the task (Smith et al., 1996, Braver et al., 1997, Cohen et al., 1997). Cohen et al. (1997) used fMRI to investigate the temporal dynamics of the n-back task, by examining the difference between transient activations believed to be associated with sensory and motor processes (i.e. not specifically involved in working memory), and more sustained activation associated with working memory per se. Although the haemodynamic lag of fMRI would influence these observations, they reasoned that the temporal information of fMRI, taken together with the fact that activation associated with sensory and motor processes should not increase as a function of memory load, should assist in identifying processes associated with working memory. Indeed, Cohen et al. (1997) identified a distributed network involved in working memory, including the DLPFC, more posterior and inferior regions of the frontal cortex, and the posterior parietal cortex. In the same year, Braver et al. (1997) directly investigated the parametric nature of the n-back task to examine the effect of increasing memory load on working memory related activations. They observed that the n-back activated a distributed region consistent with that described by Cohen et al. (1997), with a linear relationship between activity and working memory load observed in a number of regions activated by the n-back, including the dorsolateral and left inferior regions of PFC.
In one of the earliest studies to examine SWM using the n-back, Smith et al. (1996) used PET to investigate both a visuospatial and verbal version of the letter n-back task; in the spatial version, participants were required to remember the location of the letters presented whereas in the verbal version participants had to remember the actual letter. This study identified hemispheric differences between spatial and verbal working memory, with the spatial task observed to activate a right hemisphere dominant network of right DLPFC and parietal cortex. These regions fit well within the spatial attention network (Mesulam, 2000).

There have consequently been a range of studies to investigate the n-back task for visuospatial information (Carlson et al., 1998, Casey et al., 1998, Owen et al., 1999, Nystrom et al., 2000, Hautzel et al., 2002) and other stimulus types including verbal and non-spatial modalities (Awh et al., 1996, Jonides et al., 1997, Martinkauppi et al., 2000, Druzel and D'Esposito, 2001, Rama et al., 2001, Kim et al., 2002, Ragland et al., 2002, Zurowski et al., 2002, Kim et al., 2003). These tasks range in interstimulus intervals from less than 1 second (Hautzel et al., 2002) to nearly 10 seconds (Cohen et al., 1997), and in terms of SWM tasks, the stimuli have ranged from the location of different letters (Smith et al., 1996, Nystrom et al., 2000), to shapes such as squares (Carlson et al., 1998) or dots (Casey et al., 1998, Owen et al., 1999, Hautzel et al., 2002). However, the findings of these studies have demonstrated that the n-back produces a network of activations which is relatively consistent between studies. A recent meta-analysis examined the activation coordinates of 24 n-back studies (Owen et al., 2005), and demonstrated that that regardless of the type of task (i.e. modality), the n-back activates a robust network which included 6 cortical regions. This network comprised the bilateral and medial posterior parietal cortices, including precuneus and inferior parietal lobules (approximately BA 7/40); bilateral premotor cortices (BA 6, 8); dorsal cingulate/medial promoter cortex, including supplementary motor area (SMA; BA 32, 6); bilateral rostral PFC (BA 10); bilateral DLPFC (BA 9, 46); and bilateral mid-VLPFC or frontal operculum (BA 45/47). While this network was observed regardless of information type, there is evidence suggesting that the modality of information does influence the network with subregional and hemispheric lateralisation. Consistent with a recent meta-analysis of all working memory task types (Wager and Smith, 2003), SWM tended to cause greater activation in the right
DLPFC. Specifically, the SWM n-back reveals augmented activation of the lateral premotor cortex and posterior parietal cortex (Owen et al., 2005).

In summary, evidence suggests that the n-back task produces a robust and consistent working memory network, including the PFC, premotor cortex, cingulate cortex and parietal cortex. Further, there is some indication that SWM n-back working memory produces greater activation in the right hemisphere than the left hemisphere.

1.5 THE DOPAMINERGIC SYSTEM

Until the mid 1950’s, dopamine was considered as a precursor to noradrenaline due to its intermediate role in synthesis of the other catecholamines, noradrenaline and adrenaline (the latter in the peripheral system). The demonstration that dopamine was present in almost equal amounts and had a quite distinct distribution in the brain to noradrenaline (Dahlstrom and Fuxe, 1964, Ungerstedt, 1971), led to extensive research demonstrating the unique and independent nature of dopamine as a neurotransmitter. The discovery that dopamine is important in the pathogenesis and/or treatment of both Parkinson’s disease and schizophrenia further emphasised the importance of dopamine as a neurotransmitter. Dopamine is now considered a critical neurotransmitter within the human brain, not only in terms of motor function but in a range of processes including attention and cognition (for a review, see Nieoullon, 2002), and reward and addiction (Di Chiara et al., 2004, Kalivas and Volkow, 2005). Further, as will be outlined in Chapter 2, there is also strong evidence to suggest a role for dopamine in modulating SWM. Below a brief overview of dopamine synthesis and metabolism, dopamine pathways and dopamine receptors is presented (for a detailed description of the dopaminergic system, see Szabo et al., 2000, Cooper et al., 2003).
1.5.1 Synthesis and metabolism of dopamine

Dopamine is synthesised from the aromatic, neutral amino acid, L-tyrosine (tyrosine) by a sequence of enzymatic steps (first postulated by Blaschko, 1939). The amino acid tyrosine is first converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (Nagatsu et al., 1964). The conversion of tyrosine to L-DOPA is considered the rate limiting step in the synthesis of dopamine (discussed in Chapter 3 in reference to acute tyrosine depletion as a methodology of depleting dopamine). L-DOPA is subsequently converted to dopamine by the aromatic L-amino acid decarboxylase which is found in the cytoplasm. This conversion occurs at a rapid rate, thus L-DOPA levels remain relatively negligible within the brain (Blaschko, 1939). Following synthesis, dopamine is transported to storage vesicles or is metabolised in the cytoplasm. Figure 1.3 summarises the synthesis of dopamine. The predominant mechanism for catecholamine release from nerve terminals is by exocytosis of storage vesicles via a calcium-dependent mechanism (Moore and Bloom, 1979). The extent of dopamine release appears to be a function of the rate and the pattern of firing, with dopamine autoreceptors also prominent regulators of dopamine release (for further discussion, see Grace, 2002).

The main enzymes involved in the metabolism of dopamine are monoamine oxidase (MAO) and catechol-\textit{O}-methyltransferase (COMT). COMT is thought to be of importance in the metabolism of released dopamine in the PFC, where dopamine transporters and lacking (Lewis et al., 2001, Moron et al., 2002). These enzymes convert dopamine to its main metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and in small amounts 3-methoxytyramine (3-MT). Accumulation of HVA has been used as a marker of dopaminergic activity with the brain (Cooper et al., 2003).
Tyrosine is converted to DOPA by the enzyme tyrosine hydroxylase (TH), which is subsequently converted to dopamine (DA) by L-aromatic amino acid decarboxylase (L-AAD). Tyrosine is metabolised by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), which converts dopamine to its main metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and in small amounts 3-methoxytyramine (3-MT). Figure is adapted from Cooper et al. (2003) and Szabo et al. (2004).

1.5.2 Dopamine Pathways

There are three major dopamine pathways in the human brain: 1) the nigrostriatal, 2) mesolimbic, and 3) mesocortical systems (Lindvall and Bjorklund, 1978) (see Figure 1.4, below). The nigrostriatal pathway originates from neuronal cell of the substantia nigra (A9) (for description of lettered nomenclature, see Williams and Goldman-Rakic, 1998), and primarily innervates the caudate nucleus and putamen (known together as the striatum). Neurons within this pathway are assumed critical for
movement as destruction of these neurons is associated with motor dysfunction in Parkinson’s disease.

The mesolimbic system originates in the ventral tegmental area (A10) which is located just medial to the A9 cells, and shares similarities with the nigrostriatal circuit in that it is a parallel circuit consisting of axons that make up much of the medial forebrain bundle (Szabo et al., 2000). However, these axons ascend through the lateral hypothalamus, and project to primarily mesial components of the limbic system including the nucleus accumbens, the nuclei of the stria terminalis, parts of the amygdala and the hippocampus, the lateral septal nuclei and the mesial frontal, anterior cingulate and entorhinal cortex and tuberculum olfactorium (Szabo et al., 2000, Cooper et al., 2003).

The neuronal cell group A10 is also the predominant source of the mesocortical pathway which innervates the PFC and other cortical areas, the septum, amygdala and hippocampus. Other dopamine pathways are present within the brain, such as the mesopontine and tuberhypophyseal pathways, but will not be discussed in this thesis.

In summary, the striatum (including the caudate, putamen and nucleus accumbens) is a major projection target of the dopamine neurons of the substantia nigra. However the cerebral cortex is also highly innervated with dopamine neurons, with these projections arising from cells of the substantia nigra dorsalis, ventral tegmental area, and retrorubral area (Lewis et al., 1988, Williams and Goldman-Rakic, 1998).
1.5.3 Dopaminergic receptors

There are 6 dopamine receptor subtypes currently identified (D$_1$, D$_{2a}$, D$_{2b}$, D$_3$, D$_4$, D$_5$) (Kebabian and Calne, 1979, Stoof and Kebabian, 1984, Kebabian, 1993). These receptor subtypes are divided into two families: the D1 and D2 families. The subtypes were originally based on differing relationships to adenylyl cyclase (AC), the enzyme that converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (Cooper et al., 2003). The D1 family comprises the D$_1$ and D$_5$ receptor subtypes which are generally considered as excitatory (stimulating AC). In contrast, the D2 family comprises the D$_2$, D$_3$ and D$_4$ receptor subtypes which are generally considered as having an inhibitory effect on AC. Due to a relative lack of sensitivity and specificity of dopaminergic agonists/antagonists between the specific receptors in humans, practically all research into humans has focussed on co-activation or
blockade of D1 or D2-like receptors. Consistent with the much of the literature, this thesis will use the terminology D1 and D2 receptors, but the specificity of the effects investigated within the experimental chapters will generally be limited to D1-like and D2-like receptor families, respectively (unless otherwise stated).

The distribution of D1 and D2 receptors within the human brain differs. The PFC is primarily innervated with D1 receptors (Lidow et al., 1991). D1 receptors within the PFC are visible in all cortical layers, but particularly layers II, III and V (Goldman-Rakic et al., 1996). Both pre- and post-synaptic D1 receptors are found in the PFC, although post-synaptic receptors are most common. While D2 receptors are found in the PFC, they are 20-fold less abundant than D1 receptors. D2 receptors are more highly expressed in the striatum, hippocampus, amygdala and other parts of the cerebral cortex (Lidow et al., 1991). Dopamine autoreceptors which are present on the dopamine neuron itself can be classed as D2 autoreceptors, and act as inhibitors to control the rate of firing of the neuron and release of dopamine by the action potential and the terminal (Grace, 2002). Nevertheless, while D1 and D2 receptors can be clearly distinguished from each other, there is also evidence to suggest that there may be interactions between D2 and D1 receptors, such that modulating the D2 receptors may affect D1 receptor function (Lidow et al., 1991, Lidow and Goldman-Rakic, 1994).
Chapter Two

2 The pharmacology of working memory

2.1 INTRODUCTION

As outlined in the previous chapter, dopamine has been recognised as a neurotransmitter for little more than 50 years. However, during this time, the presence of cognitive impairments in patients suffering Parkinson’s disease first raised the suggestion of a contribution of dopamine in controlling non-motor aspects of behaviour (Nieoullon, 2002). There is now considerable evidence of working memory deficits in patients with Parkinson’s disease (e.g. Lange et al., 1992, Kulisevsky et al., 1996, Postle et al., 1997a, Bublak et al., 2002). Further, findings suggest that working memory deficits are a cardinal symptom of schizophrenia (e.g. Park and Holzman, 1992, Weickert et al., 2000, Meyer-Lindenberg et al., 2001, Abi-Dargham et al., 2002, Callicott et al., 2003), and a strong indicator of poor clinical outcome (Green et al., 2000). While the precise nature of working memory deficits in these clinical disorders is far from clear, neuroimaging studies have demonstrated patients with schizophrenia show working memory impairments which are related to deficits in prefrontal function (for review, see Manoach, 2003) and D$_1$ receptor availability within the PFC (Abi-Dargham et al., 2002).

Following the identification of cognitive deficits in clinical groups with suggested dopaminergic abnormalities, studies began to investigate the possible role of dopamine in working memory within non-clinical samples. This chapter will present what is known about the role of dopamine in working memory from acute drug challenge studies, beginning with an overview of the non-human primate literature and leading to a review of pharmacological challenge studies in healthy humans. The dopamine system does not however exist in isolation. While a detailed review of the pharmacology of working memory is beyond the scope of this thesis, the current chapter will attempt to present a basic blueprint of the pharmacology of working memory in humans through the presentation of key findings from acute drug
challenge studies of other (non dopamine) neurotransmitter systems. Evidence from animal studies and clinical studies will be presented when literature on acute human challenges is limited. While the majority of studies reviewed are of SWM tasks (specifically within the dopaminergic section of this review), for completeness this review will also include N-SWM tasks. An earlier version of this review has been published in the International Journal of Neuropsychopharmacology (Ellis et al. 2001; see Appendix 4 for reprint).

The current chapter will culminate in the aims and research questions addressed in this thesis, drawing from the reviews presented in Chapters 1 and 2.

2.2 THE DOPAMINERGIC SYSTEM

2.2.1 Non-human Primate Studies

Based on convergent evidence from lesion studies (Funahashi et al., 1993), regional depletion studies (Brozoski et al., 1979, Roberts et al., 1994), and administration of dopamine receptor agonists and antagonists (for reviews, see Goldman-Rakic et al., 1996, Arnsten, 1997), it is now well established that the integrity of the dopaminergic system within the PFC is critical for working memory performance in non-human primates. For example, dopamine lesions (using 6-hydroxydopamine) of the PFC in primates cause SWM impairments (Brozoski et al., 1979, Roberts et al., 1994), and it has also been demonstrated that such impairments in performance can be restored by the injection of dopamine agonists such as apomorphine or L-dopa into the PFC (Brozoski et al., 1979). Further, iontophoretic application of dopamine enhances delay activity of neurons in the PFC (Sawaguchi et al., 1990). Studies have also shown that during the performance of a delayed-response task, midbrain dopamine neurons in the non-human primate become more active (Schultz et al., 1993) and dopamine levels within the PFC increase (Watanabe et al., 1997).

A series of studies in the early 1990’s suggested a preferential role for the D₁ receptor within the PFC in modulating working memory performance. These studies revealed that local administration of D₁ receptor (and not D₂ receptor) antagonists within the PFC modulated working memory performance (Sawaguchi and Goldman-Rakic, 1991, Sawaguchi and Goldman-Rakic, 1994, Williams and Goldman-Rakic, 1995).
Evidence further indicates that the effect of the D₁ receptor within the PFC follows an inverted U dose-related response (see Figure 2.1, below). Sawaguchi and Goldman-Rakic (1994) reported that injecting higher doses of the dopamine antagonist SCH39166 (a selective D₁ antagonist) into the dorsal PFC of rhesus monkeys was associated with greater impairments on an oculomotor delayed-response task (Sawaguchi and Goldman-Rakic, 1994). In contrast, Williams and Goldman-Rakic (1995) observed that lower concentrations of a D₁ antagonist administered by iontophoresis were associated with improved performance (Williams and Goldman-Rakic, 1995). This enhancement could be reversed by iontophoresis of the partial D₁ receptor agonist SKF 38393, and the effect was specific to the D₁ receptors; the D₂ receptor antagonist raclopride had no delay-specific effect on neuronal responsivity when intophoresed onto prefrontal neurons. The results may best be described in terms of the importance of optimal stimulation of D₁ receptor in PFC, with either insufficient or excessive D₁ receptor stimulation leading to SWM performance impairment (Williams and Goldman-Rakic, 1993, Williams and Goldman-Rakic, 1995).

![Figure 2-1](image)

**Figure 2-1** Schematic representation of the inverted U shaped function as it may relate to delayed-response performance in the monkey.

While direct infusion of D₂ antagonists into the non-human primate PFC does not modulate working memory, a role for the D₂ receptor is still suggested based on evidence that systemic administration of the D₂ agonist quinpirole has been observed to improve working memory performance (Arnsten et al., 1995). While the precise mechanism of these effects is unclear, they are potentially the result of effects within
the striatum or through activation of other dopamine receptor cites (i.e. D₃/D₄ receptors) (Arnsten et al., 1995). Further, lower doses, which presumably act pre-synaptically (thus decreasing dopamine release), actually impair SWM function consistent with the dose-related responses observed with the D₁ agonists/antagonists. A recent study by Wang et al. (2004) also demonstrated a possible dissociation between D₁ and D₂ receptor modulation of working memory. Specifically, these authors observed that modulation of the D₂ receptor (using the D₂ antagonists raclopride or eticlopride, and the D₂ agonist quinpirole) selectively modulated the neural activities associated with memory-guided saccades during an oculomotor delayed-response task (i.e. the response process), yet had little or no effect on the persistent mnemonic-related activity. In contrast, D₁ receptors (as modulated by the agonist SKF38393 and antagonist SCH39166) did not influence response activity, but instead modulated delay related activity consistent with previous findings, as discussed above (for example, see Williams and Goldman-Rakic, 1995).

2.2.2 Human Dopamine Studies

**Dopamine receptor agonists**

While it is generally believed that dopamine plays a prominent role in PFC functions in humans, evidence is inconsistent (Ellis and Nathan, 2001, Kimberg and D'Esposito, 2003). This is in part due to the lack of appropriate pharmacological tools for specifically probing the human D₁ receptors. Direct dopamine receptor challenge studies in humans have therefore been required to employ agonists/antagonists of the D₂ receptor, or combined D₁/D₂ agonists and antagonists. Studies with D₂ antagonists/agonists have yielded inconsistent results, with some studies observing changes in working memory performance (Luciana et al., 1992, Luciana and Collins, 1997, Mehta et al., 2001), and other studies observing no effect (Kimberg et al., 1997, Muller et al., 1998, Bartholomeusz et al., 2003). The effects of the combined D₁/D₂ receptor agonist pergolide have also been inconsistent, with evidence for enhancing working memory performance (Muller et al., 1998), improving performance only in some individuals, dependent on working memory capacity (Kimberg and D'Esposito, 2003), or having no effect on performance at all (Bartholomeusz et al., 2003, Roesch-Ely et al., 2005). Table 2.1 summarises the key findings from dopamine receptor agonist studies in humans.
### Table 2-1  Summary of findings for acute dopamine agonist studies in humans

<table>
<thead>
<tr>
<th>Author</th>
<th>Drug/dosage</th>
<th>Sample</th>
<th>Tasks</th>
<th>Change in performance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct receptor agonists studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luciana et al. 1992</td>
<td>2.5mg BROM</td>
<td>N=8</td>
<td>SWM delayed-recall</td>
<td>Performance ↑</td>
</tr>
<tr>
<td>Luciana and Collins 1997</td>
<td>1.25 and 2.5mg BROM</td>
<td>N = 66</td>
<td>SWM delayed-recall + N-SWM delayed-recognition</td>
<td>SWM effects only: 1.25mg dose ↑ 2.5mg dose (no effect)</td>
</tr>
<tr>
<td>Luciana et al. 1998</td>
<td>1.25mg BROM</td>
<td>N=38</td>
<td>SWM delayed-recall</td>
<td>Performance ↑</td>
</tr>
<tr>
<td>Kimberg et al. 1997</td>
<td>2.5mg BROM</td>
<td>N=31</td>
<td>SWM delayed-recall</td>
<td>Baseline dependent. High baseline participants ↓ Low baseline participants ↑</td>
</tr>
<tr>
<td>Kimberg et al. 2001</td>
<td>2.5mg BROM</td>
<td>N=11</td>
<td>N-SWM 2-back</td>
<td>No performance changes; but modulation of task-related brain activity</td>
</tr>
<tr>
<td>Müller et al. 1998</td>
<td>2.5mg BROM 0.1mg PERG</td>
<td>N=32</td>
<td>SWM delayed-recognition</td>
<td>PERG ↑ BROM no effect Low baseline participants: ↑ performance on SWM span task</td>
</tr>
<tr>
<td>Mehta et al. 2001</td>
<td>1.25mg BROM</td>
<td>N=20</td>
<td>SWM self-ordered strategic search, spatial span task, SWM delayed-recognition</td>
<td>No effects</td>
</tr>
<tr>
<td>Bartholomeusz et al. 2003</td>
<td>2.5mg BROM 0.05mg PERG</td>
<td>N=12</td>
<td>N-SWM n-back</td>
<td>High baseline participants ↑ Low baseline participants ↓</td>
</tr>
<tr>
<td>Kimberg et al. 2003</td>
<td>0.1mg PERG</td>
<td>N=31</td>
<td>SWM + N-SWM delayed-recognition</td>
<td>No effects</td>
</tr>
<tr>
<td>Roesch-Ely et al. 2005</td>
<td>2.5mg BROM 0.1mg PERG</td>
<td>N=40</td>
<td>SWM delayed-recall</td>
<td>No effects</td>
</tr>
<tr>
<td><strong>Indirect dopamine agonist studies (stimulant studies)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elliot et al. 1997</td>
<td>40 and 60mg MPH</td>
<td>N=28</td>
<td>SWM Self-ordered strategic search, spatial span</td>
<td>Performance ↑</td>
</tr>
<tr>
<td>Mehta et al. 2000</td>
<td>40mg MPH</td>
<td>N=10</td>
<td>SWM Self-ordered strategic search, spatial span</td>
<td>Performance ↑</td>
</tr>
<tr>
<td>Mattay et al. 2000</td>
<td>0.25mg/kg D-AMPH</td>
<td>N=10</td>
<td>N-SWM n-back</td>
<td>High baseline participants ↓ Low baseline participants ↑</td>
</tr>
<tr>
<td>Mattay et al. 2003</td>
<td>0.25mg/kg D-AMPH</td>
<td>N=27</td>
<td>N-SWM n-back</td>
<td>COMT genotype effects: Met/Met ↓ ; Val/Val ↑</td>
</tr>
<tr>
<td>Cooper et al. 2005</td>
<td>5, 15 and 45mg MPH</td>
<td>N=32</td>
<td>Modified continuous performance task (CPT)</td>
<td>Performance ↑ (+ changes to ERP and autonomic arousal measures)</td>
</tr>
</tbody>
</table>

Key: BROM = bromocriptine, PERG = pergolide, MPF = methylphenidate, D-AMPH = dextroamphetamine
The first examination of the effect of dopamine receptor agonists on SWM performance in healthy humans was conducted by Luciana et al. (1992). Eight young healthy females performed a visuo-spatial delayed-response task following an oral dose of 2.5mg of bromocriptine, a D₂ agonist. The task was a classic delayed-recall paradigm, with a spatial cue (a black dot) presented as stimulus, followed by a delay of both 0 seconds (i.e. motor control condition) and 8 seconds, and a response requiring the participant to indicate the location of the cue with a fine-pointed light pen. Consistent with authors’ hypothesis there was a 44% improvement in the accuracy of identifying the cue location in the 8-second bromocriptine condition, compared to placebo, with no significant improvement in the 0-second delay. However the small sample size (N=8) of the study limited the generalisability of these results.

In a subsequent study, using a larger sample of 66 young adults (aged 19 – 37 years), Luciana et al. (1997) provided additional evidence for a facilitating role of D₂ receptor agonists in SWM (using a delayed-recall task), but found no effect on object working memory (using a delayed-recognition task). While these findings may suggest specificity for dopaminergic modulation of SWM, Luciana et al. (1997) also suggested that the possible differences in response preparation and execution between the delayed-recall (high motor response requirements) and delayed-recognition (low motor response requirements) may also underlie the apparent modality dissociation.

In this study the acute dose of 2.5mg bromocriptine, which had facilitated SWM performance in the earlier study (Luciana et al., 1992) was not replicated, although performance accuracy on the spatial task was improved following administration of a smaller dose of bromocriptine (1.25mg). More recently, Luciana et al. (1998), in a sample of 38 volunteers, again observed a facilitating effect of 1.25mg of bromocriptine on SWM when behavioural testing occurred between 3.5 and 5.5 hours after drug administration. The authors suggested that the discrepancy between studies might be due to differences in the time of cognitive testing (Luciana and Collins, 1997). In the first study (Luciana et al., 1992), the delayed-response task was administered between 2.5 and 3.5 hours after drug administration, while in the subsequent studies (Luciana and Collins, 1997, Luciana et al., 1998) the delayed-response task was administered between 3.5 and 5.5 hours after drug administration. Luciana et al. (1998) argued that in the former study (which used a high dose of
2.5mg and tested one hour earlier), testing may have taken place while bromocriptine levels were “sub-maximal”, and the cognitive effects may be comparable to those for “maximal” levels of 1.25mg of bromocriptine in the later studies.

Such a suggestion would be consistent with an inverted U dose-related response of bromocriptine on SWM performance, with low doses facilitating performance and higher doses having no effect or an impairing effect. However, it should be noted that an apparent inverted U shape response may also be explained as the superimposition of an inverse dose-related sedative effect, in addition to the putative dose-related cognitive effects. Sedation has been widely associated with increased dopamine levels (Canales and Iversen, 2000, Schapira, 2000). Indeed, Luciana and Collins (1997) noted in their study that adverse effects of bromocriptine at “high” levels (2.5mg) resulted in a 50% withdrawal rate of participants.

Mehta et al. (2001) further suggested a role for the D_2 receptor in modulating SWM. In this study, 20 healthy participants were administered 1.25mg bromocriptine, and performed three working memory tasks: a SWM span task, a SWM delayed-recognition task, and a SWM self-ordered strategic search task (all taken from the CANTAB battery). Mehta et al. (2001) observed improvements in performance on the spatial span task following bromocriptine (compared to placebo), but no effect on the other two SWM tasks. Consistent with the proposal of Luciana et al. (1997), Mehta et al. (2001) suggested that differences in response preparation and execution demands between the spatial span task (requiring response preparation during delay and execution of spatially guided motor response) and the other two tasks (not requiring response preparation until after the delay) may underlie the differences in results.

In contrast to possible dose and task-related effects, Kimberg et al. (1997) demonstrated the importance of individual difference on the effect of bromocriptine on working memory performance. Their sample was divided into two groups, either high working memory span or low working memory span, based on verbal working memory (as assessed by reading span). Thirty one participants performed a variety of tasks sensitive to prefrontal function (a card sorting task, associative memory task, context memory task, and a Stroop task), in addition to a SWM task similar to the delayed-response task used by Luciana et al. (1992). Following an acute dose of
2.5mg of bromocriptine, performance changes were observed to be dependent on the baseline working memory capacity of the subject. Participants with a high baseline working memory performed more poorly while under the influence of bromocriptine compared to placebo, while participants with low baseline working memory performed better following bromocriptine administration compared to placebo. In one of only a few studies using neuroimaging to examine the effect of dopaminergic manipulation of working memory, Kimberg et al. (2001) further examined the effect of bromocriptine on working memory and associated brain activity, and observed evidence of a decrease in task-related activation following bromocriptine in the left posterior parietal cortex, a region previously identified as part of the working memory network (Wager and Smith, 2003). While D₂ receptors are found in areas such as the PFC, they are 20-fold less abundant than D₁ receptors (Lidow et al., 1991), and Kimberg et al. (2001) suggest that the relative scarcity of D₂ receptors in the PFC and other neocortical areas may indicate that the effect of bromocriptine on working memory is not a direct effect. Given the abundance of D₂ receptors on layer V of the PFC, it was suggested that the down stream effects of bromocriptine (from areas rich in D₂ receptors) through projections to the cortical areas (via layer V), may dominate the cortical effects of D₂ receptor stimulation (Kimberg et al., 2001). However, there is also evidence to suggest that there may be interactions between D₂ and D₁ receptors, such that modulation of D₂ receptors may affect D₁ receptor function (Lidow et al., 1991, Lidow and Goldman-Rakic, 1994). This indicates that D₁ receptors may play a more prominent role in directly modulating human working memory, which may be highlighted further with the development of an appropriate D₁ receptor agonist for use in humans.

A number of more recent studies have attempted to investigate the effects of both the D₁ and D₂ receptor on working memory performance, using the combined D₁/D₂ agonist pergolide. Muller et al. (1998) performed the first of such studies, with a cleverly designed pharmacological subtraction design in which both pergolide (D₁/D₂ receptor agonist) and bromocriptine (D₂ receptor agonist) were administered (with dosages of pergolide and bromocriptine thought to be comparable in terms of biological and therapeutic action). Thirty-two healthy young adults received either an acute oral dose of 0.1mg pergolide (a combined D₁/D₂ receptor agonist) or 2.5mg of
bromocriptine (a D₂ receptor agonist) and performed a SWM delayed-recognition task designed to minimise motor control, with delay lengths of 2, 8 or 16 seconds. Consistent with Luciana et al. (1992), the main experimental task was performed between 2.5 and 3.5 hours after drug intake. The findings of this study demonstrate that while bromocriptine (2.5mg) failed to facilitate SWM, pergolide improved performance at delays of 16 seconds. Based on a pharmacological subtraction, the authors concluded that this demonstrated evidence for D₁ but not D₂ receptor involvement in SWM.

As discussed by Muller et al. (1998), pharmacological subtraction technique is reliant on a number of assumptions, such as equal dosage. An additional assumption is that pergolide and bromocriptine must have similar affinities for D₂ receptors, while only pergolide should have an affinity for D₁ receptors. However, the ratio of D₁/D₂ receptor affinity for pergolide and bromocriptine has been reported to be comparable for the orally administered agonists (pergolide = 67nM and bromocriptine = 60nM) (De Keyser et al., 1995), and pergolide has been shown to have a greater affinity for D₂ receptors than bromocriptine (Clemens et al., 1993, Miyagi et al., 1996), and is up to 650 times more potent than bromocriptine at D₂ receptors (Zhang et al., 1995). Nevertheless, Muller et al. (1998) demonstrated that prolactin secretion (an indicator of D₂ receptor activity) was not significantly different between the two treatment conditions in the study of Muller et al. (1998), suggesting that from a pharmacodynamic perspective, both pergolide and bromocriptine may have similar D₂ receptor efficacy, and the conclusion that D₁ receptors mediated the effects is most likely.

Not all studies have observed an effect on working memory following pergolide administration. In the largest study to date, Roesch-Ely et al. (2005) tested 40 healthy participants following pergolide (0.1mg) and bromocriptine (2.5mg) on a SWM delayed-recall task (in addition to other tests of executive function). These findings revealed no effect of either drug on performance. Similarly, Bartholomeusz et al. (2003) assessed the possible effects of pergolide and bromocriptine on object working memory using the object n-back task. No significant effects of either drug on performance were observed, and Bartholomeusz and colleagues (2003) suggested that these findings, taken together with the findings of Luciana et al. (1997) (in which
bromocriptine failed to modulate object working memory, but improved SWM) may suggest a specificity of dopamine for SWM.

Kimberg and D’Esposito (2003) also examined the effect of pergolide (0.1mg) on thirty one participants who performed an object and SWM delayed-response tasks (in addition to other cognitive tasks). While there was no main effect of pergolide on performance, participants with greater verbal working memory capacity demonstrated improved performance following pergolide, while low-capacity participants performed more poorly following the pergolide. The effect did not differentiate between spatial and object working memory. This interaction between working memory span and pergolide on working memory performance was opposite to that observed by the same authors in an earlier study with bromocriptine (Kimberg et al., 1997). In that study, low span participants showed improved performance following dopaminergic receptor stimulation. The authors suggested that these results are not directly conflicting since both drugs target different receptors; pergolide targets both D<sub>1</sub>/D<sub>2</sub> receptors, while bromocriptine at low concentrations and early in its time course has predominantly presynaptic inhibitory effects on cortical activity of D<sub>2</sub> receptor, whereas at higher concentrations, or later in its time course, its effects are predominantly postsynaptic, facilitatory effects and may also be an agonist for D<sub>1</sub> receptors (Kimberg et al., 1997). Nevertheless, Roesch-Ely et al. (2005) and Bartholomeusz et al. (2003) also examined baseline dependent effects and observed no differences between groups. Indeed, while baseline working memory has previously been related to performance changes following dopamine manipulation (Kimberg et al., 1997, Mehta et al., 2000, Kimberg and D’Esposito, 2003), these effects have been inconsistent and sometimes contradictory, and may be dependent on factors such as concentrations of drug, and time of cognitive testing (in respect to kinetic effects of the drug), as highlighted by Kimberg and D’Esposito (2003).

There is some indication that individual differences between participants may be more reliably linked to functional polymorphism (val-met) in the catechol O-methyltransferase gene (COMT) (of which baseline-dependent behaviour may be a reflection). COMT is an enzyme involved in regulating synaptic dopamine levels (as discussed in Chapter 1), and studies indicate that COMT appears to play a unique role in regulating dopamine flux in the PFC, while having little or no effect on dopamine
levels in the striatum (Lewis et al., 2001, Moron et al., 2002). The COMT gene contains a functional polymorphism that codes for a substitution of Methionine (Met) for Valine (Val) at codon 158, with the Met allele having one-fourth the enzymatic activity of the Val allele (Lachman et al., 1996). Recent evidence suggests that COMT genotype variation is related to frontal cortex function, with evidence that participants with the Val/Val allele perform more poorly on the Wisconsin card sort test of executive function than participants with the Met/Met allele (Egan et al., 2001, Malhotra et al., 2002). Further, examination of whether COMT genotype may be associated with schizophrenia is a topic of considerable research interest (for a meta-analysis, see Fan et al., 2005), with recent evidence suggesting that COMT genotype may influence the effects of sub-chronic olanzapine treatment on n-back task performance in patients with schizophrenia (Bertolino et al., 2004).

A recent study by Mattay et al. (2003) suggested that COMT genotype may influence the effects of the indirect dopamine agonist dextroamphetamine on working memory performance. Using fMRI, these authors demonstrated that participants with the Val/Val genotype (and presumably less prefrontal dopamine) showed improved efficiency of PFC function associated with a N-SWM n-back task following amphetamine, while participants with Met/Met genotype (and presumably higher prefrontal dopamine) were impaired on the 3-back task and revealed less efficient PFC task-related function.

Evidence that stimulant drugs such as dextroamphetamine and methylphenidate may modulate working memory performance has previously been established. Methylphenidate and amphetamine are indirect dopamine agonists, which act to increase the synaptic concentration of both dopamine and noradrenaline by blocking their reuptake, and studies suggest that these drugs may enhance working memory performance. For example, Cooper et al. (2005) recently demonstrated that administration of methylphenidate to 32 healthy young men appeared to improve performance, as assessed by changes in behavioural, autonomic arousal (heart rate, skin conductance) and psychophysiological (evoked-response potential; ERP) measures. Increasing dose (5 mg, 15 mg or 45 mg) was associated with a reduction in reaction time and omission errors, and an associated reduction in latency of the target P3 (an ERP peak occurring between 220–550ms post-stimulus) and increase in
background P3 amplitude. Such findings are consistent with an earlier report by Elliot et al. (1997) who observed a significant improvement in performance of SWM as assessed by the CANTAB spatial span task and strategic search task (Elliott et al., 1997). Mehta et al. (2000) also observed methylphenidate-induced improvements on the SWM strategic search task, and using PET imaging these authors further demonstrated that methylphenidate-induced improvements in SWM performance were associated with task-related reductions in rCBF in the DLPFC and posterior parietal cortex (Mehta et al., 2000). Consistent with direct dopamine agonist studies in which performance changes were dependent on baseline working memory capacity (Kimberg et al., 1997, Kimberg and D'Esposito, 2003), Mehta et al. (2000) also observed greater effect on working memory in participants with lower baseline working memory capacity. Similar findings were reported by Mattay et al. (2000) using fMRI following administration of dextroamphetamine. These authors demonstrated that dextroamphetamine increased task-related blood oxygen level dependent (BOLD) signal within the right DLPFC during the n-back task. However, improved performance was only in those participants who had relatively low working-memory capacity at baseline, with participants who had high working-memory capacity at baseline revealing performance impairment. And as stated above, Mattay et al. (2003) have recently demonstrated that COMT genotype may influence the effects of dextroamphetamine on working memory performance.

It is important to note that the effects of indirect dopamine agonists such as methylphenidate and amphetamine may be related to noradrenergic mechanisms. While methylphenidate blocks reuptake of dopamine more effectively than it blocks noradrenaline (NA) and much more than it blocks 5-HT (Gatley et al., 1996), the importance of noradrenaline modulation in altering working memory performance cannot be ruled out. Indeed, recent evidence in the rat has demonstrated that both idazoxan (a noradrenergic α2 adrenoceptor antagonist,) and SCH23390 (a dopamine D1 antagonist) can reverse the beneficial effects of methylphenidate on working memory performance (Arnsten and Dudley, 2005). This indicates that both noradrenergic α2 adrenergic and dopamine D1 receptor stimulation may contribute to working memory enhancing effects of methylphenidate. There are also a number of studies demonstrating noradrenergic modulation of working memory, which will be outlined below (Section 2.3).
In summary, findings with direct dopamine agonists (e.g. bromocriptine and pergolide) and indirect catecholamine agonists (e.g. stimulant drugs) are suggestive of a facilitatory effect on working memory performance, however the findings are inconsistent. Differential effects between dosages (e.g. Luciana et al., 1992, Luciana and Collins, 1997, Luciana et al., 1998), baseline working memory status (e.g. Kimberg et al., 1997, Kimberg and D'Esposito, 2003), and possibly COMT genotype (e.g. Mattay et al., 2003) indicate that “optimal” levels of dopamine functioning are related to optimal performance, similar to the finding that either excessive or insufficient D₁ receptor modulation disrupts performance in non-human primates (Williams and Goldman-Rakic, 1993, Williams and Goldman-Rakic, 1995). Inconsistencies within the literature may be due to a number of issues, such as differences in task demands (including response preparation), differences between individuals (such as baseline working memory, which may be best reflected by differences in COMT genotype), and/or complex effects between D₁ and D₂ receptors.

**Dopamine receptor antagonists**

There are a limited number of studies examining the effects of dopamine receptor antagonists on working memory performance conducted in healthy humans. Mehta et al. (1999, 2003, 2005b) have conducted a series of studies examining the effects of the D₂ dopamine antagonist sulpiride on working memory; the study conducted in 2003 including functional brain imaging (PET). In the first study (Mehta et al., 1999), these authors employed a sample of 34 young healthy males, and reported that SWM was impaired following both 200mg and 400mg doses of sulpiride (compared to placebo). However, in their latter studies (Mehta et al., 2003, Mehta et al., 2005b), these authors failed to observe an effect of 400mg of sulpiride on SWM performance. In addition, despite sulpiride having a main effect on blood flow (as predicted), there was also no effect of sulpiride on SWM n-back task-related brain activity (Mehta et al., 2003).

In the study outlined above by Luciana and Collins (1998), which investigated the effects of bromocriptine on a SWM delayed-response task, the effect of the D₂ receptor antagonist haloperidol was also examined. Following a 3mg oral dose of haloperidol, a decrease in performance was observed on the SWM delayed-recall task. This decrement was observed at delays of 8 and 16 seconds, but not at a delay of 5
seconds (Luciana et al., 1998). As far as can be ascertained, there have been no other studies investigating the effects of haloperidol on working memory in healthy human participants. In non-human primates, evidence suggests that haloperidol can impair both SWM and N-SWM, and this effect can be reversed by the selective D₁ receptor agonist ABT 431. It is unclear whether the effects of the D₁ and D₂ receptors on performance in this study were independent, or through interactions between D₁ and D₂ receptors (Lidow et al., 1991, Lidow and Goldman-Rakic, 1994).

Typical antipsychotics such as haloperidol have been shown to impair working memory in patients with schizophrenia, whereas atypical antipsychotics with less D₂ antagonistic properties have been shown to improve working memory in schizophrenia (Honey et al., 1999). However, research investigating patients with schizophrenia has generally involved chronic administration of dopamine antagonists, and interpretation has been difficult as patients with schizophrenia are generally regarded as having abnormalities in their dopaminergic systems. In addition, these antipsychotics also have other pharmacological properties, including cholinergic receptor antagonism, which may independently influence working memory functioning (see Section 2.4.1 below, for discussion of cholinergic modulation of working memory).

**Acute Tyrosine Depletion (TPD)**

An alternative technique for decreasing dopaminergic transmission system has been global depletion of dopamine, through acute tyrosine/phenylalanine depletion (TPD). A full description of tyrosine depletion as a method of depleting dopamine levels is presented in Chapter 3 (General Methods). Briefly, as outlined in the previous chapter, the synthesis of dopamine is dependent on the availability of its precursor, the amino acid tyrosine (and tyrosine’s precursor, phenylalanine). Evidence indicates that reducing the availability of tyrosine and phenylalanine consequently reduces the synthesis and release of dopamine in rats (Milner et al., 1986, Tam and Roth, 1997, McTavish et al., 1999a, McTavish et al., 1999b, McTavish et al., 1999c). Studies in humans further show reduced dopamine release within the human striatum (Montgomery et al., 2003), and reduced d-amphetamine-induced dopamine release following TPD in humans (Leyton et al., 2004b), as assessed by changes in

Within the last 5 years, studies have investigated the effect of TPD on SWM, and in line with the dopamine agonist/antagonist studies the findings have also been inconsistent. The first study to assess the effects of TPD on working memory was conducted by Harmer et al. (2001), who administered both a SWM delayed-recognition task and the self-ordered strategic search task to a sample of 12 healthy participants. This study revealed TPD-related SWM deficits on both tasks following TPD (compared to placebo/balanced). These findings were supported in a subsequent study by Harrison et al. (2004), who observed impaired accuracy on a SWM delayed-recognition task in a sample of 13 healthy females. This latter study further suggested modality specificity, with no TPD-related performance impairment observed on a N-SWM task. However, two recent studies conducted by McLean (2004) and Roiser et al. (2004) have failed to observe a detrimental effect of TPD on the self-ordered search task used by Harmer et al. (2001), while Lythe et al. (2005) failed to observe impairment on a delayed-response task following TPD (see Table 2.2 for summary of studies).

**Table 2-2**  Summary of studies examining the effects of TPD on SWM in humans

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Tasks</th>
<th>Change in performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmer et al. 2001</td>
<td>N=12</td>
<td>SWM Self-ordered strategic search, SWM delayed-recognition</td>
<td>Performance ↓</td>
</tr>
<tr>
<td>Harrison et al. 2004</td>
<td>N=13</td>
<td>SWM delayed-recognition N-SWM digit sequence</td>
<td>SWM: Performance ↓ N-SWM: No effect</td>
</tr>
<tr>
<td>Lythe et al. 2005</td>
<td>N=12</td>
<td>SWM delayed-recognition</td>
<td>No effect</td>
</tr>
<tr>
<td>McLean et al. 2004</td>
<td>N=49</td>
<td>SWM Self-ordered strategic search, SWM spatial span</td>
<td>No effects</td>
</tr>
<tr>
<td>Mehta et al. 2005</td>
<td>N=14</td>
<td>SWM delayed-recall</td>
<td>No group performance effect. Greater dopamine depletion ↓</td>
</tr>
</tbody>
</table>
Nevertheless, research into the role of TPD on working memory is still in its infancy. A recent study demonstrated the advantages of combined neuroimaging and behavioural performance measures in examining TPD effects on working memory, by demonstrating that although TPD did not alter performance at a group level, there was a correlation between the magnitude of striatal dopamine depletion (as indexed by striatal [11C]raclopride binding changes) and performance changes (Mehta et al., 2005a). Specifically, only participants with a high dopamine depletion level (i.e. increased [11C]raclopride binding) within the striatum revealed performance impairment, with virtually no change (and/or subtle improvement in performance) observed in participants with minimal dopamine depletion levels.

**Alternative methods of dopamine depletion**

Dopamine can also be experimentally depleted via α-methyl-paratyrosine (AMPT). This technique involves inhibiting tyrosine hydroxylase, the rate limiting enzyme for dopamine and noradrenaline synthesis (see Chapter 1 for overview of catecholamine synthesis). However, as AMPT inhibits the first step in catecholamine synthesis (via tyrosine hydroxylase), this method depletes both dopamine and noradrenaline. Further, AMPT has been observed to cause side effects including mild Parkinsonian symptoms and akathisia (an inability to remain still) in some participants (Verhoeff et al., 2003). Nevertheless, Verhoeff et al. (2003) demonstrated that AMPT caused subtle impairments in working memory, however these trends were not significant.

Exposure to acute stress, such as cold temperature, may also disrupt the sustained release of catecholamines (Bandaret and Lieberman, 1989), and has been previously observed to cause impairment on delayed-response task performance (Thomas et al., 1989, Shurtleff et al., 1994). Interestingly, Shurtleff et al. (1994) demonstrated that administration of 150mg/kg per body weight of the catecholamine precursor tyrosine, 90 minutes before exposure to the cold protected against working memory performance. However, tyrosine only had an effect on performance in the cold environment and not in participants who performed the task in normal conditions. These findings were subsequently supported by a study assessing the effects of acute stress related to a complicated test battery (compared to simple test battery), and demonstrated that tyrosine may sustain working memory when competing
requirements to perform other tasks simultaneously degrade performance (Thomas et al., 1999).

### 2.3 THE NORADRENERGIC SYSTEM

A role for noradrenaline in arousal, attention and reinforcement has long been established, based on studies in rodents and non-human primates (for reviews, see Aston-Jones et al., 1991, Sara et al., 1994, Coull, 1998, Sara, 1998, Berridge and Waterhouse, 2003, Arnsten and Li, 2005). Similarly, research in non-human primates suggest a role for noradrenaline in working memory. A series of studies have demonstrated α2 adrenergic agonists to improve working memory in non-human primates, particularly in aged animals (Arnsten et al., 1988, Arnsten and Goldman-Rakic, 1990, Rama et al., 1996, Franowicz and Arnsten, 1998). Arnsten and colleagues have further demonstrated that noradrenaline has a beneficial effect on working memory within the PFC through its actions at post-synaptic α2 adrenergic receptors, but impairs PFC function through actions at α1 adrenergic receptors (for reviews, see Arnsten, 1997, Arnsten and Li, 2005).

Findings from human studies have supported a role for the α2 adrenoceptor in working memory. Studies using the α2 adrenoceptor agonist clonidine suggest that the effects on working memory are dose-dependent. For example, Coull et al. (1995) reported that administration of clonidine (which effectively decreases noradrenaline in normal healthy humans) impaired SWM performance on the self-ordered SWM task, with 2.5µg/kg producing a greater deficit in performance than 1.5µg/kg. Similarly, Jakala (1999) observed doses of 0.5µg/kg and 5µg/kg of clonidine to impair SWM (on the self-ordered SWM task), with no effects were observed following a dose of 2µg/kg. Jakala (1999) further observed clonidine to impair working memory performance on a delayed-response task. Dose response effects following clonidine may be related to the fact that lower doses of α2 agonists act pre-synaptically, while higher doses of α2 agonists are thought to have greater action at post-synaptic (and post-junctional) receptors (Arnsten, 1997). In addition to the effects of clonidine, Jakala (1999) also observed that 29µg/kg dose of guanfacine (also an α2 adrenoceptor agonist) improved SWM performance. These authors suggesting that the greater selectivity of guanfacine for α2 adrenoceptor subtype may underlie its differences from clonidine.
A recent study extended the examination of noradrenergic modulation of working memory to include the beta receptor. Muller et al. (2005) examined the effect of the beta-receptor antagonists (“beta-blockers”) propranolol and atenolol on numeric working memory. Propranolol is a lipophilic drug and crosses the blood brain barrier (BBB), while atenolol is a hydrophilic drug and is less likely to cross the BBB than propranolol (Lopez-Sendon et al., 2004). Propranolol induced impairments in performance, with no effect of atenolol and as both beta-blockers induced a comparable decrease of blood pressure and pulse, Muller et al. (2005) suggested that the propranolol effect was likely to be a central effect, presumably acting at the level of the PFC. Muller et al. (2004) have also examined the effect of modafinil, a non-amphetamine psychostimulant drug purported to have therapeutic potential in Attention Deficit Hyperactivity Disorder (ADHD), on working memory performance. While the mechanism of action of modafinil is still unclear, evidence indicates that the behavioural effects of the drug can be antagonised by noradrenergic and not dopaminergic antagonists (Duteil et al., 1990, Lin et al., 1992). Muller et al. (2004) examined the effects of 200mg modafilin on performance of a delayed-response task and working memory task involving manipulation of information, and observed subtle enhancing effects on task performance, with greater effects in lower performing participants.

In summary, evidence suggests that manipulation of the noradrenergic system, specifically the $\alpha_2$ receptor, modulates working memory performance in humans in a dose dependant manner. However, the overlapping of attentional and working memory processes in working memory tasks must be given specific consideration when considering the sizeable literature suggesting noradrenergic modulation of attention (for discussion, see Muller et al., 2004). Further, there is evidence that $\alpha_2$ agonists and antagonists (including clonidine) produce concomitant changes in both extracellular dopamine and NA in the PFC of rodents (Gresch et al., 1995, Devoto et al., 2001, Kawahara et al., 2001), hence the effects of modulation of $\alpha_2$ receptors on working memory may be at least partly related to dopamine mechanisms.
2.4 OTHER NEUROTRANSMITTER SYSTEMS

Further to the effects of catecholamines, other neurotransmitter systems are known to influence working memory performance, both directly and through proposed interactions with the dopaminergic system. While a detailed review is outside the scope of this thesis, key evidence indicating a role for acetylcholine, serotonin, Gamma-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) will be briefly reviewed below.

2.4.1 Acetylcholine

The relationship between human memory and the cholinergic neurotransmitter system is well established in the literature with early reports of the role of acetylcholine (ACh) in learning and memory (Drachman and Leavitt, 1974), and there is extensive literature detailing learning and working memory deficits in Alzheimer’s disease (for review, see Germano and Kinsella, 2005). A number of studies have demonstrated that scopolamine (a muscarinic receptor antagonist) can impair working memory performance (Mewaldt and Ghoneim, 1979, Rassmusson and Dudar, 1979, Duka et al., 1996, Robbins et al., 1997, Ellis et al., 2005a, Green et al., 2005), and there has also been evidence of a dose related effect, with increased dosages related to greater deficits in performance (Duka et al., 1996, Robbins et al., 1997). Consistent with studies of dopaminergic involvement in working memory, there is some indication that the effect of scopolamine on working memory may be related to the type of behavioural task used, with tasks requiring greater processing requirements of the working memory central executive being potentially more susceptible to scopolamine than more passive working memory tasks (Kopelman and Corn, 1988, Rusted, 1988, Rusted and Warburton, 1988, Rusted et al., 1991).

In a series of important studies into working memory pharmacology, Furey et al. (1997, 2000a, 2000b) used PET and fMRI imaging to investigate the effect of cholinergic enhancement on working memory. It was observed that increased acetylcholine levels (via administration of physostigmine, an anti-cholinesterase inhibitor) were associated with a decrease in task-related rCBF within the PFC during working memory tasks, and this decrease in rCBF was correlated with an improvement in working memory performance (Furey et al., 1997, Furey et al.,...
2000a). Based on this evidence, Furey and colleagues suggested that cholinergic enhancement of working memory performance appears to be the result of increases in neuronal activity in regions associated with early perceptual processing, and decreases in activity in regions associated with memory maintenance. This hypothesis was supported in a more recent fMRI study, in which enhancement of visual processing in the ventral occipital cortex during encoding, and decreased activity in the anterior PFC during maintenance of information, was observed (Furey et al., 2000b). The authors concluded that enhancement of cholinergic activity improves working memory by focusing perceptual processing in extra-striate visual cortices, particularly during encoding. They suggested that by producing a more robust visual percept during encoding, working memory maintenance is simplified and less effort is required by the PFC to maintain the information. This research has demonstrated the usefulness of neuroimaging in not only establishing the possible regional/spatial effects of pharmacological manipulation within the brain during working memory, but identifying that the effect may be specific to a temporal stage of the task.

There is also evidence that nicotine administration can improve working memory performance (Rezvani and Levin, 2001). However, in addition to its effect as a non-selective cholinergic agonist, nicotine also causes the release of dopamine in the basal ganglia and nucleus acumbens (Pidoplichko et al., 1997), and evidence in rats indicated that working memory deficits induced by nicotinic antagonists might be reversed by administration of the dopamine agonist quinpirole (Levin and Rose, 1995). Thus, the results of nicotine administration are difficult to attribute purely to cholinergic mechanisms. Recently, our laboratory has investigated the effect of blockade of the nicotinic receptor using the nicotinic receptor antagonist mecamylamine. Evidence indicated that mecamylamine (15mg) had no effect on working memory performance (Ellis et al., 2005a, Green et al., 2005). However, concurrent administration of mecamylamine and scopolamine was observed to result in greater impairments in working memory performance than administration of scopolamine alone, on both delayed-response and n-back tasks (Ellis et al., 2005a, Green et al., 2005). These findings suggest a synergistic effect of muscarinic and nicotinic receptor antagonism, comparable with animal studies that have shown evidence for synergistic effects of these receptors on tests of attention and working memory (Levin et al., 1990, Mirza and Stolerman, 2000, Leblond et al., 2002).
Taken together, these findings suggest that cholinergic processes, particularly the cholinergic muscarinic system, may modulate working memory performance. Overall, the evidence presented indicates decreases in cholinergic function are associated with impaired performance, while increases in function appear to improve performance. Further, there is evidence to suggest that acetylcholine may modulate working memory primarily during the encoding period of a task, through influences on the creation of a working memory percept.

2.4.2 Serotonin as an Inhibitory Modulator

The role of serotonin (5-HT) in working memory is unclear. Luciana et al. (2001) observed tryptophan (a precursor of serotonin) loading to impair affective working memory and digit span backwards tasks. In contrast however, a number of studies have investigated the effects of tryptophan depletion (TD) on cognition, and generally report that while TD impairs cognitive functions including memory consolidation and delayed recall (over many minutes), working memory remains relatively spared (Riedel et al., 1999, Schmitt et al., 2000, Riedel et al., 2003, Harrison et al., 2004, Riedel, 2004).

Luciana et al. (1998) have demonstrated that a 60mg dose of fenfluramine, a 5-HT re-uptake inhibitor and releasing agent which effectively increases serotonin levels, impaired working memory on the same visuo-spatial delayed-response task discussed in Section 2.2, above. Luciana et al. (1998) suggested that serotonin may have constrained SWM through an inhibitory effect on dopamine, based on evidence suggesting serotonin and dopamine have opposing roles with respect to emotional and motor behaviours (although this interaction effect was not explicitly tested). There is also evidence that serotonergic-dopaminergic interactions may be important in changes in cognitive functions such as vigilance following SSRI administration (Schmitt et al., 2002). In addition, evidence suggests that serotonin may modulate the cholinergic system and therefore have an indirect effect on cognition (for a review, see Steckler and Sahgal, 1995). Indeed, studies in rodents have indicated an interaction between the serotonergic and cholinergic systems in working memory functions (Richter-Levin and Segal, 1989, Miura et al., 1993, Ohno and Watanabe, 1997). At present, there is insufficient empirical evidence to permit clear conclusions about the role of serotonin in working memory functions. However, the evidence
suggests that future research investigate whether serotonin may interact with the dopaminergic and cholinergic systems in modulating working memory in humans.

2.4.3 GABA and NMDA

It has previously been suggested that inhibitory processes may be important in the regulation of working memory in non-human primates (see Chapter 1, Section 1.4 for discussion), and since the majority of interneurons use the inhibitory neurotransmitter GABA, a role for GABA in working memory functions is indicated (Goldman-Rakic, 1995b) There is evidence to suggest that the GABA_A receptor may play an important role in working memory, however the nature of this effect is unclear. Direct application of bicuculline methiodide (BMI) (a GABA_A antagonist) onto spatially tuned neurons within the DLPFC resulted in a loss of spatial tuning, mediated by disinhibition, in non-human primates performing an oculomotor delayed-response task (Rao et al., 2000). However, administration of benzodiazepines (GABA_A agonists) in humans has also been observed to impair cognitive processes, including working memory performance, in both healthy volunteers as well as in patient groups (for reviews, see Curran, 1991, Buffett-Jerrott and Stewart, 2002, Chouinard, 2004, Verster and Volkerts, 2004). Evidence suggests that while benzodiazepines also increase sedation and impair attentional processes, these impairments do not fully account for the widespread memory deficits caused by benzodiazepine administration (Buffett-Jerrott and Stewart, 2002). Therefore, direct application of GABA_A receptor antagonists within the DLPFC appears to impair working memory due to neuronal disinhibition and resultant loss of spatial tuning in both pyramidal cells and interneurons of the DLPFC. In contrast, the benzodiazepine studies involve systemic administration of GABA_A receptor agonists and therefore may act on GABA_A receptors throughout the brain (not only the DLPFC), causing non-selective and global inhibitory effects that appear to also impair function. More research is required to ascertain the nature of the relationship between GABA and working memory, but it appears probable that modulation of GABA_A receptors may influence working memory performance, potentially through altering neuronal inhibition levels.

A role for NMDA receptors/glutamate in working memory is suggested based on interactions between NMDA receptors and the central dopaminergic system (for a review, see Lee and Liu, 2004), and evidence that chronic NMDA receptor
antagonism may reduce extracellular dopamine levels, modulate dopamine neurotransmission in the PFC and alter working memory (Jentsch and Roth, 1999). However, there have been limited examinations of acute pharmacological challenges on working memory. A recent study examined the effects of administration of 0.27 mg/kg ketamine, a non-competitive NMDA receptor antagonist, in a sample of 10 healthy male participants and reported subtle impairments in SWM on a visual Morris water maze task (although no effects on a verbal working memory task were observed). These findings are consistent with evidence in the rat that selective and competitive NMDA receptor antagonists, which block NMDA receptor activity, increase the number of errors in working memory (Pontecorvo et al., 1991, Ohno et al., 1992, Ohno et al., 1993, Gutnikov and Rawlins, 1996, Puma et al., 1998, Puma and Bizot, 1998). Recent studies have examined the effect of d-cycloserine, a partial agonist of the glycine site of NMDA receptors (administered in conjunction with antipsychotic treatment) on cognition performance and negative symptoms of patients with schizophrenia. However, two recent studies found no evidence of a positive effect of d-cycloserine on working memory performance (Goff et al., 1999, Evins et al., 2002). Nevertheless, based on the limited research conducted to date, and with specific consideration of the interactions between NMDA receptors and dopamine, it remains probable that with additional research a role for the NMDA receptor in working memory may become clear.

2.4.4 Summary of non-catecholamine neurotransmitter systems

Evidence suggests that acute challenge of the cholinergic system may modulate working memory. The evidence presented indicates that decreases in cholinergic function are associated with impaired performance, while increases in function appear to improve performance. Further, there is evidence to suggest that acetylcholine may modulate working memory primarily during the encoding period of a task, through influences on the creation of a robust working memory percept. There is some evidence to suggest that serotonin may play a role in working memory, with evidence to date indicating that any effect is most likely inhibitory, perhaps through modulation of other neurotransmitter systems such as the dopaminergic and cholinergic systems. Currently there is insufficient evidence to conclude as to whether GABA and glutamate modulate working memory. However, with consideration of the importance of the inhibition processes in working memory, and the interactions between
Chapter 2: Summary of Chapters 1 and 2

The first two chapters of this thesis presented reviews which introduced the concept of working memory, outlined the dopaminergic system in humans, and reviewed the literature investigating the pharmacology of working memory. The first chapter suggested that while working memory is hard to define, it can be generally characterised as a process of maintaining information “online” during a delay, for further manipulation or to guide behaviour. Also highlighted in the first chapter was the fact that working memory can be measured by a range of tasks, which can generally be classed as either delayed-response paradigms or working memory tasks with additional executive demands (such as the n-back). Evidence from lesion and neuroimaging studies highlighted a critical role for the PFC in working memory, which has now long been accepted in modern neuroscience. In addition, neuroimaging studies have established that SWM activates a distributed network of regions, with the commonly used n-back task demonstrating a robust network generally comprising six key regions (the parietal cortex, premotor cortex, dorsal cingulate/medial premotor cortex, rostral PFC, DLPFC, and mid-VLPFC).

In the current chapter, acute dopaminergic challenge studies in non-human primates suggest a critical role for dopamine within the PFC during working memory. It appears evident that the effect of dopaminergic manipulation on SWM performance is related to the so called inverted U response curve, in which “optimal levels” of dopamine are required for optimal performance. A preferential role for the D$_1$ receptors within the PFC has been demonstrated with evidence that local administration of D$_1$ receptor (and not D$_2$ receptor) antagonists modulate working memory performance (Sawaguchi and Goldman-Rakic, 1991, Sawaguchi and Goldman-Rakic, 1994, Williams and Goldman-Rakic, 1995). However, a role for D$_2$ receptors in working memory is also suggested based on evidence that systemic administration of D$_2$ receptor agonists can modulate performance, potentially through effects within the striatum or through activation of other dopamine receptor cites (i.e. D$_3$/D$_4$ receptors) (Arnsen et al., 1995). In humans, there is evidence that dopamine...
agonists may improve working memory performance, and dopamine antagonists or
global dopamine depletion via TPD may impair SWM performance. However, the
behavioural findings to date are inconsistent, which may be related to factors such as
differences in task demands (including response preparation) and differences between
individuals. This review suggests that the precise role of D1/D2 receptors in SWM in
humans remains unclear, and highlights a relative paucity of studies examining the
effects of dopamine modulation of SWM networks in humans.

2.6 AIMS AND OVERVIEW OF THIS THESIS

The general aim of this thesis was to extend upon the understanding of the effects of
dopamine in modulating SWM in healthy humans by conducting a series of
behavioural and neuroimaging studies. The primary dopaminergic manipulation used
in this thesis was acute tyrosine depletion (TPD). Acute tyrosine depletion is a
relatively new technique for decreasing dopamine levels and examining the role of
dopamine in working memory in humans. TPD appears to be a useful method of
modulating the dopaminergic system as it reveals an apparent specificity for
dopamine depletion (over noradrenaline), and allows the examination of depleting
global dopamine levels (in contrast to examining the effects on the D2 receptor alone,
such as with D2 antagonists). Over the last five years, behavioural studies have
examined the effect of TPD on working memory performance; however the results
have been inconsistent. Furthermore, there are currently no studies examining the
effects of TPD on working memory related brain neurophysiology. This thesis also
examined whether the proposed effects of TPD on SWM performance and associated
task-related brain activity could be reversed by stimulation of the D1/D2 receptors
using the dopamine D1/D2 receptor agonist pergolide.

Therefore, the aims of this thesis were:

1) To examine the effects of TPD on behavioural performance on a range of
   SWM tasks with different task demands.

2) To conduct the first functional imaging studies examining the effects of TPD
   on: a) neural networks (as assessed by changes in rCBF using PET), and b)
   cortical electrophysiology (as assessed by changes in SSVEPs using SSPT).
3) To examine whether stimulation of D$_1$/D$_2$ receptors under conditions of TPD would reverse or attenuate TPD related effects on SWM performance and associated brain activity.

The current thesis contains four experimental chapters. The first experiment of this thesis had two aims: 1) to examine whether TPD-related impairment on the “Sternberg” SWM delayed-recognition task (as observed by Harrison et al. 2004) could be replicated in a larger sample, and 2) to extend upon previous research and examine whether stimulating D$_1$/D$_2$ receptors under dopamine depleted conditions would modulate SWM by “reversing” the proposed negative effects of TPD on SWM performance. This experiment has been published in the peer reviewed journal Psychopharmacology (see Appendix 5 for reprint).

The second experiment examined, for the first time, the effects of TPD on neural networks associated with SWM by examining changes to regional cerebral blood flow (rCBF) during a SWM n-back task using H$_2$O PET. The SWM n-back task was used in all neuroimaging studies within this thesis as it activates a well established and robust network including the PFC and posterior parietal cortex (as reviewed in Chapter 1) and patients with schizophrenia show performance impairments which have been correlated with rCBF within the PFC (for a review, see Manoach, 2003) and PFC D$_1$ receptor availability (Abi-Dargham et al., 2002). In addition, as the effects of TPD on SWM behavioural performance have yielded inconsistent results to date which may be related to response demands of tasks, this experiment also examined whether differences in response preparation and execution demands of delayed-response tasks resulted in differential effects of TPD on performance. Two versions of the classic delayed-response paradigm were specifically designed to be matched on all parameters excluding response requirements. Evidence of TPD-related effects on performance of one task and not the other may indicate the nature of TPD effects on different aspects of the SWM process. Data from this experiment has been published as an abstract in Neuroimage and International Journal of Neuropsychopharmacology, following presentation at the Human Brain Mapping meeting in Hungary (June 2004) and the Collegium Internationale Neuropsychopharmacologicum (CINP) meeting in France (June 2004), respectively (see Appendix 6 for poster).
In contrast to Experiment 2, Experiments 3 and 4 (presented in Chapters 6 and 7) examined temporal aspects of electrophysiological activity associated with the SWM n-back task, using SSPT. In Experiment 3, the temporal aspects of the SWM n-back task were examined under normal (no drug) conditions, for the first time. This experiment has been presented at the Australasian Society for Psychiatric Research (ASPR) meeting in Canberra (December 2002) (see Appendix 7), and submitted for publication within Neuroimage. Experiment 4 extended upon these findings, and due to the fact that the number of SSPT sessions each volunteer can participate in is not limited by radioactive levels (as in PET), examined the effects of both TPD, and D1/D2 stimulation (under TPD conditions) on the SSVEP associated with the SWM n-back task. It was predicted that dopaminergic modulation of working memory may be related to changes in cortical electrophysiology during the delay within the PFC (and/or the associated working memory network), consistent with previous electrophysiological studies in primates (i.e. Williams and Goldman-Rakic, 1993, Williams and Goldman-Rakic, 1995, Wang et al., 2004), and evidence in humans that bromocriptine modulates task-related activity within the parietal cortex during the n-back task (Kimberg et al., 2001).

Within each experimental chapter, the findings and possible implications of the individual experiment are discussed. This thesis concludes with a general discussion of the broader context of all findings, with the results of this thesis discussed in terms of the aims presented above, and the proposed theory of an “optimal level” of dopamine (the so called inverted U response curve) (Chapter 8).
Chapter Three

3 General Methods

This chapter outlines general methodology used in this thesis, and has three sections. Section one overviews methodological issues common to all experimental chapters, including participant selection, neuropsychological testing and statistical analysis. Section two introduces the pharmacological manipulations used in this thesis and the rationale for their selection. Finally, section three outlines the two neuroimaging modalities used in this study, providing a basic description of both modalities and discussing the rationale for their selection and use. Full methodological details specific to each experiment are provided within each experimental chapter.

3.1 PARTICIPANTS

3.1.1 Inclusion Criteria

All participants in all experiments were adult males. The maximum age of participants was limited to 65 years. Due to radiation committee requirements, the minimum age of participants within the PET experiment (Chapter 5) was 30 years, with all remaining participants having a minimum age of 18 years. A male sample was employed for homogeneity of the sample, and due to evidence that mood effects of amino acid depletion to be more pronounced in females, and population prevalence indicates that females may be more susceptible to lowered mood states than males (Ellenbogen et al., 1996, Nishizawa et al., 1997). All participants were right handed, assessed by the Edinburgh inventory (Oldfield, 1971). Only one participant in one study was a smoker (within PET experiment, Chapter 5), however this participant abstained from the normal one cigarette a day for a minimum of 48 hours before testing sessions. All participants provided written informed consent before participating.
3.1.2 Exclusion criteria

All participants were healthy at the time of testing and free of chronic medical, psychiatric or neurological medical conditions. Exclusion criteria comprised a history of neurological or psychiatric disorders (including history of depression or anxiety disorders in first degree relatives), chronic physical illness, medication and/or drug use, or excessive alcohol consumption. Suitability for the study was ascertained through 3 pre-screening steps. First, a pre-study telephone screening was conducted. Second, participants were assessed with a clinical evaluation scale [either the Prime-MD or the Structured Clinical Interview for the DSM-IV (SCID) were used, as detailed within each chapter; both are semi-structured clinical exam based on the DSM-IV]. Third, a subsequent semi-structure clinical examination was performed by a physician.

3.1.3 Design

All studies in this thesis using pharmacological manipulation employed a double blind, placebo controlled, repeated measures design. Each session was separated by a minimum five-day washout period, with order of condition randomised using a counterbalanced or quasi-latin squared design as appropriate (details are provided within each experimental chapter). The repeated measures placebo-controlled design is particularly useful for pharmacological studies as it reduces the variance caused by differences in performance between participants, with each participant effectively acting as their own control. The repeated measures placebo controlled design allows for increased statistical power, particularly when the number of participants is relatively small. In the alternative between-subjects design, in which participants are allocated to either drug or placebo condition, variability due to non-specific differences, such as education levels, motivation, intelligence levels and alternative strategies may mask drug related changes, as it reduces the sensitivity of detecting a statistical differences. Administration of cognitive tasks was randomised, as detailed in specific experimental chapters, in an attempt to avoid order related effects (such as fatigue) confounding results. Participants attended pre-study practice sessions to minimise practice effects, as described within each chapter.
3.2 NEUROPSYCHOLOGICAL/COGNITIVE ASSESSMENT

All neuropsychological/cognitive tasks were presented via computer displayed on a high-resolution VGA colour monitor, and all responses were made using an external button box (yes/no), via touch screen, or using a critical flicker fusion (CFF) tube. Computerised cognitive assessment offers a number of advantages over traditional ‘pencil-and-paper’ tests, including precision of measurement (particularly for response latencies/reaction time) and consistency of presentation and feedback. Further, computerised assessment enables concurrent behavioural assessment and neuroimaging with millisecond accuracy.

The button box was hand held with thumbs resting upon the respective button. Participants were instructed to respond “as quickly as possible but with accuracy as their priority” on all tasks in all experiments (response instructions discussed below). For all cognitive tests not performed within the PET camera, participants were seated approximately 1 metre from the computer monitor in a dimly lit room and were requested to sit upright throughout the task. Within the PET camera, participants were supine with the computer monitor suspended above them and responded using the touch screen with their right hand.

The experiments in the following chapters use a variety of psychological tests. A number of tasks were taken from the Cognitive Drug Research (CDR Ltd, Goring-on-Thames, UK; www.cdr.org.uk) computerised assessment suite, regarded for its validity as a measure of memory and attention and its proven sensitivity in studies of acute tyrosine depletion (Harrison et al., 2004). This battery is specifically designed for pharmacological manipulation studies, and all tasks within this battery have been susceptible to improvements and impairments following pharmacological agents. The CDR has the advantages of sufficient parallel forms for use in repeated measures studies. This thesis also developed versions of the delayed-response task paradigm and the n-back task with sufficient parallel forms for repeated measures testing (detailed within each experimental chapter). All tasks were extensively tested for reliability in a series of pilot studies. In a separate series of experiments not included in this thesis, performance on the n-back task designed and used in Chapter 6, and all tasks taken from the CDR battery used in this thesis have been demonstrated to be
sensitive to pharmacological manipulation in studies performed within the same laboratory as the corresponding experiments in this thesis (Ellis et al., 2005a, Green et al., 2005).

### 3.2.1 Reaction time vs. accuracy

The behavioural measures of performance within this thesis are accuracy and reaction time (latency/speed). For well over 100 years it has been understood that accuracy and reaction time are linked measures (Woodworth, 1899), with an improvement in either measure generally related to a decrement in the other.

It is generally held that task instruction bias responses. Instructions which emphasise speed (e.g. respond as quickly as possible) or accuracy (e.g. respond as accurately as possible regardless of speed) shift the relationship between accuracy and reaction time on the speed-accuracy trade-off curve (see Figure 3.1). Within the experiments detailed in the current thesis, the aim was to limit the variability in speed accuracy trade-off between participants by using a consistent task instruction, and to consider the statistical ramifications of speed-accuracy trade-off as a possible confound when considering changes in both measures in different drug conditions.

![Figure 3-1 Speed-accuracy trade-off curve](image-url)
The task instruction “respond as quickly as possible but with accuracy as your priority” was used in this thesis, and this instruction was chosen for a number of reasons. First, if speed was emphasised with a disregard to accuracy, it is rarely the case that the accuracy of all conditions suffer equally; participants under speed stress tending to make far more errors in the difficult conditions than in the easy conditions of an experiment (for discussion, see Ruthruff, 1996). This is of particular importance in tasks with differing levels of difficulty such as the n-back task (which has a parametric increase in memory load and task difficulty). Second, while the commonly used instruction of “respond as quickly and accurately as possible” could have been employed as it seemingly emphasises both speed and accuracy, this instruction can be ambiguous and may introduce variability between participants. In contrast, while the task instruction employed in this thesis gives a small emphasis to accuracy, it is suggested that this will reduce variability in the speed accuracy tradeoff both between and within participants (Ruthruff, 1996).

There have been a number of statistical techniques suggested for accounting for speed-accuracy trade-off, all with advantages and disadvantages (for a review, see Salthouse and Hedden, 2002). These techniques generally either compare changes in accuracy to changes in reaction time to examine if they are related, or attempt to avoid this by consider both measures at the same time by generating an accuracy/latency composite score. While this latter technique is useful, it also makes the assumption that both aspects are of equal importance, and reduces the sensitivity of observing drug related changes to either one or the other measure independently (Salthouse and Hedden, 2002). Therefore, analysis within the current thesis is conducted on both accuracy and reaction time measures separately. In the situation of a significant effect on both measures, additional analysis is conducted which: a) considers accuracy as a covariate of reaction time, and visa versa, to examine whether the effects are dependent on the change in the other measure and, b) correlates accuracy and reaction time changes for each participant to examine whether changes in one are directly related to inverse changes in the other measure. While the complex accuracy-reaction time trade-off cannot be removed, such additional analyses are important in determining whether changes in performance following drug administration are purely reaction-time accuracy trade-off effects or can be attributed (at least in part) to the drug condition.
3.3 STATISTICAL ANALYSIS

All statistical analyses are detailed within the methods sections of each experimental chapter. For all experiments, analysis of cognitive data was conducted using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL). Behavioural data was analysed with parametric statistics (repeated measures analysis of variance) if all assumptions were met. If the assumption of normal distribution were violated, data were first transformed. When dealing with proportional data (e.g. percentages) the arcsine transformation was used \(Y=2 \times \text{arcsin} \sqrt{p}\), where \(p\) is the proportion correct. More marked positive skewness (as often occurs with latency data) was subjected to a logarithmic transformation \(Y=\log_{10}(X)\). Non-parametric analysis was used when, even after transformation, data did not meet the assumptions of the parametric test being used. PET data was analysed using Statistical Parametric Mapping 2 (Friston et al., 1995). SSVEP data was analysed using BrainSci, an in-house software developed at the Brain Sciences Institute, and additional software was developed in house using Matlab software for additional analyses (as discussed in experimental chapters).

3.3.1 Significance levels

All statistical tests were two-tailed with a values of \(p<0.05\) used to denote a significant difference between means, and all neuroimaging data was corrected for multiple comparisons, unless stated otherwise, according to standard procedures detailed within each experimental chapter. Where multiple comparisons were being made on cognitive/behavioural data a restricted significance threshold of \(p=0.01\) was used to avoid making a type I error (i.e. rejecting the null hypothesis when it is in fact true). The use of a Bonferroni correction was generally avoided to reduce the chance of making a type II error (i.e. failing to reject the null hypothesis when it is false).

3.4 PHARMACOLOGICAL MANIPULATION

Two methods of manipulating the dopaminergic system were used in this thesis: 1) global depletion of dopamine via acute amino acid (precursor) depletion, and 2) direct dopamine \(D_1/D_2\) receptor agonism with pergolide.
3.4.1 Acute Tyrosine Depletion (TPD)

The primary dopamine manipulation used in this thesis was acute tyrosine depletion (TPD). As outlined in Chapter 1, the synthesis of dopamine (and noradrenaline) is dependent on the availability of its amino acid precursor tyrosine (and the tyrosine precursor, phenylalanine). Therefore, restricting these amino acids has provided a novel technique for experimentally depleting dopamine levels and probing the effects on working memory performance. Acute amino acid depletion as a method of decreasing neurotransmitter levels was first established by the serotonin depleting effect of decreasing the serotonin precursor L-tryptophan (for a review, see Reilly et al., 1997). The mechanism by which acute amino acid depletion is believed to decrease neurotransmitter synthesis is two fold. First, by stimulating protein synthesis, amino acid depletion is purported to result in lowered plasma precursor amino acid levels. Second, by increasing competition between the amino acid precursor (i.e. tyrosine, and its precursor phenylalanine) and other large neutral amino acids (L-tryptophan, L-valine, L-isoleucine, L-leucine) for transport across the blood-brain barrier, tyrosine levels are further decreased (Oldendorf and Szabo, 1976, Pardridge, 1977).

Biggio et al. (1976) first observed that administration of an amino acid mixture lacking tyrosine (and phenylalanine) resulted in a reduction of tyrosine concentrations in serum and whole brain of the rat, with more recent studies confirming these findings (McTavish et al., 1999a, McTavish et al., 1999b, McTavish et al., 1999c, Jaskiw and Bongiovanni, 2004). McTavish et al. (McTavish et al., 1999a, 1999b, McTavish et al., 1999c) further demonstrated that TPD appears preferential for dopamine with little or no effect on noradrenaline. This is in specific contrast to the catecholamine synthesis inhibitor, α-methyl-paratyrosine (AMPT), which is a more aggressive dopamine depletion method but also causes a marked reduction in baseline extracellular noradrenaline (McTavish et al., 1999a).

In humans, Moja et al. (1996) and Sheehan et al. (1996) first demonstrated that a tyrosine-free amino acid mixture may also lowers plasma tyrosine levels in humans. However, in a seminal study of the area, Harmer et al. (2001) demonstrated evidence of an effect of TPD on dopamine transmission. Specifically, Harmer et al. (2001) demonstrated that TPD causes changes in prolactin, and as dopamine exerts inhibitory
action on prolactin release in the hypothalamus, an increase in prolactin levels are indicative of decreased dopamine function (Checkley, 1980). Further, these authors suggested that TPD-related impairments in working memory task performance were indicative of decreased dopamine function (Harmer et al. 2001). A number of studies have consistently supported the findings that TPD reliably depletes plasma tyrosine levels and/or the ratio of plasma tyrosine and phenylalanine to other large neutral amino acids (Leyton et al., 2000, Harrison et al., 2004, Leyton et al., 2004a, Leyton et al., 2004b, McLean et al., 2004, McTavish et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Mehta et al., 2005a). More recently, Montgomery et al. (2003) demonstrated evidence that TPD decreases dopamine levels within the striatum. In this study, TPD caused an average increase in [11C]raclopride binding (indicative of a decrease in dopamine) of 6% within the striatum. Further, Leyton and colleagues (2004b) have demonstrated that AMPT reduces stimulated dopamine release following amphetamine, also assessed by changes in [11C]raclopride binding.

TPD has a number of advantages as a method of decreasing dopamine function. TPD is selective for dopamine, in contrast to both AMPT which depleted both catecholamines (Verhoeff et al., 2003), and antipsychotics (such as the D2 antagonist haloperidol) which also have other pharmacological properties including cholinergic and serotonergic receptor antagonism (which may independently influence working memory functioning as discussed in Chapter 2). Further, in contrast to dopamine antagonists (such as D2 antagonists sulpiride and haloperidol), TPD is also more likely to effect both D1 and D2 receptors, which is important considering evidence that the D1 receptor plays a critical role in working memory within the PFC of the non-human primate (Goldman-Rakic et al., 1996).

3.4.2 Dopamine receptor agonist

While it has been established that the D1/D2 receptors play a role in working memory in the non-human primate, findings in humans are less clear. This thesis aimed to further examine the role of these receptors in working memory by examining the effect of D1/D2 receptor stimulation under conditions of tyrosine depletion. Pergolide was selected as the dopamine agonist due to its effect at both the D1/D2 receptor sites, and previous evidence of modulatory effects on working memory in baseline (i.e. non-dopamine depletion) conditions. The affinity for pergolide at D1 and D2 receptors
within the human brain (putamen) has been reported as: $D_1$ receptor $K_i$ value = 447nM and $D_2$ receptor $K_i$ value = 10.3nM (Gerlach et al., 2003). Pergolide crosses the blood brain barrier, and hence was administered orally (Mims, 2004). When administered orally, pergolide is a rapidly absorbed (radiolabelled drug appeared in plasma 15–30 min after administration) and reaches peak plasma concentrations ($T_{\text{max}}$) between 1-3 hours (Langtry and Clissold, 1990, Markham and Benfield, 1997, Deleu et al., 2002, Blin, 2003). Pergolide is eliminated with a mean terminal half-life ($t_{1/2}$) of approximately 27 hours, and is barely detectable in 4-5 days (Deleu et al., 2002, Blin, 2003). The dose selected was based on previous studies in which 0.1mg of pergolide has been shown to improve SWM performance in healthy humans (Muller et al., 1998, Kimberg and D’Esposito, 2003).

### 3.5 NEUROIMAGING TECHNIQUES

There are a number of imaging technologies available for neuroscience researchers, each having its own specific strengths and weaknesses. Selection of a specific imaging modality involves finding the most suitable modality for each specific research question. Neuroimaging techniques can be characterised by the ability in which they distinguish details on both spatial and temporal scales, know as spatial and temporal resolution. Spatial resolution refers to the ability to distinguish two separate objects that are positioned in close proximity to each other. In contrast, temporal resolution refers to the ability to detect events that occur within close temporal proximity to each other (Mazziotta, 1996). Table 3.1 summarises the temporal and spatial resolution of the most commonly used neuroimaging techniques.

The current thesis aimed to examine the effect of TPD on SWM-related brain activity in terms of two aspects: 1) temporal changes in brain activity, and 2) spatial/regional changes in brain activity. Two imaging modalities were employed: Steady State Probe Topography (SSPT) and Positron Emission Tomography (PET). A brief overview of each will be presented below along with advantages of each modality for assessing the research question.
Table 3-1  Summary of common neuroimaging techniques with associated temporal and spatial resolution

<table>
<thead>
<tr>
<th>Imaging technique</th>
<th>Overview</th>
<th>Resolution</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SSPT, EPR</td>
<td>Scalp recordings of electrical activity</td>
<td>milliseconds</td>
<td>cm’s</td>
<td></td>
</tr>
<tr>
<td>MEG</td>
<td>Records magnetic fields generated by weak electrical fields</td>
<td>millisecond</td>
<td>2mm</td>
<td></td>
</tr>
<tr>
<td>fMRI</td>
<td>Records local changes in magnetic field resulting from changes in ratio of oxyhaemoglobin in deoxyhaemoglobin</td>
<td>seconds</td>
<td>&lt;1mm</td>
<td>FWHM</td>
</tr>
<tr>
<td>PET</td>
<td>Detects gamma rays as a result of emitted proton following decay of radio-labelled water</td>
<td>30-90 seconds</td>
<td>3.5-4mm</td>
<td>FWHM</td>
</tr>
<tr>
<td>SPECT</td>
<td>Detects gamma emission due to radionuclide decay</td>
<td>3-4 minutes</td>
<td>6-7mm</td>
<td>FWHM</td>
</tr>
<tr>
<td>FDG-PET</td>
<td>Detects gamma rays resulting from collision of emitted proton with an electron following decay of radio-labelled glucose</td>
<td>&gt;30 minutes</td>
<td>3.5-4mm</td>
<td>FWHM</td>
</tr>
</tbody>
</table>

FWHM (Full width half maximum) refers to the distance in which two separate foci may be distinguished. Table adapted from (Gordon, 2002, Honey and Bullmore, 2002)

3.5.1 Steady State Probe Topography (SSPT)

SSPT is based on the probe Event-related Potential (ERP) paradigm, a technique which involves recording electrophysiological responses to task irrelevant or “probe” visual stimuli, and evaluating the change that occurs to these responses when a concurrent cognitive task is being performed (Papanicolaou and Johnstone, 1984). Event-related Potentials can be either transient or steady state. Transient ERPs are generated from discontinuous stimuli. In contrast, for a Steady State Visually Evoked Potential (SSVEP), the evoking stimulus must be both continuos and rapid enough to prevent the ERP from returning to baseline.

Thus SSPT involves eliciting SSVEPs from a continuos task irrelevant flicker during the performance of cognitive tasks, which are measured from 64 electrode sites over the scalp. This technique provides a dynamic measure of brain activity as it varies
over time. SSPT has been used extensively within the Brain Sciences Institute over
the last 15 years and has investigated the SSVEP associated with a number of
cognitive tasks, including delayed-response SWM (Silberstein et al., 2001), visual
vigilance and attention (Silberstein et al., 1998, Silberstein et al., 2000b), tasks of
executive function such as the Wisconsin card sorting test (Silberstein et al., 1995),
long-term recognition memory (Silberstein et al., 2000a), mental rotation (Silberstein
et al., 2003) and emotional processing (Kemp et al., 2002). SSPT has also been used
to examine cognitive processes in clinical groups (compared to controls) including
children with ADHD (Silberstein et al., 1998) and patients with schizophrenia
(Silberstein et al., 2000b), and has been used to examine the effects of
pharmacological manipulation of emotional processing (Kemp et al., 2004).

**Stimulus**

SSPT is elicited by superimposing a 13Hz sinusoidal white flicker over the visual
field using a pair of specially designed goggles. The frequency of 13Hz falls within
the high alpha or low beta bandwidth, and use of this frequency has a number of
advantages. First, the alpha bandwidth has traditionally been viewed as a measure of
activity with the cortex (Ray and Cole, 1985). The 13Hz bandwidth is however
distinct from the alpha peak, so as to optimise the signal to noise ratio (where “signal”
refers to the driven 13Hz activity by cognitive tasks and “noise” refers to the
prominent alpha peak observed at approximately 10Hz in healthy humans during EEG
recordings). Indeed a distinct advantage of SSVEP imaging over EEG is enhanced
signal to noise ratio (discussed further below). The selection of a frequency of the
order of 13Hz has an advantage over higher frequencies (e.g. 20Hz or 40Hz), as it
produces rhythmic activity over widespread areas of the brain, rather than being
restricted to the visual cortex (Speckreijse et al., 1977). In summary, a 13Hz
frequency is employed as the “driving” stimulus in SSPT as it produces SSVEPs in
widespread areas of the cortex within the high alpha bandwidth, but is far enough
away from the alpha peak as to optimise the signal to noise ratio (Silberstein et al.,
1995), and has consistently shown robust changes to both amplitude and latency
measures during cognitive tasks.
Recording

The SSVEP is recorded from 64 monopolar electrode leads attached to a lycra electrode cap, with electrodes positioned according to the international 10/20 system as well as additional sites midway between (see Figure 3.2 for electrode placement). The averaged potential of both earlobe electrodes is used as reference, after each earlobe electrode signal is separately buffered with unity gain, low noise amplifiers to remove the problems of unbalanced electrode impedance in linked earlobe electrode references (Silberstein et al., 1995). The nose electrode is used for ground. In this thesis, electrode gel was inserted into all electrodes to enhance the signal and the impedance of each electrode, which was examined both via an impedance metre (with impedance level on all electrodes generally below 5kΩ), and an examination of the power spectra for each electrode to assess for interference and/or dud electrodes before testing commenced.

Specially designed goggles are attached, and provide a visual white 13Hz flicker subtended a horizontal angle of 160° and a vertical angle of 90°, with a modulation depth of 45% when viewed against the background. Recorded brain electrical activity was band pass filtered from 0.74 to 74Hz and digitised at a rate of 500Hz with 16-bit accuracy, consistent with previous studies (e.g. Silberstein et al., 1998, Silberstein et al., 2001, Kemp et al., 2002, Gray et al., 2003, Kemp et al., 2004, Silberstein et al., 2004).

Signal Processing

SSVEP signal processing has been discussed previously (Silberstein et al., 1990, Silberstein et al., 1995). Fourier analysis is employed to extract the SSVEP from the brain electrical data for each electrode by calculating the 13Hz Fourier coefficients (FC) for each stimulus cycle. This yields information on the SSVEP amplitude and SSVEP phase. Changes in phase are generally expressed as changes in latency (in milliseconds) following initial extraction of the data, using the formula: (change in phase/2 x Π) x (1000/13). For clarity, all changes in phase will be discussed in terms of latency values throughout this thesis.

The FC time series is smoothed by averaging overlapping blocks of 10 FCs, also known as a “10 unit window”. A 10 cycle smoothing window provides excellent
temporal resolution of 0.77 seconds, while still maintaining high signal to noise ratio through the rejection of brain activity which is not centred on the stimulus frequency (i.e. 13Hz). For each task and for each participant, both amplitude and latency data is normalised to account for large inter-subject variations. For each participant, amplitude is normalised by averaging the magnitude for each electrode which creates a normalisation factor. The magnitude of each individual electrode is then divided by this “normalisation factor”. Latency is normalised at each individual electrode with reference to the control task used in each experiment (Silberstein et al., 1990).

![Figure 3-2](image.png)

**Figure 3-2** Position of 64 scalp electrodes used in SSPT recordings in this thesis

**Artefact detection and correction**

One of the advantages of the SSVEP methodology is that it is highly insensitive to artefacts and noise (Regan, 1989, Silberstein et al., 1995). However, the SSVEP amplitude and latency for each electrode within each task was still checked individually for artefact (Silberstein et al, 1995). This is done by first examining the data for gross deviation from normality, and secondly comparing each electrode to its nearest topographical neighbour, as closely spaced electrodes are expected to be highly correlated (Nunez, 1981). “Dud” electrodes are replaced with a weighted average of four adjacent electrodes which passed inspection. Participants were
excluded from analysis if more than eight electrodes of that session were listed as replacements.

**Rationale for use of SSPT**

In summary, SSPT was selected to investigate whether dopamine manipulation during working memory performance is associated with changes in temporal brain activity. SSPT was selected for a number of reasons. First, electrophysiology remains the technique with the highest temporal resolution (in the order of hundreds of milliseconds). Second, the SSVEP is highly insensitive to artefact and noise. As the Fourier analysis is effectively a narrow band pass filter at the stimulus frequency of 13Hz, the data is virtually unaffected by noise and common artefacts such as the electro-oculogram (EOG), the electromyogram (EMG) and eye blinks (Silberstein et al., 1990). This is because the signal power of artefacts such as the EOG and eye blink is located primarily at low frequencies and is negligible above 8Hz and muscle activity is distributed over a range of frequencies. In contrast, the SSVEP power is concentrated almost exclusively at the stimulus frequency of 13Hz and its harmonics (Regan, 1989). Mains electrical interference is also effectively filtered out by Fourier analysis, as all mains power is at 50Hz. Previous research has shown SSPT to reveal robust changes in both latency and amplitude during a number of cognitive tasks, including the delayed-response SWM task, and yield important information about the temporal nature of cognitive tasks.

**3.5.2 H$_2^{15}$O Positron Emission Tomography (PET)**

The second research question addressed by neuroimaging in this thesis was whether dopamine manipulation during working memory performance is associated with changes in spatial aspects of brain activity - that is, which regions of the brain are affected. This question was addressed by Positron Emission Tomography (PET). PET is a commonly used noninvasive radiotracer-based neuroimaging technique which has been explained in detail previous (Frackowiak and Friston, 1994, Grasby, 2002) and therefore only a brief description is provided below.

Changes in neural brain activity are almost invariably associated with changes in local cerebral blood flow (for discussion, see Raichle, 1998, Arthurs and Boniface, 2002). PET can be used to examine changes in regional cerebral blood flow (rCBF) by the
introduction of water labeled with the radioactive isotope oxygen-15 (15-O) into the blood stream of participants via peripheral injection. The radioactive nuclei emits positrons that annihilate with electrons in the tissue, and this annihilation event results in two gamma photons being emitted in almost 180 degrees and with energy of 511 keV each. The gamma photons are detected in coincidence in a detector ring (or several detector rings) within a PET scanner, allowing reconstruction of the geometry of the source. This is interpreted as an indirect measure of local synaptic activity within the brain. Oxygen-15 labelled water has a short half life of 2.1 minutes (Frackowiak and Friston, 1994). As displayed in Table 3.1, the spatial resolution of PET is in the order of millimeter due to the resolution of the scanner.

**Rationale for use of PET**

The most common technique used to examine the effect of pharmacological agents during cognitive task-related activation within the brain is H$_2^{15}$O PET$^1$. This neuroimaging technique is a reliable and well proven method of examining changes in task-related activation following pharmacological manipulations with good spatial resolution throughout the brain. Non-direct measurements of brain activity [i.e. “blood flow changes”; rCBF or blood oxygen dependent signal (BOLD)] following pharmacological manipulation rely on the assumption that coupling between blood flow and metabolism remains intact, and evidence suggests that PET rCBF measurements following dopaminergic manipulation are not significantly effected by neurovascular (NV) uncoupling. Indeed, the dopaminergic system is the most studied neurotransmitter system on this matter with evidence suggesting no significant neurovascular (NV) uncoupling following DA modulation (see for example, McCulloch, 1982, McCulloch et al., 1982, Arthurs and Boniface, 2002). For the purpose of assessing changes in activation in different regions of the brain following dopaminergic manipulation during working memory performance, H$_2^{15}$O PET imaging was deemed a highly suitable technique for use in this thesis.

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$^1$ Over the past 5 years, functional magnetic resonance imaging (fMRI) has also been used increasingly to examine the modulatory effects of psychopharmacological agents on cognitive activation, although this technique is still in its relative infancy.
Chapter Four

4 Experiment 1: Examining the effects of dopaminergic modulation on SWM behavioural performance.

4.1 INTRODUCTION

In Chapter 2, evidence was presented which suggests that the integrity of the dopaminergic system within the PFC is critical for working memory performance in non-human primates, based on convergent evidence from lesion studies (Funahashi et al., 1993), regional depletion studies (Brozoski et al., 1979, Roberts et al., 1994) and administration of dopamine receptor agonists and antagonists (for reviews, see Goldman-Rakic et al., 1996, Arnsten, 1997). A preferential role for the D₁ receptors within the PFC has been demonstrated with evidence that local administration of D₁ receptor (and not D₂ receptor) antagonists modulate working memory performance (Sawaguchi and Goldman-Rakic, 1991, Sawaguchi and Goldman-Rakic, 1994, Williams and Goldman-Rakic, 1995). However, a role for D₂ receptors in working memory is also suggested based on evidence that systemic administration of D₂ receptor agonists can modulate performance by potentially affecting response preparation within the PFC (Wang et al., 2004), or through effects within the striatum or via activation of other dopamine receptor sites (i.e. D₃/D₄ receptors) (Arnsten et al., 1995).

Deficits in working memory performance in clinical populations such as schizophrenia (Park and Holzman, 1992, Weickert et al., 2000, Meyer-Lindenberg et al., 2001, Abi-Dargham et al., 2002, Callicott et al., 2003) and Parkinson’s disease (Lange et al., 1992, Kulisevsky et al., 1996, Bublak et al., 2002, Callicott et al., 2003) further support dopamine as a modulator of working memory performance. Evidence has also linked impairments in D₁ receptors in the PFC to working memory deficits in patients with schizophrenia (Abi-Dargham et al. 2002). However, experimental evidence in healthy humans has failed to clearly elucidate the role of dopamine in human working memory performance, due in part to the lack of an appropriate D₁
receptor agent for use in humans. Research using the D² receptor agonist bromocriptine has produced inconsistent results, with some evidence of a positive modulatory effect on performance (Luciana et al., 1992, Luciana and Collins, 1997, Mehta et al., 2001), but other studies showing no effect (Kimberg et al., 1997, Muller et al., 1998, Bartholomeusz et al., 2003). Similarly, the D² receptor antagonists sulpiride and haloperidol have been observed to impair working memory performance in some studies (Luciana and Collins, 1997, Mehta et al., 1999, Mehta et al., 2004), but other studies have failed to show an effect (Mehta et al., 2003). The combined D₁/D² receptor agonist pergolide has also demonstrated inconsistent effects, with evidence that it may enhance working memory performance (Muller et al., 1998), may have a beneficial effects on performance in only some individuals, dependent on working memory capacity (Kimberg and D'Esposito, 2003), or have no effect on performance (Bartholomeusz et al., 2003, Roesch-Ely et al., 2005). While it has been suggested that baseline working memory capacity could mediate the effect of dopamine agonists on working memory performance, the nature of these effects has also differed between studies (Kimberg et al., 1997, Mehta et al., 2000, Kimberg and D'Esposito, 2003).

A number of recent studies have examined the effects of global depletion of dopamine on working memory performance. As outlined in the previous chapter, dopamine (and noradrenaline) relies on an available source of its amino acid precursor tyrosine (and tyrosine’s precursor, phenylalanine) for synthesis within the brain. Restricting these amino acids has provided a novel technique for experimentally depleting dopamine levels and probing the effects on working memory performance. The first study to assess the effects of TPD on SWM revealed TPD-related deficits on two SWM tasks; a delayed-recognition task (impaired accuracy was observed) and a self-ordered strategic search task (impaired strategy was observed) (Harmer et al., 2001). These findings were supported by Harrison et al. (2004) who observed TPD-related deficits on a variation of the Sternberg working memory task (a SWM delayed-recognition task). Harrison et al. (2004) also showed a modality specific selectivity for TPD, with no deficits observed on a N-SWM delayed-recognition task, consistent with findings that suggest that spatial memory tasks may be more sensitive than non-spatial tasks to dopaminergic medication used in PD (e.g. Lange et al., 1992, Kulisevsky et al., 1996, Postle et al., 1997a, Postle et al., 1997b, Cools et al., 2002). However, two subsequent
studies (McLean et al., 2004, Roiser et al., 2004) were unable to replicate impairments on the self-ordered strategic search task, while Lythe et al. (2005) failed to observed impairment on a delayed-response task following TPD. Mehta et al. (2005a) also failed to observe working memory deficits at a group level following TPD, although there was some suggestion of impairments in participants with greater depletion of striatal dopamine levels (as indicated by changes in \([11C]\)raclopride binding).

Therefore, this experiment aimed to further examine the role of dopamine in SWM behavioural performance in healthy humans. Due to the inconsistent behavioural effects of TPD on SWM behavioural performance reported to date, the first aim of this experiment was to examine whether TPD-related impairment on the “Sternberg” SWM delayed-recognition task (as observed by Harrison et al. 2004) could be replicated in a larger sample. The second aim of this experiment was to extend upon previous literature and examine whether stimulating D₁/D₂ receptors, under dopamine depleted conditions, would modulate SWM by “reversing” the proposed negative effects of TPD on SWM performance.

The possible interaction of baseline working memory capacity on the modulatory effects of dopamine on working memory was examined between participants with “high” and “low” baseline, based on evidence of previously baseline-dependent effects (Kimberg et al., 1997, Mehta et al., 2000, Kimberg and D’Esposito, 2003). Additionally, tests measuring reaction time and sedation were included in order to examine whether any changes in working memory reaction time reflect drug related effects on reaction time per se.

### 4.2 METHODS

#### 4.2.1 Participants

Twenty-three healthy male participants were recruited for the experiment through advertisements within local universities and general community. One participant withdrew from the experiment due to faintness following an initial blood sample, and four participants withdrew from the experiment due to nausea and/or vomiting following consumption of the amino acids. The resulting sample comprised 18 males (mean age 22.9 ± 6.4 years). All participants were healthy, right handed, and non-
smokers. Exclusion criteria comprised a history of neurological or psychiatric disorders (including history of depression or anxiety disorders in first degree relatives), chronic physical illness, medication and/or drug use, or excessive alcohol consumption. Medical and psychiatric suitability to participate in the experiment was ascertained following an initial screening by telephone (including administration of the Prime-MD, Pfizer, 1996), general screening questionnaire (see Appendix 1) and a consequent semi-structured clinical assessment with a physician.

4.2.2 Design
The experiment was conducted using a double-blind, balanced-drink (placebo) control, repeated measures design over three separate sessions: 1) 104.4g balanced control condition (BAL condition), 2) an equivalent mixture deficient in tyrosine and phenylalanine (TPD condition), and 3) TPD mixture + pergolide (0.1mg) condition (TPD+P condition). Each session was separated by a minimum five-day washout period, with order of condition randomised using a quasi-latin-square design. The experiment was approved by the Swinburne University Human Research Ethics Committee. All participants gave written informed consent.

4.2.3 Procedure
All participants attended a pre-study practice day, during which they completed the cognitive battery four times (separated into two sessions, a minimum of two hours apart), based on evidence that performance stabilises following four training sessions (Wesnes and Pincock, 2002).

On each testing day, participants arrived at the laboratory at 0815 hours, having consumed a low protein diet (less than 25g) in the preceding 24 hrs and fasting from 1900 hrs. Participants were also asked to refrain from alcohol the day before testing, and to arrive well rested. Each participant was contacted by phone to encourage compliance with the pre-experimental protocol. Following arrival, the participant sat quietly for 15 minutes, before commencing baseline testing (described below). At approximately 0900 hrs (time 0) the amino acid drink and capsules were administered (details below), and at +3 hrs post-drink (approximately 1200 hrs) an oral dose of pergolide (or placebo capsule) was administered. Post-drug testing commenced at
+5 hrs post drink, to coincide with the peak behavioural effects of TPD (Harmer et al. 2001, Harrison et al. 2004) and pergolide (Markham and Benfield, 1997). To reduce the potential side effects of pergolide, two doses of the peripheral dopamine receptor antagonist domperidone were administered during each testing session (at +30 mins and +2 hrs post-drink; 10mg per administration/20mg total). Domperidone is a peripherally acting D2 antagonist which was administered to minimise possible side effects, specifically nausea. Administration of domperidone to reduce side effects is established in the treatment of Parkinson’s disease (e.g. Storch et al., 2005), and has been previously employed in dopamine agonist studies in healthy humans (e.g. Muller et al., 1998, Bartholomeusz et al., 2003). At +2.5 hrs post-drink a carrot was also provided to reduce hunger. Subjective side effect questionnaires were administered at +1 and +3 hrs post-drink (see Appendix 2). Visual analogue scales (Bond and Lader, 1974) were administered at baseline (pre-drink) and at +5 hrs (post-drink/pre-testing) to measure subjective feelings. Two 20ml venous blood sample were taken per session for analysis of plasma amino acid concentrations; the first preceding baseline testing (i.e. before time 0) and the second preceding post-drink testing (at +4 hrs, 45 mins). Testing concluded +5.5 hrs post-drink, and participants were provided with a high protein snack before departing.

### Table 4-1 Timeline of experimental procedure

<table>
<thead>
<tr>
<th>Time</th>
<th>Relative time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0815 hrs</td>
<td></td>
<td>Arrival</td>
</tr>
<tr>
<td>0830 hrs</td>
<td></td>
<td>Baseline testing</td>
</tr>
<tr>
<td>0900 hrs</td>
<td>“time 0”</td>
<td>Amino acid administration</td>
</tr>
<tr>
<td>0930 hrs</td>
<td>+ 30 min</td>
<td>First domperidone dose</td>
</tr>
<tr>
<td>1100 hrs</td>
<td>+ 2 hrs</td>
<td>Second domperidone dose</td>
</tr>
<tr>
<td>1130 hrs</td>
<td>+ 2.5 hrs</td>
<td>Low protein snack (carrot)</td>
</tr>
<tr>
<td>1200 hrs</td>
<td>+ 3 hrs</td>
<td>Pergolide administration</td>
</tr>
<tr>
<td>1400 hrs</td>
<td>+ 5 hrs</td>
<td>Post-treatment testing</td>
</tr>
</tbody>
</table>

### Amino acid suspension

The composition of the balanced amino acid mixtures was based on the original 100g balanced suspension developed by Young et al. (1985): L-alanine 5.5g; L-arginine
4.9g; L-cystine 2.7g; glycine 3.2g; L-histidine 3.2g; L-isoleucine 8.9g; L-leucine, 13.5g; L-lysine monohydrochloride, 11g; L-methionine 3g; L-proline 12.2g; L-serine 6.9g; L-threonine 6.9g; L-valine 8.9g; L-tryptophan 2.3g; L-tyrosine 6.9g and L-phenylalanine 5.7g (with both L-tyrosine and L-phenylalanine excluded for TPD). Drs were prepared within a few minutes of oral administration by mixing the powdered amino acids with 180ml orange juice. Due to the unpleasant taste, L-cysteine, L-methionine and L-arginine were encapsulated in gelatine capsules and administered separately. Participants were instructed to swallow the suspension in as short a time as possible. A nose plug was provided during ingestion to reduce olfactory cues.

All cognitive tasks were taken from the Cognitive Drug Research computerised assessment suite (CDR Ltd, Goring-on-Thames, UK; www.cdr.org.uk), regarded for its validity as a measure of memory and attention and its proven sensitivity in studies of acute tyrosine depletion (Harrison et al., 2004). Tasks were presented via computer and all responses were made using an external button box (yes/no) or critical flicker fusion tube. The button box was hand held with thumbs resting upon the respective button. Participants were instructed to respond “as quickly as possible but with accuracy as their priority” on all tasks. Participants were seated approximately 1 metre from the computer monitor in a dimly lit room (consistent between sessions) and were requested to sit upright throughout the task.

Accuracy was recorded as: 1) percentage accuracy in correctly identifying the original stimuli, and 2) percentage accuracy in correctly identifying the new stimulus. The main accuracy measure, the sensitivity index, was calculated using these two values, and is a measure of overall task efficiency ranging between 0 (chance level accuracy) and 100 (perfect recognition of all stimuli) (Frey and Colliver, 1973). Specifically, the sensitivity index assesses the participants ability to discriminate between original and novel stimuli, with a high sensitivity index representing both accuracy in correctly identifying the original stimulus and avoidance of falsely identifying novel/distracter stimuli. Reaction time in milliseconds was recorded for all tasks. The duration of the test battery was approximately 20 minutes.
**SWM delayed-recognition**

This is a modified version of the Sternberg maintenance task for working memory (Sternberg, 1966), which requires both intact spatial recognition and the manipulation of stored information to discriminate ‘original’ from ‘novel’ spatial cues. The image of a house front displaying nine windows was presented for 10 seconds, with four of the nine lights turned on (‘original’ stimuli). Participants were shown 36 consecutive presentations of the house front, with an inter-stimulus interval of 1 second, with only a single window lit up. Participants were required to identify whether the light was in a matching ‘original’ position by pressing “yes”, or in a novel location to one of the four original lights by pressing “no” as quickly as possible.

**Reaction time**

This task involved 50 trials of responding to the word “yes” by pressing the appropriate response button as quickly as possible (inter-stimulus interval ranged between 1 and 3.5 seconds).

**Critical flicker fusion (CFF)**

This task is a traditional psychophysical threshold measure of alertness and attention and was used as a measure of drug-induced sedation (Hindmarch and Parrott, 1977). Participants held the CFF unit, and fixed their gaze on two red lights at the base. The flicker ranged from 25Hz to 65Hz in alternating ascending and descending mode, with three trials ‘up’ and ‘down’. Participants responded when they perceived the light to either start or stop flickering, by pressing “yes” on the button box, or to discriminate between the two lights by indicating which one is flickering.

**Side effect questionnaire**

Participants rated how they felt on an 11 point symptom checklist in response to 11 questions (for example “I have a headache”, “I feel nauseous”, “I have stomach pains”, “my heart is beating faster than normal”), on a range from 1 (not at all) to 5 (very much so). A minimum score of 11 indicated that participants felt no symptoms, with a score of 55 indicating extreme negative symptoms.
**Subjective Feeling Assessment**
Subjective feelings were obtained using a modified version of the Visual Analogue Scales (Bond and Lader, 1974) comprising sixteen 100mm horizontal lines each representing a subjective feeling dimension, with opposing words at each end, e.g. happy – sad, alert – drowsy, amicable – antagonistic. The visual analogue scales (VAS) were scored as two factors (alertness and tranquillity) consistent with the factor analysis of Herbert et al. (1976).

**Plasma amino acid level analysis**
Blood samples were collected from all participants approximately 10 minutes prior to both amino acid administration and post-treatment testing. These samples were analysed for concentrations of free amino acids tyrosine (TYR), phenylalanine (PHE), tryptophan (TRYP), valine (VAL), leucine (LEU), and isoleucine (ILE) in plasma using precolumn derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), followed by separation of the derivatives and quantification by reversed phase high performance liquid chromatography (RP-HPLC). VAL, LEU and ILE levels were analysed to calculate the ratio of plasma TYR and PHE, to other large neutral amino acids (LNAAs). Prior to derivatisation, plasma samples (100 µL) were diluted 1:1 with internal standard solution and deproteinised by ultrafiltration through a membrane with a 10 KDa nominal molecular weight cut-off (Ultrafree MC with PL-10 membrane, Millipore, MA). The filtrate (100 ml) was then subjected to 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatisation and High Performance Liquid Chromatography (HPLC) analysis using the Waters AccTag AA analysis system (Waters Corporation, Milford, MA, USA) (Cohen, 2001).

**Statistical analysis**
Data was analysed using SPSS (SPSS Inc., Chicago, IL). All data was analysed using repeated measures analysis of variance (ANOVA) if appropriate, but nonparametric statistics were used for data which violated the assumption of normal distribution. Standard errors of the means are reported in parenthesis following means.

Baseline dependent analysis was conducted by separating participants into high and low baseline working memory groups, based on their median scores in each of the baseline sensitivity index measures on the task. Additional ANOVA’s were conducted
on each working memory measure, with the baseline working memory capacity variable introduced as a between-subjects factor. Correlations were conducted between the change in performance from treatment and balanced/placebo conditions, and the corresponding changes in side effect ratings, for all significant cognitive measures in order to examine the possible contribution of side effects to any drug-induced performance impairments.

4.3 RESULTS

4.3.1 Plasma amino acid levels

Complete plasma samples were analysed for 10 participants. Separate 3 (treatment condition) by 2 (time) ANOVA’s were conducted for each amino acid, and revealed significant interactions between treatment conditions and time for tyrosine $[F(1.21,10.97)=40.39, \ p<0.001$, with Greenhouse-Geisser correction] and phenylalanine $[F(1.08,9.71)= 42.77, \ p<0.001$, with Greenhouse-Geisser correction]. Planned contrasts showed that concentrations of both tyrosine and phenylalanine decreased significantly following the TPD, and the pergolide under conditions of TPD (TPD+P) conditions, compared to the BAL condition (all $p<0.001$). The ratio of plasma tyrosine and phenylalanine to large neutral amino acids (T+P/$\Sigma$LNAA) varied significantly between treatment conditions, as revealed by a 3 (treatment condition) by 2 (time) repeated measures ANOVA $[F(2,18)=47.60, \ p<0.001]$. Planned contrasts revealed a significantly greater decrease in the ratio following both the TPD and TPD+P condition, compared to the BAL condition (both $p<0.001$) (see Table 4.2).
Table 4-2  Mean and standard error (SEM) concentrations of amino acids (μmol/l) and change in percentage from baseline to 5 hours post-drink.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Treatment</th>
<th>Baseline</th>
<th>Post</th>
<th>Percentage Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAL</td>
<td>58.47 (5.91)</td>
<td>106.77 (11.15)</td>
<td>82.61* †</td>
</tr>
<tr>
<td>Plasma L-tyrosine</td>
<td>TPD</td>
<td>62.94 (12.16)</td>
<td>20.20 (5.64)</td>
<td>-67.90* †</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>62.95 (9.13)</td>
<td>17.10 (3.68)</td>
<td>-72.84* †</td>
</tr>
<tr>
<td>Plasma L-phenylalanine</td>
<td>BAL</td>
<td>47.74 (4.56)</td>
<td>91.05 (10.82)</td>
<td>90.74* †</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>50.17 (8.24)</td>
<td>8.94 (2.77)</td>
<td>-82.17* †</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>49.78 (6.29)</td>
<td>6.88 (1.00)</td>
<td>-86.17* †</td>
</tr>
<tr>
<td>Plasma L-tryptophan</td>
<td>BAL</td>
<td>2.73 (0.55)</td>
<td>6.02 (1.03)</td>
<td>120.79*</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>2.67 (0.62)</td>
<td>6.06 (1.02)</td>
<td>126.52*</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>2.54 (0.39)</td>
<td>7.63 (1.67)</td>
<td>200.79*</td>
</tr>
<tr>
<td>Plasma L-valine</td>
<td>BAL</td>
<td>199.10 (20.16)</td>
<td>628.91 (70.62)</td>
<td>215.87*</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>221.99 (32.15)</td>
<td>722.75 (100.14)</td>
<td>225.57*</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>229.45 (28.27)</td>
<td>669.15 (91.56)</td>
<td>191.63*</td>
</tr>
<tr>
<td>Plasma L-isoleucine</td>
<td>BAL</td>
<td>74.69 (6.88)</td>
<td>296.70 (33.58)</td>
<td>297.25*</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>83.98 (11.22)</td>
<td>360.45 (53.38)</td>
<td>329.22*</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>81.56 (10.49)</td>
<td>284.74 (41.52)</td>
<td>249.12*</td>
</tr>
<tr>
<td>Plasma L-leucine</td>
<td>BAL</td>
<td>116.63 (11.56)</td>
<td>460.28 (53.40)</td>
<td>294.65*</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>129.71 (19.98)</td>
<td>580.12 (89.43)</td>
<td>347.23*</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>131.11 (17.35)</td>
<td>452.39 (68.83)</td>
<td>245.04*</td>
</tr>
<tr>
<td>TYR+PHE/ΣLNAAs</td>
<td>BAL</td>
<td>0.18 (0.004)</td>
<td>0.10 (0.006)</td>
<td>-44.13*</td>
</tr>
<tr>
<td></td>
<td>TPD</td>
<td>0.17 (0.007)</td>
<td>0.01 (0.001)</td>
<td>-93.49*</td>
</tr>
<tr>
<td></td>
<td>TPD+P</td>
<td>0.17 (0.008)</td>
<td>0.01 (0.001)</td>
<td>-93.02*</td>
</tr>
</tbody>
</table>

N=10. * indicates significant change from baseline to 5 hours post-drink (p<0.001)
† indicates significant difference between treatments (p<0.001).
BAL= Balanced condition; TPD= Tyrosine/Phenylalanine depletion;
TPD+P= Tyrosine/Phenylalanine depletion and pergolide.

4.3.2  SWM delayed-recognition task
All accuracy data was heavily skewed. Data was originally transformed using an arcsine transformation (Y=2 x arcsine √p, where p is the proportion correct). Following transformation the data remained unsuitable for parametric analysis, hence
nonparametric statistics were employed. Figure 4.1 shows the sensitivity index data for pre- and post-drug, which indicates that participants performed more poorly following TPD and TPD+P treatments. Binomial statistics reveal that there was a greater proportion of participants who performed more poorly following TPD+P than following the BAL condition (p<0.05). There was no significant difference in performance between the TPD and BAL conditions, or the TPD+P and TPD conditions (both p>0.05). However, this effect appears subtle as confirmed by a Friedman’s test of performance, which revealed no overall significant difference between all three treatment conditions for the sensitivity index ($\chi^2 = 1.075$, p>0.1), accuracy of identifying the original stimulus ($\chi^2 = 1.107$, p>0.1), or accuracy of identifying the new stimulus ($\chi^2 = 0.326$, p>0.1).

Reaction time was analysed using repeated measures ANOVA. This also revealed no significant interaction between drug and time [F(2,34)=0.63, p>0.1].

![Figure 4-1](image)

**Figure 4-1** Sensitivity Index for the SWM delayed-recognition task at baseline and post-treatment, for each treatment condition. Error bars represent standard error of the mean (SEM).

### 4.3.3 Baseline dependence analysis

Participants were classed as either “high” or “low” baseline, respectively, based on their median scores in the baseline conditions. This resulted in a group of 6 “high” baseline participants, a group of 6 “low” baseline participants, with 6 participants
excluded from the analysis, as they did not differentiate substantially between high and low baseline scores over all baseline conditions. There were no significant interactions between the baseline-working memory factor and performance on any measure (all \( p > 0.1 \))

### 4.3.4 Reaction time

There was a main effect of treatment on reaction time, with reaction time increasing after all treatment conditions \( [F(2,34)=7.00, p<0.05] \). However, there was no interaction between treatment condition and time \( [F(2,34)=1.16, p>0.1] \) (see Table 4.3 for values).

### 4.3.5 Critical Flicker Fusion (CFF)

A 3 (treatment condition) by 2 (time) repeated measures ANOVA revealed no significant interaction between treatment conditions and time on the CFF measure \( [F(2,34)=0.94, p>0.1] \) (see Table 4.3 for values).

### 4.3.6 Visual analogue scales (VAS)

TPD did not significantly influence subjective feelings scores. There was no significant interaction between drink condition and time for either alertness \( [F(2,34)=2.08, p>0.05] \) or tranquillity \( [F(2,34)=0.27, p>0.05] \). Further, there was no effect of the study day itself on subjective feelings, with no main effect of time on alertness \( [F(1,17)=1.91, p>0.05] \) or tranquillity \( [F(1,17)=0.41, p>0.05] \) (see Table 4.3 for values).

#### Table 4-3

Mean and standard error (SEM) values at baseline (pre-drug) and 5 hrs (post-drug) for simple reaction time (ms), critical flicker fusion (Hz), and subjective feelings (VAS) factors.

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Balanced</th>
<th>TPD</th>
<th>TPD+P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Reaction time</td>
<td>254.6</td>
<td>(4.7)</td>
<td>257.3</td>
</tr>
<tr>
<td>CFF</td>
<td>37.3</td>
<td>(1.2)</td>
<td>37.0</td>
</tr>
<tr>
<td>VAS: Alertness</td>
<td>26.7</td>
<td>(3.9)</td>
<td>27.0</td>
</tr>
<tr>
<td>VAS: Tranquillity</td>
<td>30.4</td>
<td>(4.9)</td>
<td>26.5</td>
</tr>
</tbody>
</table>

BAL= Balanced condition; TPD= Tyrosine/Phenylalanine depletion; TPD+P= Tyrosine/Phenylalanine depletion and pergolide.
4.3.7 Subjective side effects and correlations with cognition

The effects of TPD, and pergolide (under TPD conditions), on blood pressure and pulse were measured every hour to monitor for potential side effects. This data showed that there was no significant difference between treatment conditions on either blood pressure or pulse rate. The difference between the baseline measure (averaged over two measurements taken 5 minutes apart) and each hourly measurement did not significantly differ between sessions (all analyses p>0.05).

Four participants withdrew from the experiment due to the side effect of nausea, and their cognitive results were not analysed. However, in the remaining sample of (N=18) there was no difference between the treatment conditions in subjective side effects [F(2,34)=0.10, p>0.1]. The nausea scale was also examined separately, and similarly there was no significant difference between treatment conditions (p>0.1).

The changes in side effect rating between treatment and balanced conditions (i.e. TPD - BAL and TPD+P - BAL) were correlated with corresponding changes in working memory performance between treatment and balanced conditions (TPD - BAL and TPD+P - BAL) using Pearson’s product moment correlations. There were no significant correlations between change in working memory performance and side effect scores for either TPD (r = -0.12, p=0.63) or TPD+P (r = -0.01, p=0.9).

4.4 DISCUSSION

This experiment examined whether previous findings of impairment in SWM performance on the “Sternberg” delayed-recognition task following TPD, as previously observed by Harrison et al. (2004), could be replicated in a larger sample. The current findings did not support replication of TPD-related impairment on this SWM task. However, D₁/D₂ receptor stimulation under conditions of dopamine depletion was observed to cause subtle impairment in SWM performance.

4.4.1 TPD effects on delayed-response task

The current experiment was not able to replicate TPD-induced working memory deficits on the “Sternberg” SWM delayed-recognition task, as observed by Harrison et al. (2004), and therefore contradicts earlier reports of TPD-induced impairments of
delayed-recognition performance (Harmer et al., 2001, Harrison et al., 2004). However, these findings are consistent with two recent studies which were unable to replicate impairment on the self ordered-strategic search task as first demonstrated by Harmer et al. (2001) (McLean et al., 2004, Roiser et al., 2004), and two additional studies which failed to observe TPD-related impairments on delayed-response performance (Lythe et al., 2005, Mehta et al., 2005a). The lack of effect was unlikely to be due to insufficient plasma tyrosine/phenylalanine depletion, as the current plasma analysis revealed comparable depletion levels to that observed in both the Harrison et al. (2004) and Harmer et al. (2001) studies. While a more conservative control/placebo was observed in the current experiment than has previously been reported (indeed, by nature the TPD protocol produces a conservative control, as the ratio of tyrosine/phenylalanine to large neutral amino acid (TP/ΣLNAA) is reduced in the control, as well as the TPD, condition), there was no indication of SWM impairment in the placebo condition making it unlikely that the conservative control accounted for the lack of effect seen. However, it is probable that variation in central dopamine depletion accounted at least in part, for the lack of working memory impairments. Indeed, Mehta et al. (2005a) recently reported no TPD-related effects on working memory at a group level, but observed a correlation between central dopamine depletion (as indexed by striatal [11C]raclopride binding changes) and performance change. Specifically, impairments in performance were only observed for participants with a high dopamine depletion level, but virtually no change (and/or subtle improvement) was observed in participants with minimal dopamine depletion levels. Interestingly, a similar relationship between plasma tyrosine/phenylalanine depletion and performance was not evidence (Mehta et al., 2005a).

It is doubtful that the lack of the TPD-related effects resulted from either the type of task used, or of the experiment being underpowered. Harrison et al. (2004) demonstrated TPD-related impairments (approximately 6% impairment) on an identical SWM delayed-recognition task, in a smaller sample size of 12 participants (the current experiment employed 18 participants). Critical comparison of the methodology used in the Harrison et al. (2004) study and Experiment 1 reveals they are almost identical except for one methodological difference: gender of the sample. Harrison et al. (2004) employed a female sample, while the current experiment was conducted in a male sample. Previous research has found the mood effects of amino
acid depletion (tryptophan depletion) to be more pronounced in females (Ellenbogen et al., 1996, Nishizawa et al., 1997). In contrast to tryptophan depletion, little is known about the role of gender in TPD-related effects; however, males have previously been shown to be stronger on tests of spatial memory than females (for a review, see Kimura, 1996). While Harmer et al. (2001) reported no interaction of gender with cognitive performance in their findings of TPD-related working memory impairments, the size of the groups (7 males, 5 females) does limit the generalisability of this finding, and it remains possible that males were more resistant than females to the effects of dopamine depletion on this SWM delayed-recognition task. This emphasises the importance of attempting to maintain a homogenous sample, and therefore the experiments in this thesis continued to employ male participants.

4.4.2 D₁/D₂ receptors under conditions of TPD
Contrary to expectation, D₁/D₂ receptor stimulation under conditions of tyrosine depletion caused a subtle impairment in SWM performance. While the exact mechanism responsible for this finding is unclear, there are a number of possible reasons for this effect. Firstly, in light of the fact that TPD did not initially impair working memory, it may not be surprising that pergolide did not improve performance. Previous studies have been contradictory as to the effects of pergolide on working memory performance; while some studies have shown an improvement (Muller et al., 1998), other studies failed to observe an effect (Bartholomeusz et al., 2003, Roesch-Ely et al., 2005). It has been suggested that the lack of effect may be due to ceiling performance effects in already high performing participants (Bartholomeusz et al., 2003). In line with this suggestion, as TPD did not initially impair working memory performance in the current experiment, and participants were performing at high accuracy levels, it is possible that on average participants’ performance was already at ceiling level and hence not improved by D₁/D₂ receptor stimulation.

Secondly, pergolide would be expected to act differently within a dopamine-depleted state. It has been suggested that phasic release of dopamine within a dopamine depleted system may over-stimulate D₁ or D₂ receptors, due to sensitisation (Grace, 1991, Grace, 1993, Abi-Dargham and Moore, 2003). The postsynaptic effects of D₁ receptors are complex and can be considered as either excitatory or inhibitory,
depending on the functional status of the neuron (Yang et al., 1999), and a sensitised D1 system may shift to a more GABAergic pathway. Thus, without the presence of normal endogenous dopamine levels, pergolide may have over-stimulated the D1 system and resulted in increased inhibition (Abi-Dargham and Moore, 2003). Indeed, the importance of optimal stimulation of D1 receptors in SWM performance is well demonstrated in the non-human primate, with either insufficient or excessive D1 receptor stimulation leading to performance impairment (Williams and Goldman-Rakic, 1993, Williams and Goldman-Rakic, 1995). In addition, low doses of D2 agonists have previously been shown to preferentially stimulate D2 autoreceptors (Di Chiara et al., 1977, Tissari et al., 1983), and it is possible that pergolide's action at the D2 autoreceptor may have also influenced performance. Indeed, low doses of pergolide can augment motor deficits associated with Parkinson’s disease (Kellett and Steiger, 1999), and recent evidence suggests that L-dopa administration augmented, rather than reversed, TPD related decreases in cocaine-induced drug craving (Leyton et al., 2004a). The complexity of functional effects of dopamine augmentation within dopamine depleted states is exemplified by the inconsistent effects of dopaminergic medication on cognition in Parkinson’s disease. Dopaminergic medications have been observed to improve, have no effect, or even impair performance on a range of cognitive tasks, and the effects may be dependent on both the basal levels of dopamine within the underlying cortico-striatal circuitry and the nature of the task (Gotham et al., 1988, Cools et al., 2001, Cools et al., 2003, Fern-Pollak et al., 2004).

It is unlikely that side effects underlie the detrimental effects of pergolide on performance. First, while four participants did withdraw from the experiment due to nausea, the remaining participants showed no significant differences on the side effects measure. To more thoroughly examine the possibility that subjective side effects influenced performance, the change in side effect ratings was also correlated with change in performance for significant performance measures, and yielded no significant correlations. It could also be suggested that the working memory effect observed was actually secondary to sedation. However, there was also no interaction between treatment conditions and performance on the critical flicker fusion task, a well-regarded psychophysical threshold measure of drug-induced sedation (Hindmarch and Parrott, 1977). Furthermore, the simple reaction time measure used in this experiment showed that while the procedure itself caused an increase in
reaction time (i.e. reaction time increased following all treatments), there was no interaction with treatment condition, making it unlikely that the performance impairment observed during the SWM task was purely the result of sedation or attention deficits.

**4.4.3 Baseline working memory effects**

There was no evidence that the baseline working memory performance of participants influenced the effects of either TPD, or TPD and pergolide, on working memory performance; however it remains likely that individual differences within the current sample may have influenced results. While baseline working memory has previously been related to performance changes following dopamine manipulation (Kimberg et al., 1997, Mehta et al., 2000, Kimberg and D'Esposito, 2003), these effects have been inconsistent and sometimes contradictory, and may be dependent on factors such as concentrations of drug and time of cognitive testing (in respect to kinetic effects of the drug) (as discussed by Kimberg and D’Esposito 2003 and outlined in Chapter 2). As discussed in Chapter 2 (Pharmacology of working memory review), it is likely that individual differences may be more reliably linked to functional polymorphism (Val/Met) in the COMT gene (of which baseline-dependent behaviour may be a reflection), based on evidence that COMT genotype has been observed to influence working memory performance changes in healthy volunteers following amphetamine administration (Mattay et al., 2003), and n-back task performance in patients with schizophrenia, following olanzapine treatment (Bertolino et al., 2004).

**4.4.4 Summary**

The findings of this experiment did not replicate the findings of Harrison et al. (2004) in which a TPD-related impairment in performance was observed, which is consistent with the failure of two recent studies to replicate impairment on the SWM strategic search task as demonstrated by Harmer et al. (2001) (McLean et al., 2004, Roiser et al., 2004), and two additional studies which failed to observe TPD-related effects on delayed-response tasks (Lythe et al., 2005, Mehta et al., 2005a). However, contrary to the prediction of a positive (potentially reversing) effect of D1/D2 stimulation under dopamine depleted conditions, pergolide produced subtle impairments in SWM. These findings highlight the complexity of augmenting dopaminergic transmission within a dopamine depleted state.
The TPD-related findings, when taken together with previous studies, question the reliability of TPD to produce replicable impairments in SWM behavioural performance. However, by design, the findings of this experiment are limited to concluding that there was no evidence that TPD produced measurable effects on SWM behavioural performance. Indeed, preserved behavioural performance may mask modulation of underlying neural activity. Therefore, in the following experiments, the effect of TPD was examined with both behavioural and neuroimaging techniques to investigate whether TPD influence SWM task-related neurophysiology.
Chapter Five

5 Experiment 2: Examining the effects of TPD on behavioural performance and task-related blood flow during the SWM n-back task: A PET investigation.

5.1 INTRODUCTION

In the previous chapter, literature was reviewed which suggests that the effect of TPD on behavioural performance is inconsistent. While TPD-induced impairments of delayed-recognition performance have been shown previously (Harmer et al., 2001, Harrison et al., 2004), a number of more recent findings have failed to observe an effect of TPD on SWM behavioural performance measures (McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Mehta et al., 2005a). Consistent with these latter findings, the experiment conducted in the previous chapter demonstrated a lack of TPD-related impairment in performance (also see Ellis et al., 2005b).

It has previously been proposed that differences in response preparation and execution demands may be a possible cause of discrepant behavioural findings between studies following dopamine manipulation (e.g. Luciana and Collins, 1997, Ellis and Nathan, 2001, Mehta et al., 2001, Mehta et al., 2003). For example, Luciana and Collins (1997) observed evidence of a facilitating role of the D2 receptor agonist bromocriptine on SWM delayed-recall performance, but found no effect on performance of an object working memory delayed-recognition task. These authors noted that while the findings may indicate specificity for dopaminergic modulation of SWM, they may also reflect the fact that the delayed-recall task required higher motor response preparation and output requirements - which is specifically pertinent considering the importance of dopamine in initiating movement. Similarly, Mehta et al. (2001) observed improvements in performance on the spatial span task following bromocriptine (compared to placebo), but no effect on a SWM strategic search task or SWM delayed-recognition task. Mehta et al. (2001) suggested that differences in response preparation and execution demands between the spatial span task (requiring
response preparation during delay and execution of spatially guided motor response) and the other two tasks (not requiring response preparation until after the delay) may underlie the differences in results. Therefore, it is possible that response demands at least partly underlie inconsistent behavioural effects following TPD.

It is also possible that while a number of previous studies (including the findings reported in the previous chapter) observed no effect on behavioural performance, TPD may have influenced underlying SWM neural networks. As changes in cellular brain activity within a region are almost invariably accompanied by changes in local blood flow, measuring changes in cerebral blood flow using either PET (rCBF) or fMRI (BOLD signal) can be used infer changes in neural activity (for discussion, see Raichle, 1998, Arthurs and Boniface, 2002). Previously, Kimberg et al. (2001) observed that administration of the D₂ receptor agonist bromocriptine reduced blood flow (as assessed by changes in BOLD signal, measured using fMRI) within the parietal cortex during n-back working memory performance, despite no evidence of a changes in behavioural performance at a group level (Kimberg et al., 2001). Further, the psychomotor stimulants methylphenidate and amphetamine (indirect catecholamine agonists) have been associated with task-related changes in activity within the PFC (Mattay et al., 2000, Mehta et al., 2000, Schweitzer et al., 2004) and posterior parietal cortex (Mehta et al., 2000) which are regions consistently activated by working memory tasks (for a review, see Wager and Smith, 2003). These findings are also consistent with considerable evidence that modulation of the dopaminergic system alters underlying neural networks associated with SWM in the PFC of non-human primates (Goldman-Rakic et al., 1996), as highlighted in Chapter 2.

To date, no research has examined the effect of TPD on underlying SWM-related activity using functional neuroimaging. A recent study demonstrated the advantages of combined neuroimaging and behavioural testing in examining TPD effects on working memory, by demonstrating that although TPD did not alter performance at a group level, there was a correlation between the magnitude of striatal dopamine depletion (as indexed by striatal [11C]raclopride binding) and performance changes (Mehta et al., 2005a). Specifically, performance was worsened in participants with the greatest reduction in striatal dopamine levels, with virtually no change (and/or subtle improvement) in performance observed in participants with minimal dopamine
depletion. However, the scope of the Mehta et al. (2005a) study was limited to assessing dopamine changes within the striatum, and was unable to examine the TPD effects on task-related activation within the PFC and associated working memory networks.

Therefore, the current experiment aimed to examine the effects of TPD on rCBF within the PFC and associated SWM networks during performance of the SWM n-back task (Gevins and Cutillo, 1993, McEvoy et al., 1998). The SWM n-back was employed for the following three reasons: 1) it has a well established neuroanatomical network, consistently activating the PFC (specifically the right DLPFC), posterior parietal cortex, and anterior cingulate gyrus (for a review, see Owen et al., 2005), 2) it can be employed with a parametric variation in memory load, which linearly relates to activation within the working memory network (e.g. Braver et al., 1997, Cohen et al., 1997), and 3) patients with schizophrenia show performance impairments on the SWM n-back task, which have been correlated with blood flow changes within the PFC (for a review, see Manoach, 2003) and PFC D₁ receptor availability (Abi-Dargham et al., 2002). As far as can be ascertained, this was the first experiment to examine the effects of TPD on rCBF. Therefore, in addition to the interaction effect of TPD on task-related rCBF, this experiment also aimed to examine the main effect of TPD across all task conditions and predicted predominantly striatal increases in rCBF in line with the effects of the dopamine receptor antagonist sulpiride (Mehta et al., 2003).

Further, this experiment aimed to examine whether response demands influence the effects of TPD on performance. Two delayed-response tasks were designed, matched closely on most parameters excluding response preparation and output. Specifically, the first task - the delayed-recognition task - minimised response preparation and output, while the second task - the delayed-recall task - allowed response preparation during the delay. These tasks were administered after completion of the PET scanning and in addition to the SWM n-back task used in the scanner. Task-selective effects of TPD would inform on the nature of putative SWM impairments following TPD, and as such, create alternative hypotheses. For example, a general effect of TPD on the mnemonic aspects of SWM would predict impaired performance on all tasks. However, a greater influence of TPD on tasks requiring internal response preparation
and directionally guided fine-motor movement would predict greater impairment on the delayed-recall task, whereas a greater influence of TPD on tasks requiring the “matching” of the internal stimulus to a new external stimulus, with less focus on response preparation and execution, would predict greater impairments on the delayed-recognition task.

5.2 METHOD

5.2.1 Participants
Eleven healthy right-handed male participants [mean age = 48.27 ± SD = 11.16; mean verbal IQ = 121.01 ± 3.21 (NART - Nelson and Willison, 1991)] were recruited for the experiment through advertisements in the national press. Medical and psychiatric suitability to participate in the experiment was ascertained following an initial screening by telephone, general screening questionnaire (see Appendix 1) and administration of the Structured Clinical Interview for the DSM-IV (SCID - First et al., 2002). In addition, all participants were thoroughly screened by a trained psychiatrist, with history of psychiatric, neurological or chronic medical conditions (including cardiovascular illness), drug/medication use or substance abuse comprising exclusion criteria. Ten of the eleven participants were non-smokers, with the one smoker abstaining from his normal one cigarette per day for 48 hours prior to each day of testing. All participants gave written informed consent to participate in the experiment, which was approved by the Hammersmith, Queen Charlotte’s and Chelsea and Acton Hospitals Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee (UK).

5.2.2 Design
The experiment followed a double-blind, balanced drink (placebo) controlled, repeated measures crossover design. Each participant completed two scanning sessions, separated by a minimum 5-day washout period. Six of the eleven participants received TPD in their first session. One participant failed to successfully complete the control task during the first scanning session, and hence was removed from PET analysis and accompanying n-back behavioural data analysis. Therefore, the PET component of the experiment includes 10 participants, and the post-scan behavioural testing (delayed-response tasks) includes 11 participants.
5.2.3 Procedure

Participants arrived at the laboratory at approximately 1030 hours on the morning of each testing session, having consumed a low protein diet (less than 25g) in the preceding 24 hrs and having fasted from 1900 hrs the previous evening. On arrival, the medical physician screened participants, and if deemed suitable for the experiment an intravenous cannula was inserted in the antecubital vein in the left arm. At approximately 1200 hrs (time 0), participants were administered an amino acid drink (balanced drink comprised isoleucine 15g; leucine 22.5g; lysine 17.5g; methionine 5g; valine 17.5g; threonine 10g; tryptophan 2.5g; tyrosine 12.5g; and phenylalanine 12.5 g. TPD amino acid drink was identical to the balanced drink but lacked tyrosine and phenylalanine). Drinks were prepared within a few minutes of oral administration by suspending in water flavoured with blackcurrant. The order of drink administration for the two scans was counterbalanced. Acquisition of PET emission scans occurred +5 hrs after the amino acid mixture, to coincide with peak behavioural and physiological effects of the drink (Harmer et al., 2001, Harrison et al., 2004). Post-scan behavioural testing (delayed-response tasks) took place outside the scanner at time +6 hrs. The testing day was complete at time +6.5 hrs and participants were provided with a high protein snack before departing.

Subjective feelings and side effects were monitored during the testing sessions. Visual analogue scales (Bond and Lader, 1974) were administered at baseline (pre-drink) and at + 5 hrs (post-drink/pre-scan). A carrot was also provided to participants at +2.5 hrs post-drink to reduce hunger. Participants were provided with a standard practice session on all tasks (described below) at approximately +1 hr on both testing days.

5.2.4 PET Scans

All participants were scanned on two separate occasions. Each scanning session resulted in eight measures of rCBF, obtained using the Siemens/CTI ECAT EXACT 3D camera with the H215O bolus method (Raichle et al., 1983). The camera has a full-width half-maximum resolution (FWHM) of 4.5 x 4.5 x 4.42 mm, and a 23.4cm axial view of the field (Spinks et al., 2000). Participants lay supine in the scanner with their head secured by a foam-lined fibreglass head-holder, and strap over the forehead. Head position was monitored inter-scan, by comparing pen lines drawn on the nose and cheek with fixed laser guide lights, and participants were returned to original
position between scans if required. For each scan, approximately 6mCi H$_2^{15}$O was infused in saline for 20 seconds at a rate of 10ml/min, using the intravenous cannula in the left arm. A 30 second background frame was then acquired prior to the 90 second emission scan. There was an 8 minute inter-scan interval to allow for decay of the radiotracer and set-up of the next cognitive task. A transmission scan was collected prior to emission scans to allow correction for attenuation effects, and scans were reconstructed using a ramp filter.

5.2.5 Cognitive tasks

SWM n-back task

The n-back task is a measure of SWM with a sustained attention component (Gevins and Cutillo, 1993, McEvoy et al., 1998). This task was run on a Toshiba computer and presented on an Illyana touch screen monitor driven by a Microtouch™ touchscreen driver suspended above the PET camera. The task involved the presentation of a series of white dots on a black background (with a central white fixation cross). Each dot was presented for 250ms seconds, with inter-stimulus intervals of 2250ms. Participants were required to indicate whether each dot was in the same location as the dot “n-back” (either 1- or 2-back), by pressing the “yes” or “no” button on the touch screen. Each n-back level had the same proportion of “matching” responses, “false alarms”, and non-matches. Specifically, 60% response pairs were “matches” of the relevant n-back, 10% were “false alarm” incorrect matches (i.e. 2-back in a 1-back task), and 30% were non-matches. The visuo-spatial control task involved an equivalent task presentation (50% of dots were an n-back match, distributed amongst 1-, and 2-back), and involved participants alternating responses between the left and right response button. Alternate versions of each n-back task were constructed and randomly administered between treatment and balanced conditions.

For each scanning session, participants completed 3 x 1-back, 3 x 2-back and 2 x control tasks whilst rCBF was measured. In total, this corresponded to 120 responses elicited for the 1-back and 2-back task, and 80 responses for the control task (i.e. 40 responses per scan). Tasks were presented in a pseudorandom order that was fixed within-subjects, but varied between-subjects.
**Post scan neuropsychological testing**

Following scan acquisition, participants completed the following tasks whilst sitting upright in a semi-darkened room at arms length from a touch screen monitor. The two delayed-response tasks were completed first (counter-balanced across participants) followed by the reaction time task. The battery lasted approximately 20 minutes.

Both delayed-response tasks (recall and recognition) were run on a Dell computer connected to a Microtouch™ touchscreen and involved the presentation of black dots (displayed for 250ms) on a white screen with a black central fixation cross. Performance changes following dopamine manipulation have been observed following an 8 second delay in delayed-recall tasks (Luciana et al., 1992, Luciana and Collins, 1997, Mehta et al., 2004), and performance changes in delayed-recognition have been observed at delays of greater than 8 seconds (Muller et al., 1998). Therefore, both tasks included two inter-stimulus interval lengths: a “shorter” 4000ms delay and a “longer” 12000ms delay.

For each delayed-response task, two blocks of trials were presented, each comprising 9 trials for each delay length (in pseudo-random order – 18 trials per delayed-response task in total). Participants were instructed to focus on the central fixation cross throughout all trials, and try to remember the location of the dot during the delay.

**SWM delayed-recall task**

This task is an adaptation of the classical delayed-response task used in both experimental animal (see Goldman-Rakic, 1999) and human research (e.g. Luciana et al., 1992, Mehta et al., 2004). Three delay lengths were employed: “zero delay” control, 4000ms and 12000ms. A beep sounded at the completion of each delay, which signalled the participant to touch the screen as closely as possible to the position of the previous dot. Accuracy (distance from position touched to centre of target circle in millimeters) and response latency were recorded.

**SWM delayed-recognition task**

This task is a measure of SWM recognition with minimal motor preparation and output and is a modification of the delayed-response tasks used in human research (e.g. Sahakian et al., 1988, Muller et al., 1998, Harmer et al., 2001, Postle et al.,
Two delay lengths were employed: 4000ms and 12000ms. A beep sounded at the completion of each delay, and two dots (probes) were presented in close proximity to each other. Participants were required to indicate which of the probe dots matched the original dot, by pressing one of two buttons at the bottom of the screen.

**Reaction time**

This task was a modified version of the CANTAB reaction time task (CeNeS Ltd). Participants rested their right hand on a pressure sensitive pad in front of a touch screen monitor. In the centre of the monitor there was an empty circle and participants were instructed that on the appearance of a yellow dot within this circle, they should attempt to touch this yellow dot as quickly as possible. An initial practice session was followed by two sets of 10 responses. Both reaction time (releasing the touchpad) and movement time (touchpad to screen) were recorded in milliseconds for all correct responses (i.e. touching within circle).

**Subjective Feeling Assessment**

Subjective feelings were obtained using a modified version of the Visual Analogue Scales (Bond and Lader, 1974) comprising sixteen 100mm horizontal lines each representing a subjective feeling dimension, with opposing words at each end, e.g. happy – sad, alert – drowsy, amicable – antagonistic. The visual analogue scales (VAS) were scored as two factors (alertness and tranquillity) consistent with the factor analysis of Herbert et al. (1976).

**Statistical Analysis**

All imaging data was analysed using SPM2 (www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB version 5 (Mathworks, Sherborn, MA, USA). Each scan was realigned using the mean image of the relevant session as reference, and stereotaxically normalised into a standard anatomical space developed at the Montreal Neurological Institute (MNI 152) by using a reference template provided in SPM2. Each image was smoothed using an isotropic Gaussian kernel (FWHM 12 mm) to compensate for gyral variability between participants and improve signal-to-noise ratio. All images were rescaled to a mean global CBF of 100 ml/100g/min using ANCOVA. The condition, subject, and covariate effects were estimated according to the general linear
model at each voxel (Friston et al., 1995). Scan order was entered as a covariate of no interest to control for possible effects of time or order.

 Appropriately weighted linear contrasts were used to identify changes in rCBF on a voxelwise basis (Friston et al., 1995). The set of voxel values resulting from each contrast constitutes a statistical parametric map of the $t$ statistic. As the working memory networks have previously been well defined within the literature (see Owen et al., 2005), linear contrasts of task related effects are reported at $p<0.001$ uncorrected for multiple comparisons. In order to reduce the likelihood of type 1 error, results of contrasts examining the main effect of treatment condition and interaction effects between treatment condition and task were thresholded at $p<0.05$, corrected for multiple comparisons. Small volume correction (SVC) statistics (Worsley et al., 1996) were used to examine a priori predictions of effects within the striatum and the PFC. Specifically, the main and interaction effects of treatment condition within the striatum were examined as TPD-induced rCBF changes in this region were predicted (Mehta et al., 2003); with the striatum defined by Mawlawi (2001). Further, interaction effects between treatment condition and task were examined within the PFC, with PFC defined as cortical regions anterior to the precentral gyrus using a maximum probability atlas (Hammers et al., 2003). Simple linear regression was used to examine possible relationships between changes in task-related rCBF and corresponding change in cognitive performance (between BAL and TPD conditions). The cluster threshold was set to 5 for all figures. Peak voxels within significant clusters are reported using both MNI and Talairach co-ordinates. Talairach coordinates were estimated using MNI2TAL (courtesy of Matthew Brett, www.mrc-cbu.cam.ac.uk/Imaging). Brodmann areas are associated with activated clusters where appropriate as estimated by Talairach and Tournoux (1988).

 All behavioural data was analysed within SPSS (SPSS Inc., Chicago, IL), using repeated measures analysis of variance (ANOVA). The standard error of the difference of the means (SED) (the index of variation commonly used in the crossover design) is reported for all data, as defined by Cochran and Cox (1957): $\text{SED} = \sqrt{(2 \times MSe)/n}$, where $MSe$ is the mean square error (or residual) term and $n$ is the total number of observations made. Standard errors of the means are also reported in parenthesis following means.
5.3 RESULTS

5.3.1 SWM n-back performance

The main performance measures of this task were accuracy (percentage correct) and latency/reaction time, and are presented in Table 5.1. A 2 (treatment condition) by 3 (n-back level) ANOVA revealed that there was no significant interaction between amino acid mixture (TPD or BAL) and n-back level (control, 1-back, 2-back) for either latency [F(1.95,17.58)=0.19, p=0.82, with Greenhouse-Geisser correction] or percentage correct [F(1.32,11.88)=0.11, p=0.81, with Greenhouse-Geisser correction].

One-way repeated measures ANOVAs were conducted to examine the effect of drug on the accuracy and latency of the 1- and 2-back tasks individually. There was no significant effect of drug on either 1-back accuracy [F(1,9)=0.85, p>0.1] or latency [F(1,9)=0.37,p>0.1]; or 2-back accuracy [F(1,9)=0.39,p>0.1] or latency [F(1,9)=0.10,p>0.1]. As predicted, there were no differences between treatment condition in performance on the control task (accuracy: F=(1,9)=0.26,p>0.1]; latency: [F(1,9)=0.01, p>0.9].

As expected, participants were slower and made more errors with increasing load on the n-back task; accuracy [F(2,18)=12.98, p<0.001] and latency [F[2,18]=34.19, p<0.001]. Planned contrasts revealed a parametric memory load effect in which performance decreased (indexed by a reduction in accuracy and increase in latency) as memory load increased, between the control task and 1-back (accuracy: [F(1,9)=4.50,p=0.06]; latency [F(1,9)=39.22, p<0.001]) and 1-back and 2-back (accuracy: [F(1,9)=21.00,p<0.001]), latency [F(1,9)=5.38,p<0.5]

The possible influence of baseline working memory performance was examined by separating participants based on their median 2-back score (under BAL conditions) and entering this variable as a between participants factor. This analysis revealed no significant effects for any of the above measures (all p>0.1).

5.3.2 SWM delayed-recall performance

The main performance measures for this task are shown in Table 5.1. As expected, there was a main effect of delay on accuracy, [F(2,20)=15.91, p<0.001], with the distance from response to target increased with increased delay, which was supported
by a planned contrast statistics (“0 second” vs. 4 second [F(1,10)=17.70, p<0.01]; 4 sec vs. 12 sec [F(1,10)=7.54, p<0.05]). However, no interaction between amino acid mixture and delay condition (0, 4, 12 second) was observed for either accuracy [F(2,20)=0.60, p>0.1] or latency [F(2,20)=2.14, p<0.1]. The baseline performance analysis revealed no interaction between baseline working memory level (high or low, as defined above) and change in performance, in either accuracy or latency (both p>0.05).

### 5.3.3 SWM delayed-recognition performance

The main performance measures for this task (percentage accuracy and latency) are shown in Table 5.1. There was a main effect of accuracy, with task difficulty increasing with increased delay period [F(2,20)=15.91, p<0.001], but no interaction between amino acid mixture and delay for either accuracy [F(1,10)=1.79, p=.21] or latency [F(1,10)=1.60, p=.23]. The working memory baseline analysis revealed no significant effects on any measure (all p>0.1).

#### Table 5-1
Mean and standard error (SEM) values for behavioural data: n-back accuracy (percentage correct), delayed-recall accuracy (distance from target in mm), delayed-recognition accuracy (percentage correct), and latency (msec) for all tasks, following TPD and BAL treatment conditions.

<table>
<thead>
<tr>
<th>Task and delay</th>
<th>Accuracy</th>
<th>Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAL</td>
<td>TPD</td>
</tr>
<tr>
<td>N-back task</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control task</td>
<td>98.2 (0.6) 97.8 (1.1) 0.6</td>
<td>768.4 (93.2) 768.2 (99.9) 34.2</td>
</tr>
<tr>
<td>1-back</td>
<td>94.1 (1.9) 93.2 (1.7) 0.7</td>
<td>1278.7 (61.9) 1253.6 (67.7) 29.1</td>
</tr>
<tr>
<td>2-back</td>
<td>87.4 (2.9) 86.1 (2.5) 1.6</td>
<td>1383.5 (56.7) 1346.9 (63.3) 25.9</td>
</tr>
<tr>
<td>Recall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 sec</td>
<td>8.2 (1.0) 9.4 (0.9) 0.4</td>
<td>1249.7 (83.9) 1313.2 (104.8) 32.8</td>
</tr>
<tr>
<td>4 sec</td>
<td>9.8 (1.1) 11.8 (1.0) 0.7</td>
<td>1160.3 (60.7) 1185.4 (68.2) 44.2</td>
</tr>
<tr>
<td>12 sec</td>
<td>12.3 (1.4) 13.2 (1.5) 1.0</td>
<td>1246.0 (57.0) 1200.6 (70.1) 39.5</td>
</tr>
<tr>
<td>Recognition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 sec</td>
<td>90.9 (3.4) 89.4 (5.2) 2.7</td>
<td>2219.6 (75.6) 2204.7 (132.8) 77.6</td>
</tr>
<tr>
<td>12 sec</td>
<td>88.9 (4.7) 92.4 (3.5) 1.5</td>
<td>2172.9 (73.1) 2224.8 (108.1) 70.3</td>
</tr>
</tbody>
</table>
5.3.4 Reaction time/Movement time performance

The data for 2 participants were incomplete, and therefore analysis was performed on 8 participants. One way repeated measures ANOVA was conducted on both reaction time and movement time measures. No significant difference was observed between amino acid drink conditions on either measure [reaction time: $F(1,7)=0.42$, $p>0.1$; movement time: $F(1,7)=0.43$, $p>0.1$] (see Table 5.2 for values).

<table>
<thead>
<tr>
<th>Task</th>
<th>BAL</th>
<th>TPD</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time</td>
<td>300.9 (13.6)</td>
<td>310.5 (20.3)</td>
<td>9.8</td>
</tr>
<tr>
<td>Movement time</td>
<td>411.7 (26.8)</td>
<td>436.7 (39.7)</td>
<td>24.0</td>
</tr>
</tbody>
</table>

5.3.5 Visual analogue scales

TPD did not significantly influence subjective feeling scores. There was no significant interaction between drink condition and time for either alertness [$F(2,20)= 0.95$, $p>0.05$] or tranquillity [$F(2,20)=1.6$, $p>0.05$]. Further, there was no effect of the experiment day itself on subjective feelings, with no main effect of time on alertness [$F(2,20)=0.39$, $p>0.05$] or tranquillity [$F(2,20)=0.21$, $p>0.05$] (see Table 5.3 for values).

<table>
<thead>
<tr>
<th>Task</th>
<th>BAL Pre</th>
<th>BAL Post</th>
<th>TPD Pre</th>
<th>TPD Post</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alertness</td>
<td>73.3 (5.8)</td>
<td>74.1 (5.9)</td>
<td>75.9 (5.3)</td>
<td>72.7 (6.2)</td>
<td>1.7</td>
</tr>
<tr>
<td>Tranquillity</td>
<td>72.9 (2.5)</td>
<td>73.8 (3.4)</td>
<td>70.8 (2.8)</td>
<td>69.6 (3.6)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

5.3.6 Radioactivity levels between treatment condition

Repeated measures analysis of variance was conducted to examine whether radiation levels varied between treatment sessions, and revealed no significant difference in injected level of radioactivity [$F(1,9)=0.52$, $p>0.1$] or recorded head counts [$F(1,9)=1.16$, $p>0.1$] (see Table 5.4 for values).
Table 5-4  Mean and standard error (SEM) of injected radioactivity and head counts (average for all scans in each session).

<table>
<thead>
<tr>
<th>Injected radioactivity</th>
<th>Recorded head counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPD</td>
</tr>
<tr>
<td>Injected radioactivity</td>
<td>6.3 (0.2)</td>
</tr>
</tbody>
</table>

5.3.7 Task-related activations

The effects of the SWM task on rCBF were examined in the BAL condition alone (to avoid confounding task activations with the effects TPD) using a multisubject condition and covariates PET model in SPM2. Glass brain images for the SWM n-back task activations (2+1 back, minus control task) are presented in Figure 5.1a, with details of the associated maxima presented in Table 5.5. As expected, the task activated a distributed network including the right middle frontal gyrus (BA 9/46), the left supplementary motor cortex (BA 6), both superior and inferior regions of the parietal cortices, bilaterally (BA 7/40), and the cingulate gyrus, in line with previous neuroimaging studies and a recent meta-analysis of the n-back task (Cohen et al., 1997, Jansma et al., 2000, Zurowski et al., 2002, Owen et al., 2005).

5.3.8 Memory load effects

Table 5.5 presents maxima of memory load effects, with glass brain images presented in Figure 5.1b. As expected, a number of regions within the working memory network (as defined using the task-related activation identified above) were sensitive to memory load. Load-related increases in activation were located within the PFC (BA 9/46, right hemisphere; BA 6, left hemisphere) and bilaterally within the parietal cortex (BA 7). Analysis of possible load effects over the whole brain revealed an additional and dominant load-related effect in the rostral frontal cortex (BA 10/11).
Table 5-5  Peak increases in rCBF during the SWM n-back task (1- and 2- back tasks), and memory load related rCBF increases for the BAL condition scans.

<table>
<thead>
<tr>
<th>Region*</th>
<th>MNI coordinates</th>
<th>Talairach Coordinates</th>
<th>Cluster Size</th>
<th>Peak t value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Task activation (1&amp;2 back)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R DLPFC (9/46)</td>
<td>50,34,26/ 52,16,32</td>
<td>50,34,22/ 52,17,29</td>
<td>1425</td>
<td>5.36/4.63</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>L MFG (6)</td>
<td>-34,2,54</td>
<td>-34, 4,50</td>
<td>240</td>
<td>4.51</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>L Inferior Parietal lobe (7/40)b</td>
<td>-32, -56,44</td>
<td>-32,-52,43</td>
<td>912</td>
<td>4.81</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Superior Parietal lobe (7)c</td>
<td>42,-62,54</td>
<td>42,-58,52</td>
<td>2611</td>
<td>4.53</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Cingulate Gyrus (32)</td>
<td>12,20,44</td>
<td>12,21,39</td>
<td>98</td>
<td>3.89</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>14,-92,18</td>
<td>14,-88,21</td>
<td>186</td>
<td>3.72</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Memory load (2back – 1back)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior PFC (10/11)</td>
<td>-44,50,-10/ -36,56,12</td>
<td>-44,48,-11/ -36,55,8</td>
<td>387</td>
<td>4.87</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>L MFG (6)</td>
<td>-32,6,52</td>
<td>-32, 8,47</td>
<td>23</td>
<td>3.48</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>L Inferior Parietal lobe (7)</td>
<td>-36,-64,44</td>
<td>-36,-60,43</td>
<td>14</td>
<td>3.51</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>R Superior Parietal lobe (7)</td>
<td>40,-76,44</td>
<td>40,-72,44</td>
<td>31</td>
<td>3.34</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>R DLPFC (9/46)</td>
<td>56,28,26</td>
<td>55,28,22.5</td>
<td>64</td>
<td>3.47</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

* Brodmann area given for regions in parenthesis  
  b This cluster extend posteriorly into the occipital lobe with significant sub-peaks not tabulated  
  c This cluster extends medially encompassing other significant sub peak not tabulated.  
  * denotes clusters that remain significant after correction for multiple comparisons across the whole brain.
5.3.9 Main effect of TPD on rCBF

The main effects of TPD on rCBF were examined by averaging across all tasks (to avoid confounding the main effects of TPD with task specific activations). Table 5.6 shows maxima of rCBF increases and decreases following TPD, with glass brain images presented in Figure 5.2. Small volume correction (SVC) analysis (based on the results of Mehta et al., 2003), using an MRI derived striatal region of interest map (Mawlawi et al., 2001), revealed TPD related increases in rCBF in the left putamen. Further examination across the whole brain showed signal increases in the hippocampal region, bilaterally (see Figure 5.3), and the left inferior and superior frontal gyri. TPD-related reductions in rCBF were observed in the right inferior temporal gyrus, anterior cerebellar lobe (culmen) and pons.

5.3.10 Interaction effects between treatment condition and task

TPD caused no augmentation or attenuation of the SWM-related activity within the PFC or striatum (using SVC analysis). Small volume correction analysis was also performed within the SWM network (using a mask of SWM networks created from the task-related activation identified above) and no significant signal changes were detected. In addition, no significant signal changes
were detected in areas outside the SWM network (even after exploring subthreshold changes using a statistical threshold of p<0.001 uncorrected for multiple comparisons across the whole brain).

Table 5-6  Peak changes in rCBF following TPD

<table>
<thead>
<tr>
<th>Region</th>
<th>MNI coordinates</th>
<th>Talairach Coordinates</th>
<th>Cluster Size</th>
<th>Peak t value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rCBF Increases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Putamen</td>
<td>-28,-14,0/</td>
<td>-28,-14,1/</td>
<td>170</td>
<td>9.62/9.13</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>-30,-14,-4</td>
<td>-30,-14,-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L Hippocampal region/</td>
<td>-24,-26,-6</td>
<td>-24,-25,-4</td>
<td>13758</td>
<td>13.9/10.89</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>R Parahippocampal Gyrus</td>
<td>16,-28,-12</td>
<td>-16,-28,-9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L Inferior frontal gyrus (44)</td>
<td>-54,14,10</td>
<td>-53,14,9</td>
<td>9745</td>
<td>9.75</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td><strong>rCBF Decreases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Inferior temporal gyrus (20)</td>
<td>42,-18,-34</td>
<td>42,-16,-28</td>
<td>2333</td>
<td>10.4</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Left Cerebellum/Culmen</td>
<td>-28,-56,-36</td>
<td>-28,-56,-27</td>
<td>729</td>
<td>8.26</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Pons</td>
<td>-4,-22,-32</td>
<td>-4,-23,-26</td>
<td>274</td>
<td>7.58</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

\[ a \] Brodmann area given for regions in parenthesis

5.3.11 Correlation between changes in task-related rCBF activation and cognitive performance following TPD

Possible relationships between changes in task-related rCBF and corresponding change in cognitive performance (between BAL and TPD conditions) were examined for both the 1-back and 2-back condition using simple linear regression. There were no significant correlations between rCBF changes and either performance latency and performance accuracy changes in either the 1-back or 2-back condition.
Figure 5-2  Glass-brain activation maps for main effect of TPD on rCBF: (a) increases following TPD (compared to BAL), (b) decreases following TPD. Both displayed at a threshold of $p<0.05$ (corrected for multiple comparisons).

Figure 5-3  Transverse sections (at $z = -8\text{mm}$, $-6\text{mm}$, $-4\text{mm}$) showing TPD-related increases in the area of the parahippocampus. Coloured bar shows $t$ values.

5.3.12 Additional analysis to address methodological considerations
The SPM analysis methodology used in this experiment was consistent with previous analysis performed within the Cyclotron Unit, Hammersmith Hospital, including a recent examination of the effects of sulpiride (a $D_2$ receptor antagonist) on n-back task performance (Mehta et al. 2003). However, as this was the first experiment to
examine the effects of TPD on rCBF, a series of additional analyses were conducted to assess the reliability of the results. These analyses examined:

1) Whether the results could be replicated using a design matrix that considered the TPD and BAL groups as separate (to examine whether using a “repeated measures” design biased results).

2) Whether the results remained consistent when each participant was systematically removed from the analyses (to examine whether the activations of one participant was over represented in the SPM \( t \) statistics map).

3) Whether the results remained consistent when the main effect of TPD was examined separately for each n-back level (to examine whether task-related activations confounded the drug main effect).

These findings demonstrated that the results remained highly consistent whether the TPD and BAL group were considered as “repeated measures” (and hence normalisation of blood flow was conducted considering both treatments as part of the same “session”), or whether the groups were normalised separately and compared as between groups (see Appendix 3 for examples of contrasts generated using different analyses). Second, these analyses demonstrated that no participant was over represented in results, as SPM \( t \) statistics remained highly consistent following systematically removed of each subject from the analyses (see Appendix 3). Finally, the results remained consistent when the main effect of TPD was examined separately for each n-back level – these results demonstrated a similar pattern as the main effect examined overall all task conditions (see Appendix 3 for SPM maps for each task level). These results demonstrated a reliability of the findings over different analysis designs.

5.3.13 Plasma amino acid levels
Consistent with Chapter 4, blood samples were collected at baseline, and approximately 10 minutes prior to testing (PET scans). These samples were sent for analysis to a reputable laboratory which had previously conducted similar analysis for the PET Psychiatry Unit (e.g. TPD plasma data reported in Montgomery et al., 2003, Mehta et al., 2005a). In January 2005 it was confirmed that this laboratory had accidentally disposed of the blood data (and other data not pertaining to this experiment) without analysis. It should be noted, however that all studies within the
literature report robust significant depletion of tyrosine and phenylalanine levels (or the important ratio between tyrosine/phenylalanine and other large neutral amino acids) following TPD condition compared to balanced, in the order of p<0.001 (Leyton et al., 2000, Harrison et al., 2004, Leyton et al., 2004a, Leyton et al., 2004b, McLean et al., 2004, McTavish et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Mehta et al., 2005a, Roiser et al., 2005). Further, the TPD procedure followed in Experiment 2 was identical to the procedure followed by another study conducted in the same laboratory just prior (Mehta et al., 2005a), which reported significant tyrosine depletion (over 90%). Also plasma changes have recently been found to be poor predictors of performance change (see discussion) (Mehta et al. 2005).

5.4 DISCUSSION

This was the first experiment to examine the effects of TPD on rCBF in humans. TPD was observed to produce clear and marked changes in rCBF in both cortical and limbic regions, including changes within the striatum - in line with evidence that TPD decreases subcortical dopamine transmission in humans (Montgomery et al., 2003, Leyton et al., 2004b). However the main finding of this experiment is that despite these task-independent effects of TPD on blood flow, there was no evidence of task-dependent modulations of rCBF following TPD during the SWM n-back task. In addition, TPD did not influence performance on either the n-back task, or two additional delayed-response tasks of SWM with varying response preparation and execution demands.

5.4.1 Task-related activation

These results do not support TPD-induced modulations of task-related rCBF during the SWM n-back task. These findings appear to contrast evidence of a modulatory effect of dopamine within the PFC during SWM tasks in non-human primates (for review, see Goldman-Rakic et al., 1996), and evidence in humans that increased dopamine receptor stimulation can modulate indices of working memory related neuronal activity in the parietal cortex (Kimberg et al., 2001). The current results are also inconsistent with psychomotor stimulant (indirect catecholaminergic agonist) induced changes in task-related PFC activity (Mattay et al., 2000, Mehta et al., 2000, Schweitzer et al., 2004), although these effects may be at least partly due to
noradrenergic actions. However, the current findings add to the studies that have failed to demonstrate dopamine D2 receptor agonist (Kimberg et al., 2001) and antagonist (Mehta et al., 2001) modulation of the PFC during working memory performance. Taken together with the current findings, these studies highlight the difficulty of clearly elucidating the influence of dopaminergic modulation of working memory-related neural networks in humans that is clearly predicted on the basis of work in experimental animals. The lack of TPD-related modulation of SWM neural networks was not the result of limited task-related activations, as this experiment again showed the SWM n-back task activates a robustly-defined network of brain areas including the PFC (Owen et al., 2005). This network was sensitive to memory load, with increased memory load associated with increased DLPFC and posterior parietal cortex activation, consistent with previous findings (e.g. Braver et al., 1997, Cohen et al., 1997). Additional memory load related increases were observed within the anterior PFC (BA 10), which fits well within a developing understanding of the role of the anterior PFC (for a review, see Ramnani and Owen, 2004), specifically in terms of a possible role in sub-goals within dual tasks (Koechlin et al., 1999) and in integration of sub-goals within working memory (Braver and Bongiolatti, 2002).

The lack of modulation of task-related rCBF was in the context of clear changes in rCBF following TPD. This included striatal blood flow modulation (within the putamen) consistent with previous findings of TPD-related decreases in striatal dopamine levels (Montgomery et al., 2003). However, it appears that the extent of dopamine depletion may have been insufficient to modulate SWM networks. Indeed, TPD produces less dopamine depletion within the striatum than AMPT (see Verhoeff et al., 2002, Verhoeff et al., 2003). Following TPD, changes in [11C]raclopride binding (indexing striatal dopamine depletion) are around 6% (Montgomery et al., 2003), whereas following AMPT (in the non-human primate), changes in SPECT radiotracer [123I]IBZM binding are approximately 25% (Laruelle et al., 1997a). Further, Mehta et al. (2005a) recently demonstrated, using [11C]raclopride PET, that only participants with a high level of dopamine depletion showed impaired performance following TPD. In contrast, Mehta et al. (2005a) demonstrated that there was no relationship between peripheral plasma measurement of tyrosine depletion and performance. The current experiment used an identical TPD protocol to Mehta et al. (2005a), but found no evidence of a relationship between task-related rCBF changes.
and performance changes following TPD across the whole brain. Thus, while changes in dopamine levels may predict changes in performance, there are no brain regions where rCBF changes confer a similar predictive value. The reasons for this are currently unclear, although it may relate to the lower selectivity of rCBF measures compared to [11C]raclopride imaging for dopamine-related changes, or a poor temporal specificity for potential effects that may be present within discrete subcomponents of the task.

5.4.2 Behavioural effects
The current experiment examined whether differences in task demands may be a source of discrepant behavioural findings (for discussion, see Luciana and Collins, 1997, Mehta et al., 2001, Mehta et al., 2003), and these findings suggest that this is unlikely, at least for dopamine depletion following TPD. There was no evidence to suggest that TPD influenced performance on two versions of the delayed-response task (recall and recognition) differing in the ability of participants to prepare a response during the delay, as well as the n-back task which is a complex task involving information manipulation and updating, and sustained attention. TPD-induced impairments of delayed-recognition performance have been shown previously (Harmer et al., 2001, Harrison et al., 2004). While the current findings are at odds with these early studies, they are consistent with a number of more recent findings that suggest that, overall, TPD does not have a measurable effect on working memory behavioural performance measures (McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Mehta et al., 2005a). It is unlikely that the lack of behavioural effect across all tasks in this experiment was due to insensitivity of these tasks to dopaminergic manipulations, as they are arguably the most commonly used SWM task types, and performance has been previously linked to dopaminergic levels and/or modulation in all three tasks (e.g. Luciana et al., 1992, Park and Holzman, 1992, Luciana and Collins, 1997, Muller et al., 1998, Meyer-Lindenberg et al., 2001, Abi-Dargham et al., 2002, Mehta et al., 2004). While the lack of behavioural effect may be due to under-powering of the experiment, the current sample size (N=11) is comparable to both Harmer et al. (2001) (N=12) and Harrison et al. (2004) (N=13) in which TPD-related performance effects were observed. Therefore, the lack of effects of TPD on performance of three working memory tasks appears to be in line with the
overall lack of effect on task-related rCBF across the entire brain during performance of the n-back task.

5.4.3 Main effect of TPD on rCBF

Despite failing to modulate task-related rCBF or performance, TPD induced widespread increases in rCBF, with maximal increases in the region of the parahippocampal gyrus (bilaterally) and the left inferior frontal gyrus. While the mechanisms responsible for these finding are unclear, they are likely to comprise a combination of effects, which include, but are not exclusively, dopamine-related. Firstly, rCBF or blood oxygen dependent (BOLD) signal changes following pharmacological manipulation rely on the assumption that coupling between blood flow and neuronal activity remains relatively constant across all brain regions. Despite the dopaminergic system being the most extensively studied on this matter and evidence suggesting no significant neurovascular (NV) uncoupling following dopamine modulation (for example, McCulloch, 1982, McCulloch et al., 1982, Arthurs and Boniface, 2002), the effects of TPD on NV coupling are unknown. Large neutral amino acids (LNAA) methionine, arginine, and homocysteine (which is converted to cysteine) can alter peripheral vasculature reactivity (Bellamy et al., 1998, Frame, 1999, Usui et al., 1999, Chambers et al., 2001, Rosengarten et al., 2003), and although cerebral vasculature has greater compensatory range than peripheral vasculature in minimising vascular decoupling (Rosengarten et al., 2003), TPD results in increased levels of LNAA and it remains possible that NV uncoupling contributed to the observed main effects of TPD on measured rCBF.

Notwithstanding these considerations, the pattern of TPD-related rCBF increases observed in the current experiment overlap with increases in glucose metabolism observed following AMPT (in 7 participants who did not experience relapse of depression) (Bremner et al., 2003). It is suggested that these increases (in rCBF or glucose metabolism) may be partly due to compensatory mechanisms. In rodents, loss of striatal dopaminergic terminals is accompanied by apparent increases in dopamine synthesis and release from remaining dopamine terminals, and increased activity of the rate-limiting enzyme tyrosine hydroxylase (TH) (Zigmond et al., 1984). Similarly, loss of noradrenergic terminals can results in increased TH activity specifically within the hippocampus, as a compensatory mechanism to maintain catecholaminergic
influence on target cells within the brain (Acheson et al., 1980, Acheson and Zigmond, 1981). Although these manipulations are more severe than that performed in the current experiment, the data fit well with the present findings of maximal rCBF increases in the hippocampal region, possibly reflecting similar compensatory increases in TH in order to promote dopamine/noradrenaline synthesis and release under TPD conditions. TPD may also influence interacting neurotransmitter systems. For example, anatomical and pharmacological data suggest an apparent opponent partnership between dopamine and serotonin (e.g. Azmitia and Segal, 1978, Fletcher, 1991, Kapur and Remington, 1996). Further, infusion of the serotonin precursor L-tryptophan reduces the ratio of tyrosine to large neutral amino acids (Heuther et al., 1992), biochemical markers of dopamine synthesis (Hashiguti et al., 1993), and ‘dopaminergic’ behavioural responses (i.e. increased locomotor activity) (Molina et al., 2001). It is therefore possible that dopamine depletion may similarly initiate increases in serotonergic transmission. Overall, it is suggested that the widespread rCBF increases following TPD demonstrate that globally modulating tyrosine/phenylalanine levels has a complex effect on neurophysiology, which is likely to reflect not only changes in dopamine levels but compensatory actions of the catecholaminergic systems, and interactions with other neurotransmitter systems.

5.4.4 Limitations of the experiment

The unfortunate loss of plasma amino acid data during analysis makes it impossible to conclusively state that the TPD protocol in this experiment depleted tyrosine levels. However, the TPD protocol has been demonstrated as a highly reliable method of depleting plasma tyrosine levels. Indeed all studies within the literature report robust significant depletion of tyrosine and phenylalanine levels (or the important ratio between tyrosine/phenylalanine and other large neutral amino acids) in the TPD condition compared to balanced in the order of p<0.001 (Leyton et al., 2000, Harrison et al., 2004, Leyton et al., 2004a, Leyton et al., 2004b, McLean et al., 2004, McTavish et al., 2004, Roiser et al., 2004, Lythe et al., 2005, McTavish et al., 2005, Mehta et al., 2005a, Roiser et al., 2005). In all experiments within this thesis, participants were contacted by phone to encourage compliance with the pre-experiment protocol. In addition, the TPD procedure followed in the current experiment was identical to the procedure followed by another study conducted in the same laboratory just prior (Mehta et al., 2005a). The Mehta et al. (2005a) study was conducted within the same
laboratory (PET Psychiatry Unit, Hammersmith hospital), used the same amino acid stock and measuring scales, and produced significant tyrosine depletion (over 90%). Therefore, taken within the context of: 1) consistent plasma depletion levels reported in all previous published studies (including significant depletion findings reported in the previous experiment; Chapter 4), 2) evidence in the present experiment that TPD resulted in a significant and widespread change in neurophysiology (as indexed by a “main effect” of TPD on rCBF), and 3) anecdotal evidence (based on informal survey performed by the author) that participants complied with the pre-experimental protocol, the most likely conclusion is that TPD caused significant depletion of plasma tyrosine levels.

In addition it should also be noted that while studies indicate TPD as highly effective in depleting plasma tyrosine levels, recent evidence suggest that plasma changes in tyrosine are not good predictors of cognitive function. Mehta et al. (2005) reported that while plasma depletion levels (i.e. plasma tyrosine and phenylalanine levels) were not related to performance changes, a correlation between central dopamine depletion (as indexed by striatal [11C]raclopride binding changes) and performance change was observed. Specifically, impairments in performance were only observed for participants with a high central dopamine depletion level, but virtually no change (and/or subtle improvement) was observed in participants with minimal dopamine depletion levels. This suggests that while plasma tyrosine levels are not good measure of changes in performance, it is probable that variation in central dopamine depletion accounted at least in part, for the lack of working memory impairments.

An additional methodological consideration is that the delayed-response tasks were conducted at 6 hours post-TPD, in contrast to the 5 hours post-TPD testing time used in previous studies (e.g. Harmer et al., 2001, Harrison et al., 2004). However, prolactin responses measured by Harmer et al. (2001) suggest no change in dopamine function between 5 and 6 hours post-TPD, and it is therefore unlikely that dopamine depletion was not at a comparable level when the delayed-response tasks were administered.
5.4.5 Conclusions

The main finding of this experiment is that despite clear task-related activations, and considerable changes in rCBF following TPD, there was no evidence of TPD-related modulation of task-related activations or SWM performance. Further, the lack of a measurable effect of TPD on SWM delayed-recall, SWM delayed-recognition and SWM n-back performance suggests that it is unlikely that task differences underlie the mixture of effects observed in previous studies to date. The implication of these findings, when taken together with previous studies, is that the degree of dopaminergic depletion achieved with TPD may be insufficient to consistently and robustly modulate SWM networks in humans.

However, the temporal resolution of PET limits these results to assessing changes over the entire working memory process, which comprises a number of sub-processes (as outlined in Chapter 1). As PET cannot detect sub-second changes in activity, it remains possible that subtle changes during the different temporal stages of the n-back process may have been undetected in this experiment. Therefore, the following experiments examined the temporal dynamics of the n-back task using a high temporal resolution ERP technique (SSPT; Chapter 6), in order to examine how the temporal characteristics of the n-back task may be altered by dopaminergic modulation (Chapter 7).
Chapter Six

6 Experiment 3: Examining the temporal aspects of the SWM n-back task using SSVEP.

6.1 INTRODUCTION

Neuroimaging studies have been useful in uncovering the neural networks associated with working memory. As outlined in Chapter 1, single cell recordings in non-human primates have identified individual neurons within the DLPFC which show elevated persistent and tuned activity (so called “memory fields”) during SWM delayed-response tasks (for a review, see Goldman-Rakic, 1996). Similarly in humans, neuroimaging studies have revealed activation of the lateral PFC in addition to more posterior brain regions during the delayed-response task (for reviews, see Courtney et al., 1998, Wager and Smith, 2003).

There is now considerable literature which has examined the neural networks associated with the n-back task. Studies using PET and fMRI have provided evidence that the SWM n-back task activates a distributed network of regions, including the DLPFC, posterior parietal cortex and anterior cingulate (Owen et al., 2005). Evidence has further demonstrated that working memory load results in increased activation generally within the working memory networks (Braver et al., 1997, Cohen et al., 1997).

Neuroimaging studies have also attempted delineate the neural networks associated with sub-processes of working memory. For example, fMRI studies have been able to separate the encoding, maintenance and response periods of the delayed-response task, demonstrating that the PFC and a number of posterior brain regions are activated during all three periods albeit to different degrees (i.e. Courtney et al., 1998, Haxby et al., 2000). Similar examination of the n-back task is more complex as there is no clear dissociation between different sub-processes of the task, in addition to the fact that the n-back task requires global task-related processes due to its continuous nature (such as
sustained attention and retention of goals). Nevertheless, studies have provided evidence to suggest that the DLPFC is more likely recruited by “executive functions” involved in complex working memory tasks (i.e. the n-back), with the VLPFC more important for simple storage tasks (for reviews, see Courtney et al., 1998, D’Esposito et al., 1998, Owen et al., 1999, Owen, 2000, Wager and Smith, 2003).

While fMRI and PET studies have given insight into the possible differentiation of working memory sub-processes, particularly in the PFC, they are limited by their temporal resolution to detect dynamic sub-second changes in neurophysiology. Electrophysiological recording remains the highest temporal resolution neuroimaging technique. The SSVEP, elicited by a task-irrelevant 13Hz visual flicker, is a particularly useful evoked potential technique for studying cognitive processes. The SSVEP has high temporal resolution (in the order of hundreds of milliseconds), enhanced signal to noise ratio above that of standard EEG techniques, and has previously demonstrated reliable and specific topographic changes during cognitive tasks (i.e. Silberstein et al., 1990, Silberstein et al., 1995, Silberstein et al., 1998, Silberstein et al., 2000a, Silberstein et al., 2000b, Silberstein et al., 2001, Kemp et al., 2002, Gray et al., 2003, Perlstein et al., 2003, Silberstein et al., 2003, Kemp et al., 2004). SSVEP signals are characterised by changes in amplitude and phase (latency) components which are reflective of the neuronal activity within pyramidal cells of the neocortex (Silberstein et al., 1995, Silberstein et al., 2001). The amplitude of SSVEP is a function of the number of pyramidal cells firing in synchrony with the visual 13Hz flicker. SSVEP phase reflects changes in latency between the SSVEP signal and the 13Hz flicker, and is indicative of the physiological delay between stimulus and response waveforms. These latency changes have been suggested to reflect summed changes in synaptic transmission time related to post-synaptic excitatory or inhibitory processes (Silberstein et al., 1995, Silberstein et al., 2000b). While PET and/or fMRI yield important information related to the location of neural activity via changes in rCBF or BOLD signal (the assumed haemodynamic correlate of neural activity) (see Arthurs and Boniface, 2002), it is unclear whether these changes index increased excitation, inhibition, or both. The ability to observe rapid changes in excitatory and inhibitory processes as measured by SSVEP latency, is a significant advantage of the SSVEP technique, specifically in light of evidence in non-human primates that inhibition of neurons within the frontal cortex is important in establishing the
“memory fields” used to hold information online (for a discussion, see Goldman-Rakic, 1996).

The SSVEP associated with the delayed-response task has been examined by two separate laboratories (with differing SSVEP methodology). These studies demonstrated that the delay of a delayed-response task is associated with increases in SSVEP amplitude within the PFC (Silberstein et al., 2001, Perlstein et al., 2003) and occipital-parietal sites (Silberstein et al., 2001), in addition to a reduction in latency in these same regions (Silberstein et al., 2001). SSVEP data has also been observed to distinguish between the delay and encoding component of a delayed-response task. While the delay period was associated with amplitude increases, early perceptual processes (or “encoding”) were associated with SSVEP amplitude reductions at prefrontal sites (Silberstein et al., 2001). These amplitude reductions appear analogous to the transient reduction in spontaneous alpha activity event-related desynchronisation associated with increased vigilance (Pfurtscheller and Aranibar, 1977, Pfurtscheller and Klimesch, 1990).

To date there has been no study of the temporal characteristics of the SWM n-back task using SSVEP. Therefore, the current experiment used SSVEP to examine the electrophysiological profile of the SWM n-back task, with 3 levels of difficulty (1-, 2- and 3-back version). The first hypothesis was that the n-back task would reveal a different SSVEP profile in the early perceptual/encoding component and the delay component, consistent with previous evidence in the delayed-response task (Silberstein et al., 2001). It was further hypothesised that the delay period would reveal amplitude increases consistent with previous findings in the delayed-response task. However, the main aim of this experiment was to examine the temporal characteristics of the delay period of the SWM n-back task. Finally, it was hypothesised that amplitude and latency changes within the PFC and parietal cortex would show memory load effects, consistent with previous neuroimaging studies (Braver et al., 1997, Cohen et al., 1997).
6.2 METHODS

6.2.1 Participants
Twenty young healthy males (mean age = 22.9 ± 6.4 years) participated in the experiment. All participants were right-handed, as assessed by the Edinburgh Inventory (Oldfield, 1971), non-smokers and medication-free for at least one month prior to testing. Medical and psychiatric screening comprised an initial telephone screening (including administration of the Prime-MD, Pfizer, 1996), a general screening questionnaire (see Appendix 1) and a consequent semi-structured clinical assessment with a physician. Exclusion criteria included history of neurological or psychiatric disorders (including history of depression or anxiety disorders in first degree relatives), chronic physical illness, medication and/or drug use, or excessive alcohol consumption. The human research ethics committee, Swinburne University of Technology approved the experiment. Participants were recruited via advertisements on university notice boards and websites, and all participants gave written informed consent.

6.2.2 Procedure
All participants were asked to refrain from alcohol for 24 hours prior to the experiment. On the day of testing, participants were asked to refrain from consuming caffeine, to consume a small breakfast before 1030 hrs, and arrive at the laboratory at 1230 hrs. All participants were provided a standardised lunch of two slices toast with jam and one small glass of orange juice on arrival, given approximately 1.5 hour prior to testing.

Participants were seated approximately 2.5 metres from a computer monitor in a dimly lit soundproofed room, and were fitted with a lycra electrode cap comprising 64 monopolar leads, positioned according to the international 10/20 system and additional sites located midway between the 10-20 locations (see Figure 6.1). Nose and linked ear electrodes were used for ground, and impedance on all electrodes was generally below 5kΩ. Participants were then fitted with a set of modified half-mirrored goggles, which superimposed the 13Hz white light flicker over the visual field to elicit the SSVEP. The visual flicker subtended a horizontal angle of 160° and a vertical angle of 90°, and had a modulation depth of 45% when viewed against the
background. This enabled participants to view the task and the sinusoidal flicker simultaneously. Recorded brain electrical activity was band pass filtered from 0.74 to 74Hz and digitised at a rate of 500Hz with 16-bit accuracy, consistent with previous studies (e.g. Silberstein et al., 1998, Silberstein et al., 2001, Kemp et al., 2002, Gray et al., 2003, Kemp et al., 2004, Silberstein et al., 2004).

Participants had attended a pre-study training session on a previous occasion to familiarise themselves with the task. On the day of testing, participants completed subsequent practice following electrode cap setup, completing each task twice under SSVEP conditions to familiarise themselves with the flicker. During testing, task n-back order (visuo-motor control, 1, 2 or 3 back) was quasi-randomised. Before each n-back was performed, participants completed a small (1 minute) practice of that n-back to ensure they were completing the correct task. Following this small practice, the subject completed 2 sets of 40 trials per n-back level (i.e. a total of 80 trials per n-back), with a small break in between. This was completed for each n-back (control, 1, 2 or 3 back) and testing was completed within 1 hour.

Figure 6-1 Position of the 64 cortical electrodes used in this experiment.
6.2.3 SWM n-back task

The n-back task is a measure of SWM with a sustained attention component (Gevins and Cutillo, 1993, McEvoy et al., 1998). This version of the n-back task was developed for this experiment using Pipscript software (which provides millisecond accuracy in stimulus presentation and response recording), and has also been administered in other studies (e.g. Green et al., 2005). The task involved the presentation of a series of white dots on a black background (with a central white fixation cross). Each dot was presented for 500ms, with inter-stimulus intervals of 3000ms. Participants were required to indicate whether each dot was in the same location as the dot “n-back” (either 1-back, 2-back or 3-back, depending on task instructions). For each n-back level, 80 responses were elicited. Each n-back level had the same proportion of “matching” responses, “false alarms”, and non-matches. Specifically, 40% response pairs were “matches” of the relevant n-back, 10% were “false alarm” incorrect matches (i.e. 2-back in a 1-back task), and 50% were non-matches. The visuo-spatial control task involved an equivalent task presentation (50% of dots were an n-back match, distributed amongst 1-, 2- and 3-back), and involved participants alternating responses between the left and right response button.

6.2.4 Steady state probe topography (SSPT) signal processing

Fourier analysis was employed to extract the SSVEP from the brain electrical data for each electrode by calculating the 13Hz Fourier coefficients (FC) for each stimulus cycle. The FC time series was smoothed by averaging overlapping blocks of 10 FCs. Each electrode within each task was checked individually for artefact, as described previously (see Silberstein et al., 1995, and also Chapter 3, Section 3.5.1). Previous research has demonstrated the SSVEP to be resistant to electromyographic (EMG) noise contamination (e.g. Gray et al., 2003). Extensive details of the principal features of SSPT signal processing are outlined by Silberstein and colleagues (1990, 1995).

6.2.5 SSVEP data analysis

Epochs of 3.5 seconds (1 epoch = 1 trial = stimulus display + delay) were extracted from the SSVEP for each task. The amplitude and phase (inverse of latency) of the SSVEP were normalised for each subject. For amplitude, this was done by averaging the amplitude of each electrode which creates a normalisation factor, and then dividing each electrode by this “normalisation factor”. Latency was normalised at
each individual electrode with reference to the control task used in each experiment (Silberstein et al., 1990). Following normalisation, individual participant’s epochs were averaged to create a cross-subject epoch (of each electrode) for each task condition (control, 1-, 2-, and 3-back). The subtraction design used in this experiment involved subtracting the mean amplitude and latency of the control task, from the time series amplitude and latency of each activation task (1-, 2- and 3-back), at each electrode. The mean amplitude and latency of the control task are used, rather than the time series, to avoid difference such as the faster button press response time (and accompanying SSVEP changes) in the control task from confounding results. SSVEP phase variations are presented in millisecond (msec) latencies: (change in phase/2 x π) x (1000/13).

6.2.6 Presentation of SSVEP data

For each subtraction, cluster maps of Hotellings $T$ statistics, amplitude differences and latency differences were generated for the entire epoch (x-axis) and displaying all electrodes (y axis), as described previously (Gray et al., 2003, Kemp et al., 2004). This is done to reduce the probability of type 1 error, as a number of randomly distributed type 1 error would be expected with point wise t-tests within a 3.5 seconds epoch, whilst examination of cluster plots increases the likelihood of a robust effects through identification of consecutive statistical spatiotemporal clusters (Guthrie and Buchwald, 1991, Murray et al., 2002, Gray et al., 2003, Kemp et al., 2004). Electrodes (presented on the y-axis of the cluster plots) are compartmentalised into regions approximately associated with frontal (electrodes 0–20, including Fp1, Fp2, F7, F3, Fz, F4 and F8), centro-parieto-temporal (electrodes 21–52, including T3, C3, Cz, C4, T4, T5, P3, Pz, P4 and T6) and occipital (electrodes 53–63, including O1, Oz and O2) regions. Based on these cluster plots, time periods of significance were selected for subsequent generation of topographical Hotellings $T$ statistical maps and difference maps.

6.2.7 Statistical analysis

Based on evidence from spatial principal components analysis, the SSVEP forms 5 independent factors, and therefore adjustment of Hotellings $T$ statistic p-values (2-tailed) by a division of 5 was employed to correct for multiple comparisons (Silberstein et al., 1995). A threshold of $p<0.05$ corrected for multiple comparisons
was employed for all primary analyses. Memory load effects for electrodes within the defined working memory region were afforded a less stringent uncorrected threshold of p<0.05. Statistics are reported as corrected unless otherwise stated in text. Behavioural data was analysed using repeated measures ANOVA within SPSS (SPSS Inc., Chicago, IL). Examination of the relationships between change in SSVEP (between control and each n-back level) and corresponding change in performance were examined using Pearson’s product moment correlation coefficient.

6.3 RESULTS

6.3.1 Behavioural data
Accuracy and reaction time (latency) data are shown in Figure 6.2 and 6.3, respectively. As expected, repeated measures ANOVA revealed that memory load significantly influenced both accuracy [F(1.5, 28.5)=79.31, p<0.001, Greenhouse-Geisser adjusted] and reaction time [F(1.7, 31.4)=83.78, p<0.001, Greenhouse-Geisser adjusted]. Planned comparisons revealed a significant increase in reaction time and decrease in accuracy between each increment in memory load [control vs. 1-back: accuracy F(1,19)=25.6, p<0.01, reaction time F(1,19)=89.2, p<0.01; 1-back vs. 2-back: accuracy F(1,19)=71.1, p<0.01, reaction time F(1,19)=82.1, p<0.01; 2-back vs. 3-back: accuracy F(1,19)=32.8, p<0.01, reaction time F(1,19)=9.1, p<0.01].

Figure 6-2  Mean accuracy (percentages correct) for all levels of the SWM n-back. Error bars represent the standard error of the means.
6.3.2 SSVEP data

Task related differences in amplitude and latency were calculated by subtracting the mean activation of the control task from the time series of each n-back (1-, 2-, and 3-back tasks). The resulting time series differences are presented in Figure 6.4 as cluster plots [time (epoch of 3.5 seconds) x electrodes] for the amplitude, latency, and associated Hotellings $T$ statistics of each n-back. Warmer colours indicate SSVEP amplitude and latency reductions relative to baseline, with cooler coolers representing relative SSVEP increases.

Figure 6.4 reveals an overall consistency between the 3 n-back contrasts, with 3 major clusters of significance corresponding to changes in both amplitude and latency. Consistent with previous research (Silberstein et al., 2001), an early component was observed while the stimulus was displayed (0 – 539ms), which differentiated from the delay component. Two further clusters were observed during the delay period: an early, shorter cluster (847 – 1386ms), and a later more sustained cluster (2541 – 3500ms). In order to examine the activations within these 3 clusters, mean topographical maps were generated for all three time clusters/epochs, shown in Figure 6.4.
Figure 6-4  Cluster Plots of amplitude, latency and Hotellings $T$ values for the 1-back (top row), 2-back (middle row) and 3-back (bottom row) for all electrodes (y axis) over time (x-axis). Warmer colours represent reductions in both amplitude and latency relative to control task. Hotellings $T$ values are corrected for multiple comparisons (see scale for corresponding $p$ values).
Figure 6-5  Topographical SSVEP maps of amplitude, latency and Hotellings T values for the 1-back (top row), 2-back (middle row) and 3-back (bottom row) for all electrodes (y axis) over time (x-axis). Three significant time periods (epochs) are presented: a) Period while the stimulus is present (Epoch 1: 0-500ms), b) Early in the delay period (Epoch 2: 850 – 1350ms) and c) Last second of the delay period (Epoch 3: 2500 – 3500ms). Warmer colours represent reductions in both amplitude and latency relative to the control task, and larger T values in the Hotellings T maps (see scale for corresponding p values).
Figure 6.5a displays the first epoch (0 – 539ms). This time period encompasses the perceptual encoding aspect of the task in which the stimulus is still visible. The most prominent significant difference was observed within frontal electrodes, which was associated with bilateral reduction in both amplitude and latency. Significant changes were also evident in temporal regions (with a bias to the left hemisphere), which appear driven by latency reductions. However, these temporal changes in latency did not significantly differ between hemispheres (p>0.05) Changes in amplitude in parieto-occipital electrodes were evident, but only reach statistical significance in the 3-back task (relative to control).

Figure 6.5b displays the second epoch (847 – 1386ms), encompassing the early delay period. The most dominant visual feature of this epoch was an increase in amplitude in the frontal region, significant at prefrontal regions in all n-back conditions (relative to control). A pattern of increased latency was observed within fronto-temporal regions, with an associated reduction in latency in more posterior temporo-parieto-occipital regions, which was dominant in the left hemisphere. This is most statistically robust in 2-back condition, although it reached significance at temporal sites in the 1-back, and in parieto-occipital electrodes in the 3-back task. Significant changes were also observed in the left temporal region, with both amplitude and latency reductions evident.

Figure 6.5c displays the third epoch (2500ms – 3500ms), encompassing the last second of the delay (late delay). This final epoch revealed a similar SSVEP pattern to that observed within the first epoch (0 – 539ms), with latency and amplitude reductions associated with significant changes in frontal electrodes. Occipital amplitude increases were also evident but only reach significance at the 3-back level.

6.3.3 Memory load analysis
To examine the effect of increasing memory load on SSVEP amplitude and latency, a direct examination of the differences between the 1- and 2-back, and 2- and 3-back task was conducted. Figure 6.6 shows the electrodes which significantly differed with memory load increases, for the domain (latency or amplitude) which primarily contributed to the significant difference. With consideration of the probability of type 1 error over 64 point wise t-tests (i.e. 64 electrodes) per comparison (i.e. 2
comparisons: 1-back vs. 2-back, and 2-back vs. 3-back), effects were reported for regions in which a cluster of electrodes reached significance (Guthrie and Buchwald, 1991, Murray et al., 2002, Gray et al., 2003, Kemp et al., 2004). Within the first epoch, there was evidence of a U shaped pattern of activation between n-backs in latency effects within the frontal area. The 2-back task showed a greater reduction in amplitude than both the 1- or 3-back task, although this only reached significance for the 2-back vs. 1-back difference (electrodes 6 and 10, p<0.05 uncorrected). Similarly, memory load had a non-linear effect on occipital amplitude, with a lower amplitude in the 2-back compared to the 1-back task (electrode 61, p<0.01 uncorrected, electrode 62, p<0.05 uncorrected), and higher amplitude in the 2-back compared to the 3-back task (electrodes 56, 57, 62, 63, p<0.05 uncorrected).

In contrast to the first (and indeed the third) epoch, memory load related effects in the second epoch appear driven primarily by SSVEP latency changes. In the frontal region, there was a greater reduction in latency in the 3-back task compared to the 2-back (electrodes 0,1,5 p<0.01 uncorrected, electrodes 2,3,4,6, p<0.05 uncorrected). In the left temporal region, there was an apparent inverted U effect in the magnitude of latency increases. The 2-back task showed significantly greater latency increase than the 1-back (electrode 21, 22, p<0.05 uncorrected) and 3-back task (electrodes 21, 29, p<0.01 uncorrected; 22, 28 corrected, p<0.05 uncorrected). However, in the right occipital region, latency reduction increased linearly with increased memory load, although this only reached significance between the 2- and 3-back tasks (electrode 61, 62 p<0.05 uncorrected).

In the third epoch, significant differences were only observed between the 2- and 3-back tasks. There was a significant attenuation of latency reduction frontally between the 2- and 3-back task (electrodes 0, 3, 4, 5, 6, 10, p<0.05 uncorrected), in addition to evidence of load related increases in amplitude in the right occipital region (electrode 57, 63, p<0.01 uncorrected, electrodes 58, 61 p<0.05 uncorrected).
6.3.4 Correlation between SSVEP and performance

The possible correlation between SSVEP and performance was examined over the entire epoch (46 time points of 77 ms duration = 3.5 seconds) for all 64 electrodes, for each n-back level, using Pearson’s product moment correlation coefficient. Due to the large number of multiple comparisons, correlations were only considered if they: 1) occurred in clusters of at least 3 successive time points (i.e. lasting above 200ms), 2) were present in at least 3 electrodes within the same region, and 3) were significant in at least 2 of the n-back levels. These analyses revealed no significant correlations between either SSVEP amplitude or latency and accuracy or reaction time which satisfied the 3 criteria above at the significance level of p<0.05.

Due to the finding that the SSVEP of the n-back clusters into 3 significant time periods, it was examined whether any of these time periods was significantly related to performance. For each of the three smaller epochs, changes in amplitude and latency were calculated (between the control and each n-back level) for the 4 regions most significantly activated by the task: frontal region (average of electrodes 0,1,2,3,4); left fronto-temporal region (average of electrodes 12,13,14,21,22); right fronto-temporal region (average of electrodes 11,19,20,26,27); and the occipital
These changes in SSVEP amplitude and latency were correlated with changes in both accuracy and reaction time (between control and each n-back level). There were no significant correlations between either SSVEP amplitude or latency and accuracy or reaction time at any of the regions tested (frontal, left fronto-temporal, right fronto-temporal, occipital), in any of the 3 epochs identified (all p>0.05).

6.4 DISCUSSION

The current experiment examined the temporal dynamics of the SWM n-back task using SSVEP. Supporting the first hypothesis, these findings demonstrated a different SSVEP amplitude pattern during the perceptual/encoding and delay components of the task, consistent with findings during the delayed-response task (Silberstein et al., 2001). Consistent with the main hypothesis, the delay period exhibited increases in amplitude in both frontal and occipital regions, also consistent with findings during the delayed-response performance (Silberstein et al., 2001, Perlstein et al., 2003). However, the main finding of this experiment is that the delay period was associated with two relatively distinct electrophysiological stages. Specifically, early in the delay (just following the stimulus disappearing from view), amplitude increases in frontal regions were observed, in addition to latency increases in the fronto-temporal regions and latency reductions more posteriorly. In contrast, late in the delay (the last second of the delay) a reduction in prefrontal amplitude and latency was observed, in addition to an increase in occipital amplitude. Importantly, all three n-back levels demonstrated a relative consistent electrophysiological profile, suggesting that this pattern is specific to the SWM n-back task. Nevertheless, these findings supported the hypothesis that memory load would modulate SSVEP within the network identified, consistent with previous neuroimaging studies (Braver et al., 1997, Cohen et al., 1997).

In terms of cortical topography, the present findings are consistent with the extensive literature outlining a distributed network associated with the SWM n-back task (Owen et al., 2005). Further, the majority of SSVEP changes across the task were within the frontal regions consistent with electrophysiological evidence in non-human primates and humans (for reviews, see Goldman-Rakic, 1996, Wager and Smith, 2003). One
difference observed in the current experiment was that latency reductions during the delay appeared biased to the left hemisphere (although hemispheric differences were not significant). This left hemispheric bias contradicts evidence suggesting SWM activates a primarily right hemisphere network (Wager and Smith, 2003). However, while the stimulus had no overt verbal content, this apparent left hemisphere bias may be related to internal verbalisation of the position of the stimulus, as tasks of verbal working memory activate a left hemisphere dominant network (Smith et al., 1996, Clark et al., 2001).

6.4.1 Encoding vs. Delay period

The first aim of the current experiment was to replicate the differing SSVEP amplitude profile between the early perceptual/encoding and delay components. The current findings supported this distinction. During perceptual encoding (when the stimulus was still present), SSVEP amplitude was reduced over frontal, temporal and parietal regions, consistent with previous SWM findings (Silberstein et al., 2001) and with reductions in parieto-occipital SSVEP amplitude during visual vigilance tasks, which have previously been interpreted as excitation of these regions related to increased visual vigilance (Silberstein et al., 1990, Nield et al., 1998).

In contrast, amplitude increased in both the frontal and occipital region during the delay. These findings support the primary hypothesis of amplitude increases during the delay, and are consistent with previous SSVEP findings during the delayed-response task (Silberstein et al., 2001, Perlstein et al., 2003). Increased amplitude within the PFC is likely to reflect neuronal activity within the pyramidal cells (Silberstein et al., 2001), and is consistent with electrophysiological studies in non-human primates which have established that pyramidal neurons in the PFC consistently fire during the hold period of delayed-response working memory tasks and that these “memory fields” hold information online in an active representation (Goldman-Rakic, 1996).

Amplitude of the SSVEP signal is assumed analogous to alpha activity within the brain. While reductions in alpha activity have traditionally been interpreted as reflecting increased “activity” or mental processing, the current findings fit well with a developing understanding of amplitude and cognition, in which amplitude (or alpha)
changes may be related to the “type” of cognitive process (Silberstein et al., 2001). Specifically, for intake tasks (where attention is paid to the external environment), findings have been associated with reductions in alpha, whereas internal tasks (where active rejection of external environment and focus on internal content) have been associated with increases in alpha (Ray and Cole, 1985). More recent findings have also demonstrated increases in alpha activity during mental imagery (Tesche et al., 1995), and increases in upper alpha range activity (10-13Hz) at frontal and temporal sites during episodic memory (Klimesch et al., 1999).

A neurophysiological model proposed by Silberstein et al. (1995, 1998, 2001) suggests that rhythmic activity in the cortico-cortico loops is an important generator of the SSVEP, and that reticulation of neural activity within these loops may provide a mechanism for holding information “online”. Therefore, during the SWM delay, an increase in amplitude could be interpreted as reflecting an increase in the efficiency or “loop gain” of these cortico-cortico loops (Silberstein et al., 2001). In contrast, the reduction of amplitude is likely to reflect a desynchronisation of these loops (Silberstein et al., 1995) and in more sensory tasks (e.g. visual vigilance or encoding), this may reflect a reduced efficiency of these loops as a direct result of enhances sensory inputs to layer V and consequent inhibition of layer I (for a full discussion of cortico-cortico loops, see Silberstein et al., 1995).

6.4.2 Delay related activity

Amplitude

The main finding of this experiment is that the delay period was associated with two relatively distinct electrophysiological stages. The early delay component (approx. 850-1400ms) was associated with amplitude increases in the frontal region, while the later delay component (approx. 2500-3500ms) was associated with amplitude reductions in the PFC region, and an associated amplitude increases in the occipital region. These findings may reflect a dual role of the PFC within a complex SWM task. While the importance of the PFC in holding working memory online is well established, there is similarly evidence indicating PFC involvement in other (more “executive”) aspects of the working memory process, such as organisation and control of the working memory “content”, implementation of strategies to facilitate memory, and updating of working memory content (Halgren et al., 2002, Bor et al., 2003).
Although the functional significance of the frontal amplitude reduction in the late delay period is unknown, prefrontal amplitude reductions have previously been associated with cognitive set changes during the Wisconsin card sort test, a well known test of executive function (Silberstein et al., 1995), and therefore the current reductions possibly reflect PFC reallocation to executive (non-maintenance) aspects of the SWM process.

The amplitude increases observed in the current experiment (i.e. early delay component: frontal increases, late delay component: occipital increases) may indicate a shift in “holding information” from frontal to occipital regions over the duration of the delay. This suggestion is in line with previous findings from a study in patients with partial epilepsy (Halgren et al., 2002). Halgren et al (2002) observed sustained co-activation of the occipital cortex with fronto-centro-parietal cortices during a working memory task. These authors inferred a directionality of relationships between cortical areas based on phase lag measures, which indicated that later in the task the flow of information was from the frontal region to the occipital region. It was suggested that such findings are highly consistent with the use of the occipital cortex as a visuo-spatial sketch pad, potentially controlled by executive functions within the frontal cortex (Halgren et al., 2002), and the current findings fit well with such a model.

**Latency**

Variations in SSVEP latency have been interpreted as reflecting variation in neural information processing speed (Silberstein et al., 2001, Gray et al., 2003, Kemp et al., 2004). An increase in latency is indicative of an increase in inhibitory processes within the cortico-cortico loops, while a reduction in latency suggestive of an increase in excitation (or reduction of inhibition) (Silberstein et al., 1995, Silberstein et al., 2001). Three major latency related changes were observed during the SWM n-back task. During the perceptual/encoding period, SSVEP latency reductions were observed bilaterally in the frontal cortex (with a general reduction in more posterior regions). These findings are consistent with latency reduction during the encoding/perceptual period of a delayed-response task (although in the Silberstein et al. (2001) study these effects failed to reach significance), and with latency reductions
during visual vigilance tasks (the A-X Continuous Performance task) (Silberstein et al., 2000b).

Latency changes were also evident during the early and late delay periods. During the early delay period, SSVEP latency was relatively increased within fronto-temporal regions and reduced in more posterior temporo-parieto-occipital regions. In contrast, during the late delay stage (2500-3500ms), latency was reduced within frontal regions. These findings suggest that early in the delay there is an increase in inhibitory processes within the fronto-temporal region. In contrast, in the later delay, latency reductions were observed in frontal regions which are suggestive of increased excitation within the PFC during the late delay period.

6.4.3 Proposed model of SSVEP changes during the SWM n-back.

While the exact mechanisms underlying the SSVEP changes are unknown, based on current understanding of SSVEP it is suggested that early in the delay, frontal amplitude increases are likely to be involved in “holding” information online, and the fronto-temporal latency increases may be involved in facilitating this online maintenance through inhibition of adjacent neurons. In contrast, during the last second of the delay both amplitude and latency are reduced in the frontal cortex (likely reflecting overall excitatory processes), and occipital amplitude is increased. It is suggested that the frontal cortex is reallocated to executive aspects of the task (which may include manipulation of information, response preparation and anticipation of the new stimulus), with a shift in the “holding” of the working memory content to the occipital region. The complexity of the n-back task, which comprises a number of separate and potentially overlapping processes, along with the probability that different participants execute the n-back task using different strategies, means that even with the high temporal resolution of the SSVEP it is not possible to delineate each working memory processes. However, while the above model is speculative, the pattern of SSVEP activations does appear specific to the processes involved in the n-back task per se, with an overall consistency in the pattern of cortical effects noted between each n-back task level. Despite the complexity of the task, the n-back exhibits a consistent temporal profile regardless of memory load.
6.4.4 Memory load

Nevertheless, the current findings demonstrated that within these three time periods, SSVEP responses exhibited memory load related changes consistent with previous PET and fMRI studies of the n-back (Braver et al., 1997, Cohen et al., 1997). For example, increased memory load was related to greater latency reduction (increased excitation) within the occipital cortex early in the delay. Consistent with the occipital cortex playing a role in “holding” information in the late delay period, increased memory load was related to increase occipital amplitude, specifically between the 2- and 3-back tasks. There was also evidence that SSVEP responses exhibited non linear, U and inverted U shaped memory load related changes in frontal and occipital regions. For example, during the early delay both fronto-temporal latency and frontal amplitude increases were greater in the 2-back task than either the 1- or 3-back task. These findings are consistent with similar inverted U shaped responses observed during the n-back task using fMRI, and it has previously been suggested that this reflects a capacity-constrained response in which activation increases within a region until capacity is reached (i.e. 2-back task), at which point activation declines (i.e. 3-back task) (Callicott et al., 1999). As expected, memory load was related to a decrease in behavioural performance. These performance changes were not correlated with SSVEP changes; however this is not entirely surprising. As the n-back task is a complex series of separate and potentially overlapping sub-processes, behavioural performance is likely to reflect the sum of these processes rather than individual time periods. These findings suggest that memory load increases result in changes in SSVEP amplitude and latency within previously activated regions, and behavioural performance is not highly related to any specific time period but more likely to the sum of sub-processes comprising the task.

6.4.5 Chapter summary

This experiment investigated the temporal dynamics of the SWM n-back task using SSVEP. The current findings demonstrate that the SWM n-back task was associated with three distinct SSVEP time periods: an early perceptual/encoding component (consistent with previous research), in addition to two distinct time periods during the delay. The different SSVEP amplitude pattern identified between the perceptual and mnemonic components of the task is consistent with previously research (Silberstein et al 2001), and fits well into a developing understanding of amplitude in reference to
the “type” of cognitive process. The two different SSVEP components during the delay appear reflective of the complexity of the task and the additional demands associated with the n-back, which appear evident specifically later in the delay. An overall consistency in the pattern of cortical effects was noted between each n-back task level. This suggests that despite the complexity of the task, the n-back exhibits a consistent temporal profile regardless of memory load. Consistent with previous fMRI and PET findings, all three time periods exhibited memory load related effects on the SSVEP response. In the next chapter, these results are extended upon by examining the effect of modulating the dopaminergic system on SWM n-back related SSVEP.


Chapter Seven

7 Experiment 4: Examining the effects of dopaminergic modulation on cortical electrophysiology during the SWM n-back task.

7.1 INTRODUCTION

The findings reported in Chapter 5 indicated that TPD did not alter rCBF during the SWM n-back task. This may reflect a failure of TPD to modulate SWM neural networks, specifically in light of the lack of TPD-related effects on behavioural performance observed throughout this thesis. However, due to the temporal dynamics of PET (in the order of seconds), changes in activation are observed over the entire working memory epoch, and subtle changes during the complex n-back process may have been undetected.

Indeed, there is an indication that different aspects of working memory may be subserved by different neurotransmitter systems (Ellis and Nathan, 2001). For example, a series of studies by Furey and colleagues suggest that acetylcholine (ACh) influences working memory performance via focusing perceptual processing in extra-striate visual cortices during encoding, thus producing a more robust visual percept which is easier to maintain during the delay (Furey et al., 1997, Furey et al., 2000a). Specifically, evidence suggests that increased acetylcholine levels (via administration of physostigmine, an anti-cholinesterase inhibitor) are associated with increased activity in the extra-striate cortex during encoding, and decreased activity in the anterior PFC during maintenance of information (Furey et al., 2000b, see Chapter 2 section 2.4.1 for review). In terms of the role of dopamine in SWM, while there is general consensus that dopamine is critical within the PFC during working memory performance, evidence in non-human primates suggests that dopaminergic action at the D₁ and D₂ receptors may have distinct functions which relate to different stages of the SWM tasks. For example, Wang et al. (2004) recently observed that modulation of the D₂ receptor (using the D₂ antagonists raclopride or eticlopride, and the D₂ agonist...
quinpirole) selectively modulated the neural activities associated with memory-guided saccades during an oculomotor delayed-response task (i.e. the response process), yet had little or no effect on the persistent mnemonic-related activity (i.e. the maintenance process). In contrast, modulation of D<sub>1</sub> receptors (using the agonist SKF38393 and antagonist SCH39166) did not influence response activity, but instead modulated delay related activity, consistent with previous findings suggesting a critical role of D<sub>1</sub> receptors in modulating PFC delay-related neural activity (Goldman-Rakic et al., 1996).

In the preceding chapter (Chapter 6), SSPT was used to examine the temporal subcomponents of the SWM n-back task, and three different time periods of significance were identified: an early perceptual/encoding period (approx. 0-500ms), an early delay period just following the stimulus disappearing from view (approx. 850-1400ms), and a late period lasting the final second of the delay and anticipation of the new stimulus (approx. 2500-3500ms). These findings provide a framework in which to examine the effects of neurochemical modulation of cortical electrophysiological activity associated with the SWM n-back task. Indeed, not only has previous research using SSPT demonstrated reliable and specific topographic changes in SSVEP during cognitive tasks (e.g. Silberstein et al., 1990, Silberstein et al., 1995, Silberstein et al., 1998, Silberstein et al., 2000a, Silberstein et al., 2000b, Silberstein et al., 2001, Kemp et al., 2002, Gray et al., 2003, Perlstein et al., 2003, Silberstein et al., 2003, Kemp et al., 2004), but changes in task-related SSVEP have been observed following drug manipulations (Kemp et al., 2004), and have been observed to differentiate between healthy participants and clinical populations (including ADHD and schizophrenia) (Silberstein et al., 1998, Silberstein et al., 2000b).

Therefore, the aim of this chapter was to extend upon both previous research and the findings of Chapter 6 and investigate, for the first time, whether dopaminergic modulation alters the temporal subcomponents of the SWM n-back task. SSPT is an electrophysiological technique and therefore allows multiple testing sessions without the constraints associated with injected radioactivity (as associated with the PET experiment presented in Chapter 5). Accordingly, this experiment took advantage of the ability to include additional testing sessions within the repeated-measures design,
and not only examined the effects of TPD on 2-back task-related SSVEP, but also examined whether simulation of $D_1/D_2$ receptors (under conditions of TPD) can reverse any effects of TPD on SWM-related SSVEPs. It was hypothesised that the cortical topography of effects were likely to be observed within the SWM network, specifically within the PFC and/or parietal region based on evidence from fMRI and PET studies in humans which demonstrate that during working memory performance, administration of bromocriptine (a $D_2$ receptor agonist) reduces task-related blood flow within the parietal cortex (Kimberg et al. 2001), and administration of psychomotor stimulants methylphenidate and amphetamine (indirect catecholamine agonists) modulates task-related blood flow within the PFC (Mattay et al., 2000, Mehta et al., 2000, Schweitzer et al., 2004) and posterior parietal cortex (Mehta et al., 2000).

7.2 METHODS

7.2.1 Participants
The volunteers who participated in this experiment also participated in Experiment 1 (Chapter 4). Full details of exclusion criteria and participant details can be found in Chapter 4. Briefly, this sample comprised eighteen healthy males (mean age $22.9 \pm 6.4$ years). All participants were healthy, right handed, and non-smokers.

The study was conducted using a double-blind, balanced-drink (placebo) control, repeated measures design over three separate sessions (1) 104.4g balanced control condition (BAL condition), (2) an equivalent mixture deficient in tyrosine and phenylalanine (TPD condition) and (3) TPD mixture + pergolide (0.1mg) condition (TPD+P condition). Each session was separated by a minimum five-day washout period, with order of condition randomised using a quasi-latin-square design. The study was approved by the Swinburne University Human Research Ethics Committee. All participants gave written informed consent.

7.2.2 Procedure
Full procedural details are provided in Experiment 1 (Chapter 4). Briefly, the amino acid drink and capsules were administered at approximately 0900 hrs (time 0), and oral dose of pergolide (or placebo capsule) was administered at +3 hrs post-drink.
(approximately 1200 hrs). Post-drug testing commenced at +5 hrs post drink, to coincide with the peak behavioural effects of TPD (Harmer et al., 2001, Harrison et al., 2004) and pergolide (Markham and Benfield, 1997).

Two doses of the peripheral dopamine receptor antagonist domperidone were administered during each testing session (at +30 mins and +2 hrs post-drink; 10mg per administration/20mg total) to reduce the potential side effects of peripheral D2 blockade, such as nausea. Subjective side effect questionnaires were administered at +1 and +3 hrs post-drink (see Appendix 2). At +2.5 hrs post-drink a carrot was also provided to reduce hunger. Two 20ml venous blood sample were taken per session for analysis of plasma amino acid concentrations: the first preceding baseline testing (i.e. before time 0), and the second preceding post-drink testing (at +4 hrs, 45 mins). Testing concluded 5.5 hrs post-drink, and participants were provided with a high protein snack before departing.

7.2.3 SSPT Procedure

SSPT protocol was consistent with the previous chapter (Chapter 6). Briefly, participants were seated approximately 2.5 metres from a computer monitor in a dimly lit soundproofed room, and were fitted with a lycra electrode cap comprising 64 monopolar leads, positioned according to the international 10/20 system. Nose and linked ear electrodes were used for ground, and impedance on all electrodes was generally below 5kΩ. Participants were then fitted with a set of modified half-mirrored goggles, which superimposed the 13Hz white light flicker over the visual field to elicit the SSVEP. Recorded brain electrical activity was band pass filtered from 0.74 to 74Hz and digitised at a rate of 500Hz with 16-bit accuracy, consistent with previous studies (e.g. Silberstein et al., 1998, Silberstein et al., 2001, Kemp et al., 2002, Gray et al., 2003, Kemp et al., 2004, Silberstein et al., 2004).

Participants had attended a pre-study training session on a previous occasion to familiarise themselves with the task. On the day of testing, participants completed subsequent practice following electrode cap setup, completing each task twice under SSVEP conditions to familiarise themselves with the flicker.
7.2.4 SWM n-back task
The SWM n-back task used in this experiment was identical to that described in full in Chapter 6. This version of the n-back has recently been shown as sensitive to cholinergic manipulation in healthy young adults (Green et al., 2005). In the current experiment, only the 2-back task was examined to reduce the number of comparisons [considering the additional treatment condition of pergolide (under TPD conditions) used in this experiment]. Selection of the 2-back was made based on evidence that n-back related activation (specifically within the DLPFC) can exhibit an inverted U related neurophysiological response from the lowest (1-back) to the highest (3-back) load, which may reflect capacity constraints (Callicott et al., 1999). In addition, the 2-back task is a commonly used level of n-back, and previous fMRI and PET studies have examined dopamine modulation of the n-back task at the 2-back level (Kimberg et al., 2001, Mehta et al., 2003). Therefore, the 2-back was considered the most appropriate n-back level as it was less likely to exceed capacity constraints if performance was impaired by TPD (i.e. as may occur with the 3-back task), and its use maintained consistency with previous research examining n-back performance following dopaminergic manipulation (e.g. Kimberg et al., 2001, Mehta et al., 2003). Three alternate versions of the task and visuo-motor control task were constructed and randomly administered between treatment and balanced control conditions. Consistent with the previous experiment, participants completed 2 sets of 40 trials (80 trials in total) for both the visuo-motor control task and 2-back task.

7.2.5 Amino acid suspension
The composition of the balanced amino acid mixtures was detailed in Experiment 1 (Chapter 4).

7.2.6 SSPT signal processing and analysis
SSPT signal processing and analysis remained consistent with the previous chapter. Briefly, the SSVEP was extracted from the brain electrical data (for each electrode) using Fourier analysis, and the resulting Fourier coefficient (FC) for each stimulus cycle was smoothed by averaging overlapping blocks of 10 FCs. Each electrode within each task was checked individually for artefact. Epochs of 3.5 seconds (1 trial = stimulus display + delay) were extracted from the SSVEP for both activation and control task, and the mean amplitude and latency of the control task was subtracted.
from the time series amplitude and latency of the 2-back task at each electrode. SSVEP phase variations are presented in millisecond (msec) latencies: \((\text{change in phase}/2 \times \pi) \times (1000/13)\). Cluster maps of Hotellings \(T\) statistic, amplitude differences and latency differences were generated for the entire epoch (x-axis) and displaying all electrodes (y axis). Based on these cluster plots, time periods of significance were selected for subsequent generation of topographical Hotellings \(T\) statistical maps and amplitude and latency difference maps.

7.2.7 Statistical analysis
A threshold of \(p<0.05\) corrected for multiple comparisons was employed for all primary analyses. Statistics are reported as corrected unless otherwise stated in text. Behavioural data was analysed using repeated measures ANOVA within SPSS (SPSS Inc., Chicago, IL). Examination of the relationships between change in SSVEP and corresponding change in performance were examined using Pearson’s product moment correlation coefficient.

7.3 RESULTS

7.3.1 SWM n-back task
The main performance measures for the n-back task were accuracy and reaction time (see Figures 7.1 and 7.2). The data for one subject contained outliers on both accuracy and reaction time measures, and was removed from the analysis; therefore the results are presented for 17 participants. Repeated measures ANOVA revealed no significant difference between treatment conditions in 2-back accuracy \([F(2,32)=0.44, p>0.1]\), however a trend towards difference between treatment conditions was observed in 2-back reaction time \([F(1.5,23.8)=3.00, p=0.08\), with Greenhouse-Geisser correction]. Planned contrasts revealed a significant increase in reaction time following TPD+P compared to the BAL \([F(2,32)=6.16, p<0.05]\), with no significant difference in reaction time between TPD and BAL conditions \((p>0.1)\).

7.3.2 Plasma amino acid levels
Plasma amino acid levels were reported in Experiment 1 for these participants. Briefly, repeated measures ANOVA revealed significant interactions between treatment conditions and time for tyrosine \([F(1.21,10.97)=40.39, p<0.001\), with
Greenhouse-Geisser correction] and phenylalanine \[F(1.08,9.71)= 42.77, \ p<0.001,\]
with Greenhouse-Geisser correction], with planned contrasts revealing that concentrations of both tyrosine and phenylalanine decreased significantly following the TPD and TPD+P condition, compared to the BAL condition (all \( p<0.001 \)). The ratio of plasma tyrosine and phenylalanine to large neutral amino acids \((T+P/\Sigma LNAA)\) varied significantly between treatment conditions \([F(2,18)= 47.60, \ p<0.001]\), with planned contrasts revealing a significantly greater decrease in the ratio following both the TPD and TPD+P condition, compared to the BAL condition (both \( p<0.001 \)) (see Table 4.2, Chapter 4 for full plasma amino acid values).

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**Figure 7-1**  Overall reaction time scores for the SWM 2-back following each treatment condition. Error bars represent the standard error of the means (SEM).

**Figure 7-2**  Overall accuracy scores (percentage correct) for the SWM 2-back following each treatment condition. Error bars represent the standard error of the means (SEM).
7.3.3 Subjective side effects and correlations with cognition
As reported in Chapter 4, there was no difference between the treatment conditions in subjective side effects [F(2,34)=0.10, p>0.1]. The nausea scale was also examined separately, and similarly there was no significant difference between treatment conditions (p>0.1). Consistent with analysis in Chapter 4, the changes in side effect rating between treatment and balanced conditions (i.e. TPD - BAL and TPD+P - BAL) were correlated with corresponding changes in SWM n-back performance between treatment and balanced conditions (TPD - BAL and TPD+P - BAL) using Pearson’s product moment correlations. There were no significant correlations between any of change in working memory performance and side effect scores for either TPD (r = -0.24, p=0.35) or TPD+P (r = -0.27, p=0.2).

7.3.4 SSVEP results
For both treatment conditions, task related differences in amplitude and latency were calculated by subtracting the mean activation of the control task from the time series of the 2-back task. Figure 7.3 displays the resulting time series differences, presented as cluster plots [time (epoch of 3.5 seconds) x 64 electrodes] for amplitude, latency, and associated Hotellings T statistics. Warmer colours indicate SSVEP amplitude and latency reductions relative to baseline, with cooler coolers representing relative SSVEP increases.

In Chapter 6, under normal (no drug manipulation) conditions, the n-back task revealed three major clusters of significance. Specifically, an early component was observed while the stimulus was displayed (0 – 539ms), with two further clusters observed during the delay period: an early, shorter cluster (847 – 1386ms), and a later more sustained cluster (2541 – 3500ms). In keeping with these findings, examination of the balanced/placebo condition cluster plot (Figure 7.3, top row) indicated that that the majority of significant activations occurred within these three time periods. Inspection of the TPD, and TPD+P cluster plot (Figure 7.3 middle row and 7.3 bottom row, respectively) similarly indicated that the majority of significant activations occurred within these three time periods for both treatment conditions. Therefore, in order to examine possible treatment related differences within these three clusters, mean topographical maps were generated for each epoch.
Figure 7-3  Cluster Plots of amplitude, latency and Hotellings $T$ values for all electrodes (y axis) over time (x-axis) for the BAL (top row), TPD (middle row) and TPD+P (bottom row). Warmer colours represent reductions in both amplitude and latency relative to control task. Hotellings $T$ values are corrected for multiple comparisons (see scale for corresponding p values).
Figure 7-4  Topographical SSVEP maps of amplitude, latency and Hotellings $T$ for BAL (top row), TPD (second row) and TPD+P (third row), for the three time periods identified. The three significant time periods (epochs) presented are: a) Period while the stimulus is present (Epoch 1: 0-500ms), b) Early in the delay period (Epoch 2: 850 – 1350ms), and c) Last second of the delay period (Epoch 3: 2500 – 3500ms). Warmer colours represent reductions in both amplitude and latency relative to control task, and larger $T$ values in the Hotellings $T$ maps (see scale for corresponding p values).
Figure 7.4a displays the first epoch (0 – 539ms), encompassing the first 500ms of the task during which the stimulus was visible. All three treatment conditions revealed a significant difference within frontal electrodes, which was associated with bilateral reduction in both amplitude and latency. There was no evidence of treatment related differences during this time period.

Figure 7.4b displays the second epoch (847 – 1386ms), encompassing the early delay period. In all treatment conditions, an increase in frontal amplitude was evident which was significant at prefrontal sites. Similarly, all treatment conditions revealed a significant reduction in latency in the occipital region. However, there was evidence of treatment related differences during this period. Visual inspection of Figure 7.4b suggested a reduction in parieto-occipital amplitude in the TPD+P condition, which was not evident in the other two conditions (TPD and BAL). To examine whether this apparent difference was significant, the Hotellings $T$ statistic could not be used because all conditions demonstrated significant Hotellings $T$ statistics within the parieto-occipital region (which may reflect changes in either amplitude or latency; hence no difference between treatment conditions could be implied). Therefore, parametric statistics were performed on the amplitude data, by generating an average amplitude of the parieto-occipital region (electrodes 48 (Pz), 61(0z), 55 and 56) for both the control and 2-back tasks (for all treatment conditions), and conducting a 3 (treatment condition) by 2 (n-back level) repeated measures ANOVA. This analysis revealed a significant difference between treatment conditions in the parieto-occipital region [$F(2,34)=3.50, p<0.5$]. Planned contrasts demonstrated greater reduction in amplitude in the parieto-occipital region in the TPD+P condition than the BAL condition [$F(1,17)=6.14, p<0.0.5$], with a strong trend in the same direction between the TPD+P and TPD conditions [$F(1,17)=3.70, p=0.07$].

The second epoch also revealed evidence of differences in latency between the treatment conditions, with the TPD+P condition demonstrating greater latency increases in the fronto-temporal region than either the BAL or TPD condition. Within the right hemisphere, inspection of the Hotellings $T$ statistic indicated there were significantly greater increases in fronto-temporal latency in the TPD+P condition than either the BAL or TPD condition (Hotellings $T$ statistics are highly likely to reflect latency changes as there were no accompanying amplitude changes in this region). In
the left hemisphere, there was some indication that all treatment conditions revealed increases in latency in posterior temporal regions (although this increase failed to reach significance in the TPD condition). However, Hotellings $T$ statistics revealed that the TPD+P condition demonstrated significant increases in left fronto-temporal latency that were not evident in either the BAL or TPD condition.

In sum, the second time period revealed a reduction in parieto-occipital amplitude and increase in fronto-temporal latency in the TPD+P condition which was significantly greater than either the BAL or TPD conditions (latency difference most prominent in the right hemisphere).

Figure 7.4c displays the third time period (approx. 2500 – 3500ms), encompassing the last second of the delay period. All three treatment conditions revealed a similar pattern of activation during this period. Significant differences were observed in the frontal region, primarily reflective of a reduction in frontal amplitude with some evidence of frontal latency reductions, specifically in the BAL condition. While there was some indication of an increase in amplitude in parieto-occipital regions in all tasks, this change did not reach significance. There was no evidence of significant differences between treatment conditions during the late delay component.

### 7.3.5 Correlations

Pearson’s moment correlations were used to examine whether the observed increase in reaction time following TPD+P (compared to BAL) was correlated with either the significant TPD+P related reduction in parieto-occipital amplitude or the increase in right fronto-temporal latency, both observed in the second epoch (early delay period: 850 – 1400ms). The change in parieto-occipital amplitude between the BAL condition (2back – control) and TPD+P condition (2back – control) was correlated with the change in reaction time between BAL and TPD+P conditions, for each participant. This analysis revealed no significant correlation ($p>0.1$). Similarly, the change in right fronto-temporal latency between the BAL and TPD+P condition was not correlated with the change in reaction time between BAL and TPD+P conditions ($p>0.1$).
7.4 DISCUSSION

This experiment examined whether modulating the dopaminergic system alters cortical electrophysiological activity associated with the SWM 2-back task, as assessed by SSPT. The first finding of this study was that administration of an amino acid load (in all conditions, including balanced control/placebo) did not disrupt the temporal pattern observed during SWM n-back task performance under “no drug” conditions. That is, the n-back task was associated with an early perceptual component and two components during the delay, consistent with the pattern demonstrated in the previous experiment (Chapter 6). However, the two main findings of this experiment were that: 1) TPD failed to significantly modulate cortical electrophysiological activity during any of the temporal components identified during the SWM n-back task, and 2) D₁/D₂ receptor stimulation (under TPD conditions) resulted in changes in fronto-temporal and parieto-occipital SSVEP within the early delay period of the 2-back task, and caused a subtle impairment in performance.

7.4.1 N-back SSVEP under amino acid load

Despite the presence of an amino acid load, the SWM 2-back task exhibited a SSVEP temporal profile during the balanced/placebo condition that was highly similar to the temporal profile observed without drug manipulation (see Chapter 6 for full discussion). Specifically, the early perceptual component which lasted during the stimulus display, was associated with a reduction in frontal amplitude and latency, interpreted as reflecting of increase in excitation related to perceptual processing (Silberstein et al., 2001). The delay period was associated with two components. Early in the delay (just following the stimulus disappearing from view), amplitude increases in frontal regions were observed, in addition to latency decreases more posteriorly. In contrast, late in the delay (the last second of the delay) a reduction in prefrontal amplitude and latency were observed, which may possibly reflect the role of the PFC in primarily non-mnemonic aspects of the n-back task during this time period (see Chapter 6 for full discussion). Further, the current results demonstrated that while D₁/D₂ receptor stimulation (under conditions of TPD) altered cortical electrophysiological activity within the early delay period, dopaminergic modulation did not alter the temporal structure of the SSVEP response to the task.
7.4.2 SSVEP following TPD
The current findings suggest that TPD did not alter either the overall temporal profile of the SWM n-back task, or influence the SSVEP amplitude or latency changes within each of the three temporal subcomponents identified. The lack of TPD-related effects on cortical electrophysiological activity associated with the SWM 2-back is consistent with the lack of TPD-related effects on task-related cerebral blood flow (as reported in Chapter 5). These findings are also consistent with the apparent failure of TPD to modulate 2-back behavioural performance (in both the current experiment and PET experiment), and a lack of TPD-related effects on three different delayed-response tasks examined in this thesis (Chapters 4 and 5). Indeed, while there are two important studies demonstrating an effect of TPD on SWM performance in healthy humans (Harmer et al., 2001, Harrison et al., 2004), the current finding fits well into a developing literature suggesting that TPD has does not consistently produce measurable effects on SWM performance (McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005). As with Experiment 2 (PET experiment reported in Chapter 5), the lack of TPD-related modulation of task-related neurophysiology is unlikely to be due to limited task-related activations, as the 2-back demonstrated task-related changes to SSVEP which were consistent with those seen under no treatment conditions (Chapter 6). Therefore, taken within the context of a developing literature of inconsistent effects following TPD, it is likely that the observed failure of TPD to modulate task-related cortical electrophysiology reflects that TPD had no discernable effect on SWM in this experiment.

7.4.3 D1/D2 receptor stimulation
Despite the lack of TPD related effects on SSVEP during the SWM n-back task, D1/D2 receptor stimulation (under conditions of TPD) was associated with impairment in performance and changes in task-related SSVEP in fronto-temporal and parieto-occipital regions during the early delay period. These findings are consistent with the behavioural results reported in Chapter 4, which demonstrated that while TPD failed to modulate performance, the addition of pergolide caused a subtle impairment in performance. While the current findings are contrary to the initial prediction of an enhancing (or reversing) effect of pergolide under conditions of TPD, modulation of fronto-temporal and parieto-occipital regions by dopaminergic manipulation is consistent with evidence of changes in task-related BOLD signal in the parietal region.
following administration of bromocriptine (Kimberg et al. 2001), and is in line with changes in rCBF within the PFC (Mattay et al., 2000, Mehta et al., 2000, Schweitzer et al., 2004) and posterior parietal cortex (Mehta et al., 2000) following psychomotor stimulants methylphenidate and amphetamine (indirect catecholamine agonists). Further, in terms of regional cortical topography the changes in fronto-temporal and parieto-occipital activity are consistent with the largely undisputed role of these regions during n-back task performance (for a review, see Owen et al., 2005).

Latency changes appear to reflect summed changes in synaptic transmission time related to post-synaptic excitation or inhibition processes (Silberstein et al., 1995, Silberstein et al., 2000b), and the observed fronto-temporal latency increases are likely to reflect an increase in localised inhibitory processes within the fronto-temporal region as a result of D_1/D_2 stimulation (under TPD conditions). While the effects of dopamine on prefrontal neurons are complex (for a review, see Abi-Dargham and Moore, 2003), an increase in inhibition within the frontal cortex is consistent with evidence that stimulation of PFC dopamine receptors located on GABAergic interneurons may promote GABA-mediated inhibition of pyramidal cells (e.g. Grobin and Deutch, 1998, Del Arco and Mora, 2000, Seamans et al., 2001, Gorelova et al., 2002), and evidence that D_1 receptors can decrease glutamate input onto cortical neurons (e.g. Gao et al., 2001, Urban et al., 2002). Further, as highlighted in Chapter 4, the postsynaptic effects of D_1 receptors can be considered as either excitatory or inhibitory, depending on the functional status of the neuron (Yang et al., 1999), and a sensitised D_1 system (in response to dopamine deletion) may shift to a more GABAergic pathway.

The current findings further demonstrated that the effect of D_1/D_2 receptor stimulation (under conditions of TPD) was restricted to the early delay period, rather than influencing all aspects of the delay. While the complexity of the n-back does not allow for clear delineation of each working memory sub-processes (as discussed in Chapter 6), these results give insight into the nature of the dopaminergic modulatory effect. The fact that the effect was in the early delay period, following the execution of a response, suggests that it is unlikely to be related to response preparation. Further, these results fit well within the model presented in the previous chapter, which suggested that during the early delay, frontal amplitude increases are likely to
be involved in “holding” information online, and fronto-temporal latency increases may be involved in facilitating this online maintenance through inhibition of adjacent neurons. Based on such a model, it could be speculated that the effects of D₁/D₂ receptor stimulation (under conditions of TPD) on SWM were at least partly related to a disruption of online maintenance.

These findings also demonstrated that D₁/D₂ receptor stimulation (under conditions of TPD) caused impairments in behavioural performance (increased reaction time) during the 2-back task. These findings are consistent with the impairment in delayed-response performance reported in Experiment 1 following the same dopaminergic manipulation (see Chapter 4 for a full discussion of impaired behavioural performance following pergolide under conditions of TPD). A slowing of reaction time fits well with the observed SSVEP latency increases. Previous research has demonstrated a correlation between mean reaction time and frontal SSVEP latency changes during the continuous performance task, in which faster reaction times were associated with a reduction in SSVEP latency (Silberstein et al., 2000b). In the current experiment, a correlation analysis was performed between the SSVEP latency and reaction time scores, but no significant correlation was observed. However, this may be due to the fact that SSVEP latency increases were accompanied by amplitude reductions in the parieto-occipital region during this same time period, and a combination of these effects may have been associated with the reaction time increase rather than either SSVEP change alone. Indeed, there was no evidence that the parieto-occipital amplitude reduction was individually correlated with performance.

The reduction in SWM-related parieto-occipital amplitude observed following D₁/D₂ receptor stimulation (under conditions of TPD) is consistent with evidence of changes in SWM-related posterior parietal cortex activity following administration of the D₂ receptor agonist bromocriptine (Kimberg et al., 2001), and the indirect catecholamine agonist methylphenidate (Mehta et al., 2000) (as measured by fMRI and H₂¹⁵O PET, respectively). Indeed, Kimberg et al. (2001) demonstrated that administration of bromocriptine was associated with reduced task-related activation in the parietal region during the 2-back task, and this was interpreted by the authors as possibly related to inhibitory effects (consistent with presynaptic effects of bromocriptine). However, with the dopamine manipulation used in the current study, reduced task-
related amplitude within the parieto-occipital region during the 2-back is more likely to be related to an increase in activation in this region. As outlined in the previous chapter, SSVEP reductions in amplitude (or alpha activity) have traditionally been interpreted as reflecting increased “activity” or mental processing, however such interpretations have been refined more recently with evidence that amplitude changes may be related to the “type” of cognitive process (Silberstein et al., 2001). Specifically, for intake tasks (where attention is paid to the external environment), findings have been associated with reductions in alpha, whereas internal tasks (where there is active rejection of the external environment and focus on internal content, such as working memory maintenance) have been associated with increases in alpha (Ray and Cole, 1985, Tesche et al., 1995). In light of the lack of increased amplitude in parieto-occipital region in the balanced/placebo condition (during the early delay time period) - indeed a small amplitude reduction was observed - it is unlikely that this region was being specifically recruited for holding information online during this time period. Rather, the reduction in parieto-occipital amplitude observed following D₁/D₂ receptor stimulation (under TPD conditions) most likely reflects a task-related increase in activation, of which the physiological significance is unclear. It could be suggested that the reduction in parieto-occipital amplitude reflects an increase in attention towards external stimulus, consistent with the proposal that amplitude reduction may reflect greater attendance to external stimulus (Ray and Cole, 1985). Increased distraction towards external stimuli in this region during the delay period may be expected to result in impaired performance, with consideration of the importance of the parietal region in SWM processes during delay periods (Wager and Smith, 2003). Equally, the reduction in parieto-occipital amplitude may also be reflective of a decrease in efficiency related to impaired performance, similar to the suggestion by Mehta et al. (2000) that a reduction in task-related activation in the parietal cortex following methylphenidate may reflect an increase in efficiency related to improved performance.²

² Furey et al. (2000a,b) proposed a similar relationship between improved performance and increased efficiency following acute modulation of the cholinergic system via physostigmine administration. Furey and colleagues observed increased activation within the visual cortex during the encoding period, and a subsequent reduction in activation within prefrontal cortex during the delay, and suggested that by producing a more robust visual percept during encoding, physostigmine may have increased efficiency of working memory maintenance (see Chapter 2 for discussion).
7.4.4 Summary

The main finding of this study is that TPD failed to modulate cortical electrophysiological activity during any of the temporal subcomponents observed during the SWM n-back task. These findings are consistent with a lack of TPD-related effects on rCBF during the SWM n-back reported in Chapter 5, and taken together with the apparent failure of TPD to modulate behavioural performance on the n-back task (in either the current experiment or the experiment reported in Chapter 5), and three different delayed-response tasks administered throughout this thesis, these findings question whether TPD has a measurable effect on SWM in the majority of participants. In contrast, despite the failure of TPD to modulate SSVEP, these findings demonstrated that pergolide (under conditions of TPD) caused an impairment in performance (consistent with the delayed-response task findings reported in Chapter 4), and altered fronto-temporal and parieto-occipital SSVEP during the early delay period of the 2-back task. Examination of the changes to the SSVEP signal suggest D_{1}/D_{2} stimulation (under conditions of TPD) increased localised inhibitory processes within the fronto-temporal region, and resulted in concurrent increases in parieto-occipital activation during 2-back task performance. The restriction of the modulatory effect to the early delay suggests that this effect is unlikely to be related to response preparation, and more likely reflects that pergolide (under conditions of TPD) interfered with mnemonic processes.
Chapter Eight

8 General discussion and conclusions

8.1 SUMMARY OF FINDINGS

This thesis presented a series of studies designed to further elucidate the role of the dopaminergic system in human SWM. Three methods of examining changes to SWM performance were employed in this thesis: behavioural cognitive testing, SSPT (to examine temporal changes in working memory related brain activity), and PET (to examine changes in activation in different regions of the brain during working memory).

The first experiment of this thesis had two aims: 1) to examine whether TPD-related impairment on the “Sternberg” SWM delayed-recognition task (as observed by Harrison et al. 2004) could be replicated in a larger sample, and 2) to extend upon previous research and examine whether stimulating D_1/D_2 receptors under dopamine depleted conditions can modulate SWM by “reversing” the proposed negative effects of TPD on SWM performance. The findings of Experiment 1 did not replicate the findings of Harrison et al. (2004) in which TPD impaired performance, which is consistent with the failure of two recent studies to replicate impairment on the SWM strategic search task as demonstrated by Harmer et al. (2001) (McLean et al., 2004, Roiser et al., 2004), and two additional studies which failed to observe TPD-related effects on delayed-response tasks (Lythe et al., 2005, Mehta et al., 2005a). However, contrary to the prediction of a positive (potentially reversing) effect of D_1/D_2 stimulation under dopamine depleted conditions, pergolide produced subtle impairments in SWM. These findings give insight into the effect of stimulating D_1/D_2 on SWM performance. Specifically, they highlight the importance of baseline dopamine levels, and likely reflect the fact that pergolide may act differently at D_1/D_2 receptors when tonic dopamine levels are lower than baseline.
The second experiment examined, for the first time, the effects of TPD on neural networks associated with SWM by examining changes to rCBF during a SWM n-back task. As the effects of TPD on SWM behavioural performance have been inconsistent to date, which may be related to response demands of tasks, this experiment also examined whether differences in response preparation and execution demands of delayed-response tasks resulted in differential effects of TPD on performance. As far as can be ascertained, this study represented the first examination of the effects of TPD on blood flow, and demonstrated that globally modulating tyrosine/phenylalanine levels had a complex effect on neurophysiology, which is likely to reflect not only changes in dopamine levels but compensatory actions of the catecholaminergic systems, and interactions with other neurotransmitter systems. However, despite widespread changes in both cortical and limbic regions following TPD, there was no evidence of specific effects on SWM neural networks or behavioural performance. Further, by demonstrating that TPD did not influence behavioural performance on three SWM tasks with different task demands, these findings suggest that task differences do not underlie the lack of effects observed.

The third and fourth experiments examined the effects of TPD on cortical electrophysiological activity during temporal subcomponents of the SWM n-back task using SSPT. Experiment 4 revealed that under baseline “no drug” conditions, the SWM n-back was associated with three distinct electrophysiological (SSVEP) time periods - an early perceptual/encoding period, when the stimulus was visible (approx. 0 – 500ms), and two addition time periods during the delay: an early period (approx. 850 – 1400ms) just following the stimulus disappearing from view, and a late period (approx. 2500 – 3500ms) lasting the final second of the delay and anticipation of the new stimulus. The cortical topography of these changes was consistent with previous studies of the SWM n-back task (Owen et al., 2005), and these findings further highlighted an important role of changes in SSVEP amplitude within the frontal cortex, which are likely to be related to underlying cortico-cortico loops. Experiment 5 demonstrated that TPD did not significantly modify 2-back task-related SSVEP (compared to placebo) during any of the three time periods. In contrast, these findings demonstrated that D₁/D₂ receptor stimulation (under TPD conditions) resulted in changes in fronto-temporal and parieto-occipital SSVEP, which were restricted to the early delay period. Examination of the changes to the SSVEP signal suggest D₁/D₂
stimulation (under conditions of TPD) increased localised inhibitory processes within the fronto-temporal region, and resulted in concurrent increases in activation of the parieto-occipital region during 2-back task performance. The restriction of the modulatory effect to the early delay suggests that this effect is unlikely to be related to response preparation, and more likely reflects interference with mnemonic processes.

In summary, the results of this thesis demonstrated that TPD failed to modulate SWM behavioural performance measures on four different SWM tasks. In addition, there was no evidence that TPD modulated either task-related rCBF or cortical electrophysiological activity during the SWM n-back task. In contrast, D<sub>1</sub>/D<sub>2</sub> receptor stimulation (under conditions of TPD) was observed to cause subtle impairments in performance on two SWM tasks, and in addition appeared to modulate SSVEP latency in the fronto-temporal region and SSVEP amplitude in the parieto-occipital region early in the delay period of the SWM n-back task.

8.2 GENERAL DISCUSSION AND IMPLICATIONS

8.2.1 Interpretation of “negative” findings

The primary dopaminergic manipulation employed in this thesis was TPD, and the dominant finding was that there was no evidence that TPD modulated SWM performance or neural networks. In terms of behavioural results, these findings are not entirely surprising, and fit within the developing literature of inconsistent behavioural effects of TPD on SWM performance. Following the first study to examine TPD effects on SWM (Harmer et al. 2004), in which TPD-related deficits were observed on both a SWM delayed-recognition task and a task requiring self-ordered strategic search, only one study has supported a clear impairing effect of TPD at group level analysis (Harrison et al. 2004; who observed impaired accuracy on the SWM delayed-recognition task used in Experiment 1 of this thesis). In contrast, a number of studies have failed to observe a measurable change in performance at group level analysis (McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Mehta et al., 2005a).

Nevertheless, it must to be considered whether the lack of behavioural result observed in this thesis actually reflects a failure of TPD to modulate SWM, or was the result of methodological issues, specifically in light of the failure to replicate the findings of
the Harrison et al. (2004) study. Examination of the methodology used in the experiments by Harrison et al. (2004) and Harmer et al. (2001) reveals that the protocols are highly similar to the experiments reported in this thesis (indeed, the Harrison et al. (2004) experiment was performed within the same laboratory as the current experiment). As discussed within Chapter 4, the lack of behavioural effect was unlikely to be due to either insufficient depletion of plasma tyrosine, insufficient sample size or insensitivity of the tasks used. Indeed, the plasma analysis in Experiment 1 demonstrated tyrosine depletion levels which were comparable to those demonstrated by Harrison et al. (2004) and Harmer et al. (2001). But perhaps more importantly, examination of the literature indicates that the TPD protocol reliably depletes plasma tyrosine levels, with all studies within the literature reporting robust significant depletion of tyrosine and phenylalanine levels (or the important ratio between tyrosine/phenylalanine and other large neutral amino acids) in the TPD condition compared to the balanced/placebo condition in the order of p<0.001 (Leyton et al., 2000, Harrison et al., 2004, Leyton et al., 2004a, Leyton et al., 2004b, McLean et al., 2004, McTavish et al., 2004, Roiser et al., 2004, Lythe et al., 2005, McTavish et al., 2005, Mehta et al., 2005a, Roiser et al., 2005). Further, Mehta et al. (2005a) recently demonstrated that while striatal dopamine levels may be related to behavioural performance, there was no correlation between the amount of plasma tyrosine depletion and performance change. It seems unlikely therefore that the lacks of effects on SWM are related to plasma tyrosine levels.

As discussed in Chapter 4, only one clear source of difference between is noted between the Harrison et al. (2004) and current studies; that is, Harrison et al. (2004) employed a female sample, while the current thesis used a male sample (Harmer et al. (2001) employed a mixed gender sample). While Harmer et al. (2001) reported no interaction of gender with cognitive performance in their findings of TPD-related working memory impairments, the size of the groups (7 males, 5 females) does limit the generalisability of this finding and it remains possible that males were more resistant than females to the effects of dopamine depletion on this delayed-recognition SWM task. The other studies reported within the literature (McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005) have tested samples comprising of both males and females, and did not report gender effects. Nevertheless, a gender related effect cannot be ruled out.
It should be noted that the lack of effects following TPD are unlikely due to insensitivity of the tasks to pharmacological manipulation. While performance on all tasks was high, this should not preclude impairment following TPD manipulation. Further, pre-treatment accuracy levels on the “Sternberg” delayed-recognition task used in this study were the same in the Harrison et al. (2004) study (96%) (which preceded a 6% worsening of performance following TPD). Similarly, accuracy on the 2-back tasks and the delayed-recall and delayed-recognition were less than 90%, which is reflective of the difficulty of these tasks and in line with accuracy levels observed in the Harmer et al. (2001) SWM recognition task (approx. 88% pre-treatment). In addition, within our laboratory the Sternberg delayed-recognition task, and the n-back task (used in the SSPT experiments) have recently been shown as susceptible to acute pharmacological (cholinergic) manipulation, with impaired performance observed following administration of 0.4mg scopolamine in healthy young participants (Ellis et al., 2005a, Green et al., 2005).

Further, it appears highly unlikely that differences in task demands underlie the lack of effects observed. Overall, the findings of this thesis demonstrated that D₁/D₂ receptor stimulation (under conditions of TPD) impaired both tasks assessed (delayed-recognition and n-back), while TPD failed to influence performance on any of the four tasks assessed (which included the two tasks modulated by the other dopamine manipulation). This indicates that the lack of effect of TPD was not related to specific task related differences such as the presence or absence of motor response requirements (specifically addressed in Experiment 2), or differences in the level of manipulation included in the task (deduced from comparison of the n-back to the delayed-response tasks). However, these findings cannot conclude that task differences do not influence the effects of dopaminergic modulation. As noted by Luciana et al. (1997) (and highlighted by patients with PD), the role of dopamine in motor activity is critical, and manipulation of dopamine may influence performance through effects on motor response. Further, Wang et al. (2004) recently presented evidence that the D₂ receptor influences working memory performance during the response stage (and not mnemonic stage) (Wang et al. 2004). Thus manipulation that more specifically target the D₂ receptor (i.e. the D₂ antagonist sulpiride or D₂ agonist bromocriptine) may be more greatly influenced by differences in task demand. Indeed it is possible that variation in task demands between the n-back and delayed-response
task may have resulted in different mechanisms of effect of D₁/D₂ receptor stimulation (under TPD conditions) on SWM performance of these tasks. Nevertheless, the current findings indicated that with the manipulations used in this study (which target both D₁/D₂ receptors and/or global dopamine depletion), task differences may be less important than the degree of dopaminergic modulation achieved by these manipulations.

In summary, this thesis demonstrated that TPD failed to modulate perhaps the three most prevalent SWM paradigms within the literature: delayed-recall, delayed-recognition (on 2 different delayed-recognition tasks, one of which was previously modulated by TPD) and the SWM n-back task, and there are no clear methodological reasons to explain the lack of effects on behaviour. The advantage of the findings of this thesis is that in addition to behavioural measures, the effect of TPD was examined with two different neuroimaging measures. Indeed, a lack of behavioural effect alone may be masking underlying effects of TPD on task-related brain activity, occurring in the underlying SWM networks at either a neural network or temporal cortical level which are not reflected in behavioural performance. However, in addition to not seeing a behavioural effect on performance on multiple task paradigms, the findings of this thesis demonstrated that TPD did not significantly disrupt the underlying neural networks or temporal cortical electrophysiological activity associated with the n-back task. This was not due to insufficient task activation, as the n-back produced robust changes in both rCBF and SSVEP in all conditions. Taken together, these results indicate that TPD did not cause measurable effects in SWM behavioural performance or underlying neural networks in this thesis.

8.2.2 The inverted U response function

**TPD-related findings**

Previous evidence in both rats and humans suggests that tyrosine depletion is a highly effective method of depleting plasma tyrosine levels (e.g. Biggio et al., 1976, Moja et al., 1996, Sheehan et al., 1996, McTavish et al., 1999a, McTavish et al., 1999b, McTavish et al., 1999c, Harmer et al., 2001, McTavish et al., 2001b, Harrison et al., 2004, McLean et al., 2004, Roiser et al., 2004, Lythe et al., 2005, Roiser et al., 2005). In addition, there is evidence that TPD-induced decreases in plasma tyrosine levels can result in decreases in dopamine levels. For example, studies show that reducing
the availability of tyrosine and phenylalanine consequently reduces the synthesis and release of dopamine in rats (Milner et al., 1986, Tam and Roth, 1997, McTavish et al., 1999a, McTavish et al., 1999b, McTavish et al., 1999c). In humans, evidence further indicates that TPD reduces dopamine release within the human striatum (Montgomery et al., 2003), and reduced d-amphetamine-induced dopamine release following TPD in humans (Leyton et al., 2004b), as assessed by changes in [11C]raclopride binding potential (BP), a measure of dopamine D₂/D₃ receptor availability. The findings of this thesis do not contradict evidence that TPD may influence dopamine levels; indeed, the findings presented in Chapter 5 suggest that TPD causes widespread changes to rCBF in both limbic and cortical regions which may be related at least in part to dopaminergic changes.

It is however suggested that the apparent failure of TPD to modulate SWM performance may reflect that TPD does not produce enough dopamine depletion to consistently and robustly modulate SWM networks in healthy humans. These findings may be best considered within the inverted U response function (also referred to as the “optimal level of dopamine” model). The inverted U is perhaps the most prominent model describing dopamine modulation of working memory and was first proposed in response to findings in the non-human primate, in which impairments in SWM performance may be observed following high dosages of dopamine D₁ antagonists, while low dosages may improve performance (an effect that could then be reversed by a partial D₁ agonist) (Williams and Goldman-Rakic, 1993, Williams and Goldman-Rakic, 1995). The critical aspect of this model is that performance is optimised when dopamine levels are within a specific or “optimal” range. Simply put, the findings of this thesis may reflect that TPD does not cause dopamine levels to fall outside of the optimal range in the majority of participants.

As discussed in Chapter 5, while TPD has been observed to deplete dopamine levels, the magnitude of depletion is significantly less than that seen following AMPT (a more aggressive method of depletion which depletes both noradrenaline and dopamine levels and results in subtle Parkinsonian like symptoms; see, Verhoeff et al. 2003). Specifically, AMPT has been observed to cause approximately 25% increase in the binding potential of the SPECT radiotracer [123I]IBZM (Laruelle et al., 1997a), and previous evidence in non-human primates suggests that a 25% change in
[123I]IBZM binding potential following AMPT corresponds to approximately 50% change in striatal extracellular dopamine concentration, as measured by microdialysis (Laruelle et al., 1997b). In contrast, TPD has been observed to cause approximately 6% change in [11C]raclopride binding, which is likely to correspond to approximately 10%–20% reduction in dopamine concentrations (Montgomery et al., 2003). In a recent study, Mehta et al. (2005a) demonstrated that worsening of performance was only evident in participants with high levels of striatal dopamine depletion. Specifically, changes in performance were only observed for participants with a high dopamine depletion level, but virtually no change (and/or subtle improvement) in participants with minimal [11C]raclopride binding changes. These findings suggest that TPD only influenced performance if the resulting dopamine depletion was of a large magnitude, and that TPD only caused depletion with great enough magnitude in some individuals. There were no brain regions identified in the PET experiment reported in Chapter 5 in which rCBF changes carry a similar predictive value. However, this may be related to the lower selectivity of rCBF measures compared to [11C]raclopride imaging for dopamine-related changes.

**Pergolide under conditions of TPD findings**

The findings of impairments following D₁/D₂ stimulation (under conditions of TPD) also fit well within the “optimal” level of dopamine for optimal performance model. Evidence in the non-human primate indicates that the spatial tuning of prefrontal neurons engaged in SWM is enhanced at moderate levels of D₁ occupancy and reduced at both lower and higher levels of occupancy (as discussed in Chapter 2; for review, see Goldman-Rakic 1996). While the exact mechanisms by which stimulation of D₁/D₂ receptors (under conditions of TPD) impaired SWM performance are unknown, as discussed in Chapter 4 it is likely that this effect was related to over activation of D₁ receptors (due to sensitisation of the system following dopamine depletion) and/or actions of pergolide at D₂ autoreceptors reducing dopamine activity, and either of these mechanisms may have disrupted spatial tuning. These findings highlight the complexity of functional effects of dopamine augmentation within dopamine depleted states, as exemplified in treatment of PD patients in which dopamine treatment impairs, improves or has no effect on performance on a variety of tasks. Cools et al. (2002, 2003) have proposed that detrimental effects of dopamine medications on some tasks may be related to overdosing the relatively intact ventral...
striatum and its connections to the ventral PFC, as early in the onset of PD, dopamine depletion is restricted to the putamen and the dorsal caudate nucleus and only later does it progress to the more ventral striatum and the mesocorticolimbic system (Kish et al., 1988, Agid et al., 1993).

The nature of the effect of D₁/D₂ stimulation (under conditions of TPD) on SWM was more clearly established in Experiment 4 (Chapter 7), which assessed changes in SWM task-related activity following dopamine modulation using SSPT. These results demonstrated that dopaminergic modulation did not disrupt the temporal SSVEP profile of the 2-back task, but altered fronto-temporal and parieto-occipital SSVEP during the early delay period of the task. Specifically, task-related SSVEP latency increases were observed in fronto-temporal regions, and SSVEP amplitude reductions were observed within the parieto-occipital region. These findings are consistent with the well established role of frontal and parietal network in SWM n-back performance (for a review, see Owen et al., 2005). Further, modulation of fronto-temporal and parieto-occipital regions by dopaminergic manipulation is consistent with evidence of changes in working memory task-related BOLD signal in the parietal region following administration of bromocriptine (Kimberg et al. 2001), and is in line with changes in rCBF within the PFC (Mattay et al., 2000, Mehta et al., 2000, Schweitzer et al., 2004) and posterior parietal cortex (Mehta et al., 2000) following psychomotor stimulants methylphenidate and amphetamine (indirect catecholamine agonists). Examination of the changes to the SSVEP signal suggest D₁/D₂ stimulation (under conditions of TPD) increased localised inhibitory processes within the fronto-temporal region, and resulted in concurrent increases in parieto-occipital activation during 2-back task performance. However, the major contribution of this finding is that the effect of this dopaminergic modulation was restricted to the early delay, not during the entire delay period. While the n-back cannot be delineated to a point at which one can identify which specific sub-processes are occurring early in the delay or late in the delay (as stated in the discussion of Chapter 6), it is likely that the early delay is related more closely to mnemonic than response preparation processes, and therefore that the stimulation of D₁/D₂ receptors (under TPD conditions) was likely to be related more closely to disruption of mnemonic function.
8.2.3 Implications for future research

The current findings do not support TPD as a reliable or consistent method of depleting dopamine and examining possible changes in SWM behavioural performance in healthy human volunteers. While there is previous evidence to suggest that TPD causes depletion of dopamine (Montgomery et al., 2003, Mehta et al., 2005a), and evidence presented in Chapter 5 suggests TPD results in widespread cortical and limbic blood flow changes, overall the level of dopamine depletion achieved with TPD does not appear to produce reliable or consistent changes in SWM performance or associated brain activity in the majority of individuals. However, TPD may prove a more useful manipulation for use in patient groups; specifically in groups in which dysfunction of the dopaminergic system is suggested. Indeed, recent evidence suggests that in recovered depressed women, TPD caused SWM impairment (McTavish et al., 2005). In addition, preliminary evidence suggests that TPD may be effective in reducing symptoms in patients with mania (McTavish et al., 2001a).

It appears likely that the contribution of underlying dopamine levels within the cortico-cortico and cortico-striatal systems, and differences between individuals in their baseline dopaminergic states may be highly influential in whether acute dopamine challenges enhance, impair or have no effect on working memory performance. A limitation of this thesis therefore is that there was no measure of baseline dopamine levels in each individual, and hence no way to assess the effect of each manipulation at an individual level. Future research which attempts to incorporate a measure of individual dopaminergic function both pre- and post-pharmacological challenge may be most successful in elucidating the effect of dopaminergic manipulations of SWM. Behavioural measures such as baseline working memory levels are perhaps the most accessible measure, and have been used with some success in discriminating between the effects observed in participants following dopamine manipulation (Kimberg et al., 1997, Mehta et al., 2000, Kimberg and D'Esposito, 2003). However there was no evidence of such effects within this thesis, or in a recent study of the effects of sulpiride on working memory (Mehta et al., 2000), and as stated by Kimberg and D'Esposito (2003) differences between individuals with high and low baseline working memory levels may be dependent on factors such as concentrations of drug and time of cognitive testing (in respect to kinetic effects of the drug). More direct assessment of dopamine functions, such as
the functional polymorphism (Val/Met) in the COMT gene, or assessment of changes in striatal dopamine levels using PET receptor imaging (as conducted by Mehta et al., 2005a) may prove more reliable measures of individual responses to dopaminergic manipulation. Indeed, Mattay et al. (2003) demonstrated that participants with the Val/Val genotype (and presumably less prefrontal dopamine) showed improved efficiency of PFC function associated with the working memory task following dextroamphetamine, while participants with Met/Met genotype (and presumably higher prefrontal dopamine) were impaired on the 3-back task and revealed less efficient PFC task-related function.

8.2.4 Concluding remarks

The findings of this thesis question the utility of TPD as a probe of dopaminergic and SWM function in healthy humans. This conclusion is based on evidence that while TPD demonstrated considerable cortical and subcortical effects on blood flow, and previous evidence suggests that TPD may deplete dopamine levels (and indeed working memory performance), it appears that the extent of dopamine depletion achieved with TPD may not be adequate to disrupt dopamine levels and therefore SWM neural networks and performance, in the majority of participants.

The current research findings have highlighted the utility of using brain imaging in conjunction with behavioural measures to assess changes in SWM neurophysiology following dopamine modulation. These findings indicate that D₁/D₂ receptor stimulation (under conditions of TPD) influences both performance and cortical neurophysiology early in the delay, and also highlights the importance of underlying levels of dopamine on the effect of acute pharmacological challenge of the system.

It appears evident that the effects of manipulating dopamine function, and consequently working memory performance, are intricately linked to the effects of the manipulation on “optimal dopamine levels” within individuals. Therefore, further insight into the role of dopamine in working memory may be best gained through research that uses a combination of behavioural and neuroimaging measures of performance, in addition to measures of changes to dopamine function of individuals.
9 References


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10 Appendices
APPENDIX 1: Personal information/Screening questionnaire.

A  Version used in experiments presented in Chapters 4, 6 and 7
B  Version used in experiment presented in Chapter 5
NOTE: 1. Personal details will remain confidential
2. For questions requiring a YES / NO response, please CIRCLE the correct response

Today’s Date ________________________________ Time ________________________________

Date of birth __________ Age __________ Sex _______ Handedness _______

Height __________ Weight __________

Have you been a subject for any type of study at Hammersmith Hospital before?  Y / N
If yes, how long ago ________________________________

Occupation ________________________________

No. of years of primary education (eg. 6) ________________________________

No. of years of secondary education (eg. 6) ________________________________

No. of years of tertiary education (eg. 3) ________________________________

Academic qualifications (Year 11, VCE, B.App.Sci., etc) ________________________________

Do you presently suffer or have you ever suffered from epilepsy?  Y / N
If yes, specify ________________________________

Do you presently suffer or have you ever suffered from hypertension?  Y / N

Do you have a colour deficiency?  Y / N
If yes, specify ________________________________

Do you have any other visual defects (short sightedness, lazy eye, etc)?  Y / N
If yes, specify ________________________________

Have you ever sustained a serious head injury?  Y / N
If yes, specify ________________________________

Do you presently suffer or have you ever suffered from any neurological or psychiatric disorders?  Y / N
If yes, specify ________________________________

Are you a smoker?  Y / N
If yes, indicate the last time you smoked ________________________________

Have you consumed tea or coffee today?  Y / N
If yes, indicate time of last consumption ________________________________

Have you had any recent illness?  Y / N
If yes, specify ________________________________
Do you currently take any prescription drugs?  
Y / N
If yes, specify ____________________________________________________________

B.

Protocol Code: TPD and H$_{2}^{15}$O

PERSONAL INFORMATION SHEET
SUBJECT CODE

NOTE:  
1. Personal details will remain confidential  
2. For questions requiring a YES / NO response, please CIRCLE the correct response

Name ____________________________________________
Today’s Date ___________________________ Time ________________
Date of birth __________ Age ___________ Sex ___ Handedness ________
Height ______________ Weight ____________.

Have you been a subject for any type of study at Hammersmith Hospital before?  
Y / N
If yes, when was participation? ____________ What was the investigators name? _______

Occupation

No. of years of primary education (eg. 6) ________________________
No. of years of secondary education (eg. 6) ________________________
No. of years of tertiary education (eg.3) __________________________
Academic qualifications (Year 11, VCE, B.App.Sci., etc) ______________

Do you presently suffer or have you ever suffered from epilepsy?  
Y / N
If yes, specify __________________________________________________________

Do you presently suffer or have you ever suffered from hypertension?  
Y / N

Do you have a colour deficiency?  
Y / N
If yes, specify __________________________________________________________

Do you have any other visual defects (short sightedness, lazy eye, etc)?  
Y / N
If yes, specify __________________________________________________________

Have you ever sustained a serious head injury?  
Y / N
If yes, specify __________________________________________________________

Do you presently suffer or have you ever suffered from any neurological or psychiatric disorders?  
Y / N
If yes, specify __________________________________________________________

Are you a smoker?  
Y / N
If yes, indicate the last time you smoked _________________________________

Have you consumed tea or coffee today?  
Y / N
If yes, indicate time of last consumption _________________________________
Have you had any recent illness?  \textbf{Y / N}  
If yes, specify

Do you currently take any prescription drugs?  \textbf{Y / N}  
If yes, specify

On average, how many glasses of alcohol would you drink a week? ________________

Have you ever used any of the following (all information is confidential)  
- Cannabis  \textbf{Y/N}  
- Amphetamines  \textbf{Y/N}  
- MDMA (Ecstasy)  \textbf{Y/N}  
- Cocaine  \textbf{Y/N}  
- Heroin  \textbf{Y/N}  

Please provide your GP’s address if you authorise us to inform your GP of your participation in this study?

__________________________________________________________________________
__________________________________________________________________________
APPENDIX 2: Side effects questionnaire.
## Symptom Checklist

SUBJECT CODE: ___________________  TIME POST AMINO ACIDS
______________
SESSION NUMBER _______  DRUG CONDITION _______
DATE: _____ / _____ / _____  TIME _____________

Please indicate how you currently feel on the symptoms listed below using the following ratings:

1 = Not at all  2 = Somewhat  3 = Unsure  4 = Moderately  5 = Very much so

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Not at all</th>
<th>Somewhat</th>
<th>Unsure</th>
<th>Moderately</th>
<th>Very much so</th>
</tr>
</thead>
<tbody>
<tr>
<td>I HAVE A HEADACHE</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I FEEL COLD</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I FEEL HOT</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I FEEL DIZZY</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I AM SWEATING MORE THAN USUAL</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I HAVE BLURRED VISION</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I FEEL NAUSEOUS</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>MY HEART IS RACING</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I HAVE A DRY MOUTH</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I HAVE STOMACH PAINS</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I FEEL TIRED</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
APPENDIX 3: Additional SPM analysis
1) Task related effects, as analysed using repeated measures design (and as reported in Chapter 5 – top row) vs. replicated analysis using a design that considers TPD and BAL as separate groups (bottom row)
2) Analysis of TPD main effects conducted on N=9 (systematically removing each participant).

TPD-related increases, minus participant 1

SPM\{T_{120}\}

SPM\{T_{120}\}

SPMresults: Abtest CINpH9W minus as1
Height threshold T = 4.85
Extent threshold k = 6 voxels

TPD-related decreases, minus participant 1

SPM\{T_{120}\}

SPM\{T_{120}\}

SPMresults: Abpost CINpH9BMM minus as1
Height threshold T = 4.85
Extent threshold k = 6 voxels
TPD-related increases, minus participant 2

SPM results: 19 subjects check global
Height threshold $T = 4.83$
Extent threshold $k = 5$ voxels

TPD-related decreases, minus participant 2

SPM results: 19 subjects check global
Height threshold $T = 4.83$
Extent threshold $k = 5$ voxels
TPD-related increases, minus participant 3

SPM results: post CN PnHBM minus ss3
Height threshold $T = 4.84$
Extent threshold $k = 5$ voxels

TPD-related decreases, minus participant 3

SPM results: post CN PnHBM minus ss3
Height threshold $T = 4.84$
Extent threshold $k = 5$ voxels
TPD-related increases, minus participant 4

SPM($T_{120}$)

SPMResults: /post CINPtHBM minus ss 5
Height threshold $T = 4.85$
Extent threshold $k = 5$ voxels

TPD-related decreases, minus participant 4

SPM($T_{120}$)

SPMResults: /post CINPtHBM minus ss 5
Height threshold $T = 4.85$
Extent threshold $k = 5$ voxels
TPD-related increases, minus participant 5

SPM\{T_{120}\}

SPMresults: /post CINPnHBM/minus ss 6
Height threshold T = 4.85
Extent threshold k = 5 voxels

TPD-related decreases, minus participant 5

SPM\{T_{120}\}

SPMresults: /post CINPnHBM/minus ss 6
Height threshold T = 4.85
Extent threshold k = 5 voxels
TPD-related increases, minus participant 6

SPM\{T_{120}\}

**SPMresults**: /post CINPnPnHBM/minus ss 7
Height threshold \( T = 4.85 \)
Extent threshold \( k = 5 \) voxels

TPD-related decreases, minus participant 6

SPM\{T_{120}\}

**SPMresults**: /post CINPnPnHBM/minus ss 7
Height threshold \( T = 4.85 \)
Extent threshold \( k = 5 \) voxels
TPD-related increases, minus participant 7

SPM\{T_{120}\}

SPMresults:/post CNFpHBM minus ss 8
Height threshold \( T = 4.85 \)
Extent threshold \( k = 5 \) voxels

TPD-related decreases, minus participant 7

SPM\{T_{120}\}

SPMresults:/post CNFpHBM minus ss 8
Height threshold \( T = 4.85 \)
Extent threshold \( k = 5 \) voxels
TPD-related increases, minus participant 8

SPM results: \( \tilde{N} \) \( \text{post} \text{ CINPnHB} \) \text{CINPnHB} \text{minus ss 9} \\
Height threshold \( T = 4.85 \) \\
Extent threshold \( k = 5 \) voxels

TPD-related decreases, minus participant 8

SPM results: \( \tilde{N} \) \( \text{post} \text{ CINPnHB} \) \text{CINPnHB} \text{minus ss 9} \\
Height threshold \( T = 4.85 \) \\
Extent threshold \( k = 5 \) voxels
TPD-related increases, minus participant 9

SPM$\{T_{120}\}$

**SPMresults:** /post CINPnHBMminus ss1.0  
Height threshold $T = 4.87$  
Extent threshold $k = 5$ voxels

TPD-related decreases, minus participant 9

SPM$\{T_{120}\}$

**SPMresults:** /post CINPnHBMminus ss1.0  
Height threshold $T = 4.87$  
Extent threshold $k = 5$ voxels
TPD-related increases, minus participant 10

SPM\{T_{120}\}

**SPMresults**: /post CNPnHBM/minus 11
Height threshold $T = 4.84$
Extent threshold $k = 5$ voxels

TPD-related decreases, minus participant 10

SPM\{T_{120}\}

**SPMresults**: /post CNPnHBM/minus 11
Height threshold $T = 4.84$
Extent threshold $k = 5$ voxels
3) Main effect of TPD (increases) for control task (first glass brain image), 1-back (second image) and 2-back (third image) only. These analyses resemble the main effect as observed for all tasks combined (as reported in Chapter 5), but with less power due to less averages.
**SPMresults** for different nus variable

Height threshold $T = 4.81$
Extent threshold $k = 5$ voxels
The pharmacology of human working memory

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Abstract

Experimental studies conducted primarily on non-human primates have begun to address the anatomical and neurochemical correlates of working memory. There is an associated growing body of experimental literature investigating whether modulating key neurotransmitters can facilitate working memory in humans. This paper reviews evidence that acute modulation of dopamine in particular, but also noradrenaline, acetylcholine and serotonin may influence working-memory performance in humans. Differences in neurochemical specificity with regard to stages of working memory, type of working memory (spatial or non-spatial) and cortical effects are also discussed. This evidence has contributed to neuropharmacological understanding of working memory in humans. The important therapeutic consequences of a better understanding of facilitation of working memory is discussed in reference to schizophrenia, Parkinson’s disease and Alzheimer’s disease.

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Key words: Acetylcholine, dopamine, human working memory, pharmacology, serotonin.

Introduction

Working memory is the ability to maintain or hold temporary, active representations of information for further processing or recall (Baddeley, 1986; Just and Carpenter, 1992). The process is thought to have two components, short-term storage (generally in the order of seconds), and executive processes that operate on the stored material (Baddeley, 1992). Working memory is mediated by a widely distributed neural system in the human brain (Baddeley, 1986). Functional brain-imaging studies of humans have identified cortical regions that are involved in spatial and non-spatial working memory including occipital, temporal, parietal and prefrontal cortical areas (Friedman and Goldman-Rakic, 1994; Haxby et al., 1995; Petrides et al., 1993). Functional imaging studies also suggest that these regions maybe involved in different stages of working memory, with the occipitotemporal cortex involved in perceptual processing, and prefrontal cortex (PFC) involved with maintaining an active representation over the delay period (Courtney et al., 1997; Funahashi et al., 1993; Haxby et al., 1995; Miller et al., 1993; Shindy et al., 1994). Evidence also suggests that individual neurons show elevated persistent and tuned activity (memory fields) during components of working memory (Goldman-Rakic et al., 1989). These memory fields may be modulated by many neurochemical systems (Goldman-Rakic et al., 1990), including dopamine (Williams and Goldman-Rakic, 1993, 1995), serotonin (Jakab and Goldman-Rakic, 1998), noradrenaline (Arnsten and Goldman-Rakic, 1986), acetylcholine (Ragozino, 2000), GABA (Rao et al., 2000) and glutamate (Seemans et al., 2001) in highly differentiated ways, suggesting that modulation of these neurochemical systems may affect different stages of working memory. There is a growing body of experimental literature focusing on the effects of acute modulation of these neurotransmitter systems on working-memory performance. To date, there has been no systematic review of the pharmacology of working memory.

This review is not intended to cover in detail the anatomical or cellular basis of working memory (for an anatomical review, see Baddeley, 1998; for a cellular review, see Goldman-Rakic, 1995, 1996), nor does it intend to focus in detail on the conceptual basis of working memory (for a review, see Baddeley and Hitch, 1974). The major aim of this review is to provide a broad overview of what is known about the pharmacology of human working memory from acute drug challenge studies in healthy human subjects. However, data from animal studies and clinical studies will be reviewed when literature on acute human challenges is limited, in order to compile a basic blueprint of the pharmacology of working memory, and to suggest a broad direction for future research in humans. This review will consider the broad construct of working memory, which encompasses both spatial and non-spatial domains. However, there are
possible differences in the neurochemical substrates that may subserve different domains, and the type of working memory used in between studies will therefore be highlighted.

The paper has three sections. The first and by far largest section of the review concentrates on a selection of neurotransmitter systems, which affect human working-memory performance when modulated by acute pharmacological agents. The most widely studied neurochemical system for working memory is the dopaminergic system, and the most commonly employed task paradigm is visuo-spatial working memory. These aspects will therefore form a majority of the review. However, there is also evidence to suggest that acute modulation of the noradrenergic, cholinergic and serotonergic systems can alter working-memory performance in humans. Animal studies suggest possible roles for the NMDA–glutamate system and GABA involvement, which will be discussed in the context of possible future directions for human studies. The second section will discuss directions for future research, with particular emphasis on the emerging importance of brain imaging in working-memory research. The final section of the review highlights the importance and possible clinical implications for individuals with schizophrenia, Parkinson’s disease and Alzheimer’s disease, of improving working-memory function with selective pharmacological agents.

The dopaminergic system

**D₁ vs. D₂ dopamine receptor agonists**

The most studied neurochemical system for working memory is the dopaminergic system. It has been widely reported that increasing dopamine levels in human subjects facilitates working-memory performance (Luciana et al., 1992, 1998; Luciana and Collins, 1997; Muller et al., 1998). However, the relative role of D₁ and D₂ dopamine receptors in modulating working memory is yet to be clarified in humans. Evidence suggesting that D₂ dopamine receptor agonists may facilitate working memory is generally based on studies using bromocriptine, a specific D₂ receptor agonist. The first of such studies was conducted by Luciana et al. (1992), who investigated the effect of an acute oral dose of 2.5 mg of bromocriptine on a sample of 8 young, healthy females, in their performance of a visuo-spatial delayed response task. The task involved presentation of a visual cue (a black dot) on a computer screen. The cue was removed for a delay of either 0 or 8 s. After the delay period, the subjects indicated the screen location of the cue with a fine-pointed light pen. The authors hypothesized that if bromocriptine influenced the working-memory component of the spatial task, bromocriptine administration would improve accuracy in the 8-s delay condition, and would have little effect in the 0-s delay condition. The authors observed a 44% improvement in the accuracy of identifying the cue location in the 8-s bromocriptine condition, compared to placebo. No improvement was seen in the 0-s delay condition following bromocriptine administration, and it was inferred that the D₂ receptor agonist might have facilitated spatial working-memory performance. However the small sample size of the study limited the generalizability of these results.

In a subsequent study, using a larger sample of 66 young adults (aged 19–37 yr), Luciana and Collins (1997) provided additional evidence for a facilitating role of D₂ receptor agonists. However in this study the acute dose of 2.5 mg bromocriptine, which had facilitated performance in the earlier study (Luciana et al., 1992), did not appear to facilitate performance in the replication study. It was observed that performance accuracy was improved following administration of a smaller dose of bromocriptine (1.25 mg). More recently, Luciana et al. (1998), in a sample of 38 volunteers, again observed a facilitating effect of 1.25 mg of bromocriptine on spatial working memory when behavioural testing occurred between 3.5 and 5.5 h after drug administration. The authors suggested that the discrepancy between studies might be due to differences in the time of cognitive testing (Luciana and Collins, 1997). In the first study (Luciana et al., 1992), the delayed response task was administered between 2.5 and 3.5 h after drug administration, while in the subsequent studies (Luciana and Collins, 1997; Luciana et al., 1998) the delayed response task was administered between 3.5 and 5.5 h after drug administration. Luciana and colleagues argued that in the former study (which used a high dose of 2.5 mg and tested 1 h earlier), testing may have taken place while bromocriptine levels were ‘sub-maximal’, and the cognitive effects may be comparable to those for ‘maximal’ levels of 1.25 mg of bromocriptine in the latter studies.

This suggests that there may be an inverted U dose-related response of bromocriptine on spatial working-memory performance, with low doses facilitating performance and higher doses having no effect or an impairing effect. However, it should be noted that an apparent inverted U shape response may also be explained as the superimposition of an inverse dose-related sedative effect, in addition to the putative dose-related cognitive effects. Sedation has been widely associated with increased dopamine levels (Canales and Iversen, 2000; Schapira, 2000). Indeed, Luciana and Collins (1997) noted in their study that adverse effects of bromocriptine at ‘high’ levels (2.5 mg) resulted in a 50% withdrawal rate of subjects, and pointed out that this decreased the statistical power of analysis in the 2.5 mg
group. Primate and non-primate studies have also described an inverted U dose-related response, with either insufficient or excessive dopamine receptor stimulation reported to be detrimental to working-memory function (Arnsten and Goldman-Rakic, 1986; Cai and Arnsten, 1997; Goldman-Rakic, 1996; Murphy et al., 1996). It is likely that the complex effects of dopaminergic modulation of working memory is related to the disruption of tuned activity or memory fields that are regulated by an optimal level of functioning of neurochemical systems, including dopamine.

In contrast to the possible dose- and time-related effects of bromocriptine on working memory, Kimberg et al. (1997) observed performance responses following bromocriptine that appear to be dependent on the baseline working-memory capacity of the subject. In this study, a sample of 31 normal human subjects was divided into two groups, either high working-memory span or low working-memory span, on the basis of their scores on a verbal working-memory task. Each subject was tested on a variety of tasks, which were sensitive to prefrontal function (a card sorting task, associative memory task, context memory task, and a Stroop task), in addition to a spatial working-memory task similar to the delayed response task used by Luciana et al. (1992). In this study, an acute dose of 2.5 mg of bromocriptine showed an interesting pattern of effects on performance of these tasks. Subjects with a high- baseline working memory, as assessed by their performance on a verbal working-memory test, performed more poorly while under the influence of bromocriptine compared to placebo, while subjects with low-baseline working memory performed better following bromocriptine administration compared to placebo. While the spatial working-memory task was not significantly affected by baseline memory capacity when analysed separately, there was a trend similar to that observed with the other tasks. However this trend was not supported in a more recent study by the same authors (Kimberg et al., 2001). In this more recent study, the opposite effects to that observed in the former study was found, with subjects with higher baseline memory capacity actually showing improved performance following bromocriptine administration.

The authors suggested that the contradictory results between the two studies might be explained by differences in the task and time of testing. However a major limitation of this study is the relatively small sample used, which might influence the results. However, the two studies might be explained by differences in the context memory task, and a Stroop task), in addition to a spatial working-memory task similar to the delayed response task used by Luciana et al. (1992). In this study, an acute dose of 2.5 mg of bromocriptine showed an interesting pattern of effects on performance of these tasks. Subjects with a high- baseline working memory, as assessed by their performance on a verbal working-memory test, performed more poorly while under the influence of bromocriptine compared to placebo, while subjects with low-baseline working memory performed better following bromocriptine administration compared to placebo. While the spatial working-memory task was not significantly affected by baseline memory capacity when analysed separately, there was a trend similar to that observed with the other tasks. However this trend was not supported in a more recent study by the same authors (Kimberg et al., 2001). In this more recent study, the opposite effects to that observed in the former study was found, with subjects with higher baseline memory capacity actually showing improved performance following bromocriptine administration.

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receptors, and the observed effects on working memory, highlights the more prominent role for D_1 receptors in working memory.

Unfortunately, the lack of an appropriate pharmacological tool, such as a potent D_1 agonist (Muller et al., 1998), has limited the ability of researchers to directly investigate the effects of D_1 receptor stimulation in humans to date. The evidence presented thus far indicates that D_2 receptor stimulation may facilitate working-memory performance. There is also an indication of an inverted U dose-related response, with facilitation of working memory more often observed for lower plasma concentrations of D_2 receptor agonists. However, it must be noted that the effects of dopamine agonists on working memory is dependent on a variety of factors including dose–response effects, time-spans for behavioural testing, difficulty of tasks, and baseline ability of individual subjects.

While evidence in non-human primates suggests that D_1 receptors are predominantly involved in modulating working memory (Sawaguchi et al., 1990a,b; Williams and Goldman-Rakic, 1995), it is interesting that the studies in humans using bromocriptine indicate a possible role for D_2 dopamine receptors. While D_2 receptors are found in areas such as the PFC, they are 20-fold less abundant than D_1 receptors (Lidow et al., 1991). The scarcity of D_2 receptors in the PFC and other neocortical areas indicate that the effects of bromocriptine on working memory may not be a direct effect (Kimberg et al., 2001). Given the abundance of D_2 receptors on layer V of the PFC (Goldman-Rakic et al., 1990), it has been suggested by Kimberg et al. (2001) that the downstream effects of bromocriptine (from areas rich in D_2 receptors) through projections to the cortical areas (via layer V), may dominate the cortical effects of D_2 receptor stimulation. However there is also evidence to suggest that there may be interactions between D_2 and D_1 receptors, such that modulating of the D_2 receptors may affect D_1 receptor function (Lidow and Goldman-Rackic, 1994; Lidow et al., 1998). Again, this indicates that D_1 receptors may play a more prominent role in directly modulating working memory in humans, which may be highlighted further with the development of an appropriate D_1 receptor agonist for use in humans.

**Dopamine receptor antagonists**

On the basis of evidence suggesting that dopamine receptor agonists may facilitate working memory, it would follow that dopamine receptor antagonists may impair working-memory performance. Unfortunately, there have been few studies conducted in healthy humans to examine the effect of acute doses of dopamine receptor antagonists on working-memory performance. Mehta et al. (1999), using a sample of 34 young healthy males, investigated the effect of the D_2 dopamine antagonist sulpiride on spatial working memory. The authors reported that spatial working memory, as assessed by a sequence generation task, was impaired following both 200 and 400 mg doses of sulpiride, compared to placebo.

In the study by Luciana and Collins (1998), which investigated the effects of bromocriptine on completion of a visuo-spatial delayed response task, the effect of the D_2 receptor antagonist haloperidol was also examined. Following a 3 mg oral dose of haloperidol, a decrease in performance was observed on the spatial working-memory task, as measured by a decrease in accuracy of identifying the location of the cue, compared to placebo. This decrement was observed at delays of 8 and 16 s, but not at a delay of 5 s (Luciana and Collins, 1998).

To our knowledge, there have been no other studies investigating the effects of haloperidol on working memory in normal human subjects. Negative effects of haloperidol on short-term memory have been reported for healthy elderly humans (Beuzen et al., 1999), although working memory was not tested. Fourteen subjects were given 3 mg of haloperidol once a day for 4 d and an acute effect was observed on day 1, with impairment in a series of memory tasks such as word recall and recognition (Beuzen et al., 1999).

Typical antipsychotics such as haloperidol, which have D_2 antagonistic properties, have been shown to impair working memory in schizophrenic patients, whereas atypical antipsychotics with less D_2 antagonistic properties have been shown to improve working memory in schizophrenia (Honey et al., 1999). However, research investigating schizophrenic subjects has generally involved chronic administration of dopamine antagonists, and interpretation has been difficult as schizophrenic patients are generally regarded as having abnormalities in their dopaminergic systems (Goldman-Rakic, 1991). In addition, these antipsychotics also have other pharmacological properties, including serotonin receptor antagonism, which may independently influence working-memory functioning (see section on serotonin).

Interestingly, in primate studies an inverted U dose-related response of dopamine antagonists has been observed by Sawaguchi and Goldman-Rakic (1994); these authors also reported that injecting higher doses of the dopamine antagonist SCH-39166 (a selective D_1 antagonist) into the dorsal PFC of rhesus monkeys was associated with greater impairments on an oculomotor delayed response task. However, Williams and Goldman-Rakic (1995) observed that lower concentrations of a D_1 antagonist were associated with improved performance. It could be argued that this may be due to the fact that at
low doses, some antagonists may have partial agonistic effects (Clifford et al., 1998; Sprouse et al., 1998).

While challenge studies in humans with D₂ antagonists suggest that blocking this receptor may impair working memory, a recent study in primates by Castner et al. (2000), showed that D₂ antagonists may induce changes to the D₁ receptor signalling pathway supporting earlier receptor studies (Lidow and Goldman-Rackic, 1994; Lidow et al., 1998). In the study of Castner et al. (2000), impairments in both spatial and object working memory induced by the D₂ antagonist haloperidol were reversed by the selective D₁ receptor agonist ABT-431. This data further highlights the importance of D₁ receptors in modulating working memory and suggests that modulation of the D₂ receptor may indirectly modulate D₁ receptor function.

The noradrenergic systems

There is some evidence suggesting that modulation of noradrenaline may influence spatial working memory. Elliot et al. (1997) administered methylenidate, a stimulant drug which increases synaptic concentration of both dopamine and noradrenaline by blocking their reuptake, to 28 young men. Following methylenidate administration, the authors reported a significant improvement in performance of spatial working memory and planning tasks, but not attentional and fluency tasks (Elliot et al., 1997). Mehta et al. (2000) also studied spatial working-memory performance following methylenidate administration. The task used in this study differed from the visuo-spatial delayed response tasks and delayed matching-to-sample task described earlier. In this study, 10 healthy male subjects were presented with red dots on a touch-sensitive computer screen. For each problem, the subject was instructed to systematically search through the array of red dots and the goal was to find blue tokens, which were obscured by the red dots. Once a token had been found behind a red dot, it was not used again to obscure a token. The task was to remember the location of dots that had been used to obscure the blue tokens. Methylenidate was observed to have a greater effect on working memory in subjects with lower-baseline working-memory capacity. This suggests that the working-memory capacity of subjects influenced the effectiveness of methylenidate on working-memory performance. These findings also support the studies on bromocriptine, which also indicated that changes in performance might be dependent on baseline working-memory capacity (Kimberg et al., 1997, 2001).

It has been reported that working-memory performance, as measured by a delayed matching-to-sample task, may be impaired by acute exposure to cold environments (Thomas et al., 1989). It has also been proposed that exposure to acute stress, such as cold, may disrupt the sustained release of the catecholamines, noradrenaline and dopamine (Bandaret and Lieberman, 1989). Shurtleff et al. (1994) investigated the effect on 8 male volunteers, of whole body exposure to 4 °C for 30 min, on a delayed matching-to-sample task. The task involved presentation of a matrix composed of red and green dots. The matrix was then removed from view for a delay period of 2, 8 or 16 s. Following the delay, two matrices were presented, of which one was identical to the original, and the subjects had to identify the original matrix. For a delay period of 16 s, exposure to cold impaired matching compared to placebo (22 °C). Shurtleff et al. (1994) also investigated the effect of administration of 150 mg/kg per body weight of l-tyrosine, a catecholamine precursor, 90 min before exposure to the cold. Subjects who were administered with l-tyrosine and were exposed to the cold did not significantly differ in performance to the placebo condition, and the authors concluded that the catecholamine precursor had a positive effect on working memory by protecting against the cold-induced memory deficits. l-tyrosine administered before exposure to the placebo condition did not influence performance, which suggests that l-tyrosine had an effect only in the cold environment.

While the effects of methylenidate and l-tyrosine may be explained in terms of changes in dopamine levels, a possible role for noradrenaline in modulating working memory cannot be ruled out. Furthermore, Coull et al. (1995) reported that administration of the α₂ adrenoceptor agonist clonidine, which effectively decreases noradrenaline in normal healthy humans, appeared to impair spatial working-memory performance. The authors also reported evidence of a dose-related effect, with 2.5 µg/kg producing a greater deficit in performance than 1.5 µg/kg.

The cholinergic systems

Cholinergic antagonists

The relationship between human memory and the cholinergic neurotransmitter system is well established in the literature. Early reports of the role of acetylcholine (ACh) in learning and memory (Drachman and Leavitt, 1974), and evidence of substantial reductions in neocortical cholinergic function in Alzheimer’s disease (Bartus et al., 1982; Nilsson et al., 1986; Perry et al., 1978), has provided evidence for a cholinergic hypothesis of memory. However the role of the cholinergic system in modulating human working memory is in its infancy.

Early evidence for a cholinergic modulation of working memory in normal human subjects derives from phar-
macological strategies using selective antagonists, particularly for the muscarinic receptors. Rassmussen and Dudar (1979) reported that oral administration of scopolamine impaired performance on a spatial working-memory task, which involved drawing a previously presented maze. In general, subjects were observed to draw extra turns in the maze while under the influence of scopolamine. The authors noted that a non-spatial working-memory task (digit memory task) was also impaired, but the impairment was less than that seen for the spatial working-memory task. However, Mewaldt and Ghoneim (1979), using a digit memory task, found an impairment in numeric working memory following administration of 8 µg/kg scopolamine in a healthy human sample. Similarly, Duka et al. (1996), in an independent group design study using 36 healthy subjects, also observed deficits in numeric working-memory task following administration of two doses of scopolamine (0.5 and 1 mg). This study also indicated that there might be a dose-related relationship between cholinergic muscarinic receptor antagonism and working-memory impairments. A dose-response relationship was supported by Robbins et al. (1997), who investigated the effects of three doses of scopolamine (200, 400 and 600 µg) on a delayed matching-to-sample visuo-spatial working-memory task. The authors reported that in a sample of 24 male volunteers, all 3 doses impaired performance compared to placebo. The authors also observed a dose-related effect, with larger doses associated with greater decreases in accuracy and greater latency in the matching of the sample.

However, it appears that, like the dopaminergic system, the effect of scopolamine on working memory may also be sensitive to the behavioural task used. In a study by Kopelman and Corn (2018), scopolamine had no significant effect on span tests or a measure of verbal short-term forgetting, which they classed as more passive types of working memory. However, cholinergic blockade appeared to produce impairment in a visuo-spatial short-term forgetting task, in addition to impairing performance on a distracter task used in a verbal memory test (Kopelman and Corn, 1988). This data suggests that the decrease in cholinergic function affected tasks with greater processing requirements.

A study by Rusted and Warburton (1988) also reported that administration of scopolamine impaired spatial working memory, but did not have an effect on a working-memory task for objects. Twenty healthy young adults completed a series of non-verbal working-memory tasks, and it was found that a subcutaneous injection of 0.6 mg/ml of scopolamine significantly impaired spatial working memory, while working memory for abstract shapes was not impaired. The addition of a concurrent articulation task used to load the working-memory articulatory loop, led the authors to interpret their results as indicative of an effect of scopolamine at the level of the working-memory central executive mechanism (readers interested in the theoretical component of working memory are directed to Baddeley and Hitch, 1974). Rusted (1988), observed similar results in a study that employed a larger dosage, administered orally (1.2 mg). In this study a semantic working-memory task was employed, and scopolamine significantly reduced the number of words recalled. A more recent study by Rusted et al. (1991) again observed scopolamine to impair working-memory performance at the level of the central executive system.

While blocking muscarinic receptors have been shown to impair various working-memory processes, it has also been shown that this impairment can be reversed by globally increasing synaptic acetylcholine levels. Ebert et al. (1998), employed a sample of 10 healthy male volunteers, and observed that following 0.6 mg of subcutaneously injected scopolamine, performance was impaired in both a spatial working-memory task (recognition of location of windows in a house front) and numeric memory task (yes/no matching of numbers to a previously presented set). The authors observed that administration of a single dose of 0.5, 1 and 2 mg of physostigmine (a cholinesterase inhibitor which increases acetylcholine levels) caused dose-dependent short-term reversal of these working-memory decrements.

Recently we have shown the nicotinic receptor antagonist mecamylamine (20 mg), to induce a delay-dependent impairment of visual recognition memory of objects, with maximum impairments found after a 12 s delay (Thompson et al., 2000). This study suggests the manipulation of the nicotinic receptor system may also modulate working-memory processes.

Taken together, these findings suggest that cholinergic processes, particularly the cholinergic muscarinic system but also the nicotinic receptor system, may modulate working memory. The available data suggests that the types of working memory most affected by muscarinic receptor antagonism are spatial and numeric working memory, while nicotinic receptor antagonism has been shown to impair object recognition working memory. However one must keep in mind that not all forms of working memory have been examined (particularly with the nicotinic receptor system) and it is possible that these systems could modulate a wider range of working-memory processes.

**Cholinergic agonists**

Studies investigating the effects of globally increasing
cholinergic function have generally focused on the acetylcholinesterase inhibitor physostigmine, which inhibits metabolism of acetylcholine and effectively increases acetylcholine levels. Furey et al. (1997) conducted one of the first studies to investigate working memory and acute increases in cholinergic transmission in healthy volunteers. In this study, working memory for faces was examined. The task involved a 4-s presentation of a face, which subjects were instructed to remember. After a delay of 6 s, two test faces appeared simultaneously, side by side, and the subjects had to identify the original face. In a sample of 13 healthy human volunteers, the efficiency of identifying the correct face (as measured by reaction time) was observed to improve following infusion of 1 mg/h of physostigmine. The control group (n = 8), which did not significantly differ in age, education or gender distribution to the experimental group, received a saline infusion and showed no change in reaction time (Furey et al., 1997). Furey et al. (2000a,b), replicated these results in two more recent studies. Again, subjects who received physostigmine showed improved working-memory performance for faces.

However, specific targeting of cholinergic receptors has not supported previous studies that have shown improvements in working memory with global enhancement of cholinergic function. Park et al. (2000) investigated the effects of the indirect cholinergic agonist nicotine on spatial working memory. The authors reported that smokers performed significantly worse after smoking a cigarette, compared to baseline. However, there are methodological weaknesses in this study that may explain the negative findings. First, the apparent cognitive effects reported in this study may be explained on the basis that subjects were asked to abstain from smoking for 24 h prior to testing. After abstaining for 24 h, subjects are in a nicotine-deficient state. If nicotine is then administered, it could be argued that this amounts to activation of a hypo-nicotinic receptor system, confounding results. Secondly, the spatial working-memory task used was quite different to those reviewed earlier. The task used in this study involves subjects fixating at the centre of the screen, while a target is presented for 200 ms. Where this task differs is in the delay period, which lasted 30 s and is nearly twice as long as most delay periods used previously. Moreover, during this delay a mathematical distracter task was presented.

A further explanation for the findings reported by Park et al. (2000) is the complexity of the nicotine effect. In addition to its effect as a non-selective cholinergic agonist, nicotine also causes the release of dopamine in the basal ganglia and nucleus accumbens (Pidoplichko et al., 1997). A study in rats indicated that working-memory deficits induced by nicotinic antagonists might be reversed by administration of the dopamine agonist quinpirole (Levin and Rose, 1995), suggesting modulation of dopaminergic transmission by the cholinergic system. Therefore, in studies using smokers who have abstained for a period of time, it is probable that there is a reduction in dopaminergic function, as a result of a hypo-nicotinic state, and this may explain the working-memory deficit observed by Park et al. (2000). Such a hypo-dopaminergic state has also been demonstrated with chronic nicotine treatment, leading to down-regulation of D2 receptor binding (Janson et al., 1992). Therefore, although nicotine is a non-selective cholinergic agonist, the effects of nicotine on working memory cannot be attributed purely to cholinergic function and may be mediated at least in part by dopaminergic processes.

To conclude, there is surprisingly limited research into the effects of cholinergic modulation on working-memory performance in healthy humans. The research by Furey et al. (2000a,b) does suggest that increasing cholinergic function improves working-memory performance. However, these studies are limited to working memory of faces, and further research is required to examine the role of the cholinergic system in other forms of spatial and non-spatial working memory. Overall, the evidence presented indicates that although the working-memory task examined appears to be highly important when considering the effect of cholinergic function on working memory, decreases in cholinergic function are associated with impaired performance, while increases in function appear to improve performance.

Serotonin as an inhibitory modulator

The putative role of serotonin in learning and memory is amongst the least understood of the monoaminergic neurotransmitters (Gold and Zornetzer, 1983; Luciana et al., 1998; Ogren, 1985). Luciana et al. (1998) investigated the effect of administering a 60 mg dose of fenfluramine, a serotonin re-uptake inhibitor and releasing agent, which effectively increases serotonin levels, on the visuo-spatial delayed response task used by same authors when examining the effects of dopamine manipulation. It was observed that at delays exceeding 4000 ms, fenfluramine appeared to impair working-memory performance. The authors suggested that serotonin may have constrained spatial working memory through an inhibitory effect on dopamine, based on evidence suggesting serotonin and dopamine have opposing roles with respect to emotional and motor behaviours. However, this interaction effect was not explicitly tested.

There is also evidence suggesting that serotonin may modulate the cholinergic system and therefore have an indirect effect on cognition (for a review, see Steckler and...
In a study by Little et al. (1995), healthy human subjects were infused with 0.08 mg/kg of m-CPP, a combined serotonin agonist/antagonist. It was observed that the infusion of m-CPP augmented deficits in word recall, word recognition and objects naming, which had earlier been induced by administration of scopolamine. Although working memory was not tested in this study, studies in rodents have indicated an interaction between the serotonergic and cholinergic systems in working-memory functions (Miura et al., 1993; Ohno and Watanabe, 1997; Richter-Levin and Segal, 1989). Richter-Levin and Segal (1989) conducted an example of such a study. In this study, a reduction of serotonin synthesis, following the administration of the specific inhibitor of tryptophane hydroxylase, was observed to exaggerate a spatial working-memory deficit (as measured by performance on a spatial water maze), which was induced by blockade of cholinergic transmission, using atropine.

At present, there is insufficient empirical evidence to permit any conclusions about the role of serotonin in working-memory functions. However, the evidence suggests that future research investigate whether serotonin may interact with the dopaminergic and cholinergic systems in modulating working memory in humans.

**Other neurochemical systems**

Evidence suggests that individual neurons show elevated persistent and tuned activity (memory fields) during working memory that may be modulated by many neurochemicals (see review by Goldman-Rakic, 1995). Little is known about the role of inhibitory mechanisms in the regulation of working memory, but since the majority of interneurons use the inhibitory neurotransmitter GABA, it has been suggested that GABA may be important for working-memory functions (Goldman-Rakic, 1995). Recent evidence from a study (Rao et al., 2000), using two rhesus monkeys has implicated GABA$_{A}$ in working-memory functioning. Rao et al. (2000) also investigated the effect of iontophoresed bicuculline methiodide (BMI), which effectively blocks GABA$_{A}$ on neurones of the PFC in monkeys performing an oculomotor delayed response task. It was reported that BMI caused disinhibition of the neurons and resulted in a loss of spatial tuning, that is, regulation of spatial working memory in the PFC.

Another system that may be important in working-memory function is the NMDA receptor complex. Although no research on modulation of glutamate–NMDA receptor complex and working memory appears to have been conducted in either human or non-human primates, research conducted in rodents indicates that the glutamate system may also be important for working-memory function. For example, blockade of NMDA receptors located at the dorsomedial PFC in rats has been reported to impair spatial working memory (Aura and Riekkinen, 1999). Selective and competitive NMDA receptor antagonists, which block NMDA receptor activity, have been reported to increase the number of errors in working memory (Gutnikov and Rawlins, 1996; Ohno et al., 1992, 1993; Pontecorvo et al., 1991; Puma et al., 1998; Puma and Bizot, 1998). On the other hand, spermidine, an agonist of the polyamine site on the NMDA receptor/channel complex, has been reported to reduce scopolamine-induced errors in working memory, as assessed by the three-panel runway task (Kishi et al., 1998). However, the NMDA agonist did not influence working memory when injected alone (Kishi et al., 1998). This again suggests that interactions between neurotransmitter systems may be important for working-memory function.

**Future research**

The research reviewed in this paper gives an insight into the pharmacology of human working memory. The current authors suggest that there are three major areas in which future research could expand. The first concerns the heterogeneity of working-memory tasks employed. Specific task sensitivity to pharmacological manipulation is not uncommon in memory research. A recent investigation of secondary episodic memory pharmacology showed that the effect that anti-cholinergic (scopolamine), anti-dopaminergic (haloperidol) and/or GABA modulators (benzodiazepines) had on memory performance depended on the exact task used, although all tasks were designed to examine ‘secondary episodic memory processes’ (Ramasayer et al., 2000). One of the limitations of the research completed to date, is the diverse range of tasks employed to examine working memory. The contentious issue of whether there are different anatomical substrates for different types of information held in working memory was alluded to in the Introduction to this review. It is possible that spatial and non-spatial working memory have different anatomical substrates, which may also result in differences in the pharmacology of the processes. However, perhaps a more important concern is the ‘within-type’ difference between tasks, as there appears to be little consistency between researchers as to what, for example, a spatial working-memory task should consist of. Therefore, it is difficult to compare differences between performance effects of a specific neurotransmitter system on spatial and non-spatial working-memory tasks when the differences between the within-type tasks are high. Development of
well-standardized tasks appears an important step for future research. It may be interesting to look at the effects of a specific drug on a number of tasks defined as testing one type of working memory, such as ‘numeric working memory’ or ‘spatial working memory’ and see how and if the results differ significantly. Further, using the same task to examine numerous neurotransmitter systems may also aid in comparing the effects of different neurotransmitter systems on working-memory performance.

Secondly, it is suggested that brain imaging may play a large role in the future research on neurochemical modulation of working memory. Overall, the evidence presented in this review indicates that acute modulation of specific neurotransmitter systems can influence human working-memory performance, but our knowledge of the cortical basis of these effects is indirect. However, with use of brain imaging it is possible to examine the direct cortical effects of pharmacological manipulation. In addition it is possible that brain-imaging techniques, like functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and various electrophysiological techniques, may be useful if identifying the cortical effects of pharmacological modulation on the various stages of working memory, including early encoding and the holding or maintenance component.

There has been limited research thus far which has employed brain-imaging technology and acute pharmacological manipulation. Indeed, the work of Furey et al. (1997, 2000a,b) who have used PET and fMRI imaging to investigate the effect of cholinergic enhancement on working memory appears to be paving the way for the new direction of research into working-memory pharmacology. Furey et al. (1997) observed that in subjects receiving physostigmine, there was a decrease in rCBF to the right PFC regions during working-memory tasks, that did not occur at rest. The decrease in rCBF to this area was also observed to significantly correlate with an improvement in working-memory function (as assessed by reaction time). In their more extensive replication study, Furey et al. (2000a) again observed a correlation between improved performance and a decrease in rCBF to the right PFC activity. Similarly, decreases in the left temporal cortex, anterior cingulate and left hippocampus rCBF were observed. This study also observed a correlation between improved performance and an increase in rCBF in the medial occipital cortex.

Based on this evidence, Furey et al. (2000b) suggested that cholinergic enhancement of working-memory performance appears to be the result of increases in neuronal activity in regions associated in early perceptual processing, and decreases in activity in regions associated with memory maintenance. This hypotheses was supported in a more recent functional magnetic resonance imaging (fMRI) study which investigated the cortical responses of 7 healthy subjects to different sub-components of the working-memory task, following administration of physostigmine (Furey et al., 2000b). As expected, enhancement of visual processing in the ventral occipital cortex during encoding, and decreased activity in the anterior PFC during maintenance of information, was observed. The authors concluded that enhancement of cholinergic activity improves working memory by focussing perceptual processing in extrastriate visual cortices, particularly during encoding. It is suggested that by producing a more robust visual percept during encoding, working-memory maintenance is simplified and less effort is required by the PFC to maintain the information.

Recently the cortical effects of working memory have also been examined for the dopaminergic system. Kimberg et al. (2001), using fMRI, observed that following bromocriptine administration, there were reductions in task-related brain activity in the parietal and occipital cortex during the maintenance component of a two-back working-memory task. These results support the study of Mehta et al. (2000) who also found that methylphenidate (which increases dopamine and noradrenaline levels) induced task-related reductions in blood flow in the PFC and parietal cortex which correlated with improvements in spatial working-memory performance.

Taken together these brain-imaging studies indicate that the manipulation of dopamine may modify brain activity during the maintenance component of working memory, while cholinergic manipulation may have more of an effect on the encoding component of working memory. Clearly, further research is required to investigate the effects of selective neurochemical modulation on different stages of working memory, but these studies highlight the importance of brain imaging as a tool to examine the effects of pharmacological manipulation on specific cortical areas, and on the different sub-components of working-memory processes.

Finally, future research on working memory in humans will be aided by the development of new and more selective agents which can be used to target specific receptor systems and a wider range of neurochemical systems. This will no doubt shed light into the pharmacology of working memory.

**Clinical implications**

Working-memory deficits have been reported in patients with clinical disorders such as schizophrenia (Goldman-Rakic, 1991), Parkinson’s disease (Sahakian et al., 1993) and Alzheimer’s disease (Levy et al., 1994; Sano et al., 1993). Dysfunction of the dopaminergic system has been
implicated in schizophrenia, and brain imaging studies of schizophrenic patients have suggested that the PFC may be particularly critical (Andreasen, 1988). Moreover, PET studies have observed decreased numbers of prefrontal dopamine D<sub>1</sub> receptors in schizophrenic patients (Okubo et al., 1997). Schizophrenic subjects have been frequently observed to be impaired in tasks thought to rely on working-memory processes, such as the Wisconsin Card Sorting Test (Kolb and Wishaw, 1983).

Individuals with Parkinson’s disease have also been observed to have abnormal dopaminergic functions, and are reported to exhibit a number of ‘prefrontal’ dysfunctions including deficits in delayed response spatial memory tasks (Freedman and Oscar-Berman, 1986). Although poverty of behaviour is a common symptom observed in Parkinson’s disease, recent evidence suggests that cognitive impairments are not necessarily explained by this symptom (Antal et al., 1998). It has been reported that while medicated Parkinson’s disease patients with severe clinical symptoms are impaired in spatial, verbal and visual working memory, medicated patients with mild clinical symptoms appear to be impaired only in spatial working memory (Owen et al., 1997). A recent review of cognitive dysfunction in Parkinson’s disease suggests that
spatial working memory, along with attentional set-shifting, appear to be selectively impaired in the early stages of the disease (Antal et al., 1998). Similarly, patients with Alzheimer’s disease (a disorder characterized in part by a degeneration of cholinergic neurons) are reported to also have working-memory deficits (Iversen, 1998). Physostigmine, which effectively increases acetylcholine levels, has been observed to improve working memory in patients with Alzheimer’s disease (Levy et al., 1994; Sano et al., 1993).

The evidence that working memory is modulated by a number of neurochemical systems including dopaminergic and cholinergic systems in humans suggests that targeting these systems with specific agents may potentially be beneficial for patients with working-memory deficits as a result of their illness.

Summary

There is evidence suggesting that human working memory may be modulated by the dopaminergic system. Stimulation of D_{1} dopamine receptors appears to facilitate spatial working-memory performance in humans. However it must be noted that the majority of evidence supporting this contention has come from one laboratory and other groups have reported conflicting evidence. A possible contributor to the conflicting evidence is the apparent lack of consistency in the behavioural tests used between studies, dose-related effects, task difficulty and baseline differences in working-memory capacity between subjects. While studies on the role of D_{2} receptors in human working memory is inconclusive, evidence supports an involvement of D_{1} receptors.

In addition to dopamine, other neurotransmitters such as acetylcholine, noradrenaline and serotonin may also modulate human working memory. Cholinergic stimulation has been observed to enhance working-memory performance, while blocking cholinergic receptors has been reported to impair working-memory performance. Increasing levels of noradrenaline has also been reported to enhance working-memory performance. There is insufficient evidence to conclude whether serotonin plays a role in working memory, although the evidence to date indicates that any effect is most likely inhibitory, perhaps through modulation of other neurotransmitter systems, such as the dopaminergic and cholinergic systems. This has yet to be explicitly tested. While other systems such as GABA and glutamate, and possible interactions between neurochemical systems. However, with the development of more selective drugs for testing in humans, we may be able to better understand the pharmacology of working memory. With the use of brain-imaging techniques, further information regarding the direct cortical effects of pharmacological manipulation, and the specific stage of working memory affected by a pharmacological agent, may also be obtained. Understanding which neurotransmitters can be modulated to enhance working memory may have treatment implications for schizophrenia, Parkinson’s disease and Alzheimer’s disease, and may ultimately play a role in specific treatments.

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APPENDIX 5: Reprint of Ellis et al. 2005 (Psychopharmacology)
Kathryn A. Ellis · Mitul A. Mehta · Keith A. Wesnes · Stuart Armstrong · Pradeep J. Nathan

Combined D₁/D₂ receptor stimulation under conditions of dopamine depletion impairs spatial working memory performance in humans

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Abstract Rationale: The mesocortical dopamine system is regarded as an important modulator of working memory. While it has been established that stimulation of the D₁/D₂ receptor in primates can improve spatial working memory performance, findings in humans are less consistent. Recent studies in humans suggest that global depletion of dopamine via tyrosine/phenylalanine depletion may impair spatial working memory performance, although these results are also inconsistent, and it has been suggested that task differences may partly underlie the inconsistent findings. Objectives: This study had two aims: (1) to investigate the effects of acute tyrosine depletion (TPD) on a number of working memory tasks and (2) to examine whether stimulation of D₁/D₂ receptors under conditions of TPD can attenuate or “reverse” TPD-induced working memory impairments. Methods: Eighteen healthy male participants performed a spatial working memory delayed-recognition task, non-spatial working memory task and spatial n-back task on three separate occasions, after TPD, TPD and pergolide (D₁/D₂ agonist), and placebo. Results: TPD did not impair working memory performance on any of the tasks administered. However, stimulation of D₁/D₂ receptors under TPD conditions caused a subtle impairment in spatial working memory performance. Conclusions: The finding that D₁/D₂ stimulation under TPD conditions impairs working memory highlights the complexity of functional effects of augmenting dopaminergic transmission within a dopamine-depleted state. The lack of TPD-related effects on a range of working memory tasks questions the reliability of TPD as a modulator of dopamine function and working memory performance in humans.

Keywords Tyrosine depletion · Dopamine depletion · Dopamine · Working memory · Pergolide · Dopamine agonists · D₁ receptor · D₂ receptor

Introduction

It is well established that the integrity of the dopaminergic system within the prefrontal cortex (PFC) is critical for working memory performance in primates, based on convergent evidence from lesion studies (Funahashi et al. 1993), regional depletion studies (Brozoski et al. 1979; Roberts et al. 1994) and administration of dopamine receptor agonists and antagonists (for reviews, see Arnsten 1997; Goldman-Rakic et al. 1996). A preferential role for the D₁ receptors within the PFC has been demonstrated with evidence that local administration of D₁ receptor (and not D₂ receptor) antagonists modulate working memory performance (Sawaguchi and Goldman-Rakic 1991, 1994; Williams and Goldman-Rakic 1995). However, a role for D₂ receptors in working memory is also suggested based
on evidence that systemic administration of D2 receptor agonists can modulate performance, potentially through effects within the striatum or through activation of other dopamine receptor cites (i.e. D3/D4 receptors) (Armsten et al. 1995).

Deficits in working memory performance in clinical populations such as schizophrenia (Abi-Dargham et al. 2002; Callicott et al. 2003; Meyer-Lindenberg et al. 2001; Park and Holzman 1992; Weickert et al. 2000) and Parkinson’s disease (Bublak et al. 2002; Kulisevsky et al. 1996; Lange et al. 1992; Postle et al. 1997a) further support dopamine as a modulator of working memory performance. Evidence has also linked impairments in D1 receptors in the PFC to working memory deficits in patients with schizophrenia (Abi-Dargham et al. 2002). However, experimental evidence in healthy humans has failed to clearly elucidate the role of dopamine in human working memory performance, due in part to the lack of an appropriate D1 receptor agent for use in humans. Research using the D2 receptor agonist bromocriptine has produced inconsistent results, with some evidence of a positive modulatory effect on performance (Luciana and Collins 1997; Luciana et al. 1992; Mehta et al. 2001), but other studies showing no effect (Bartholomeusz et al. 2003; Kimberg et al. 1997; Muller et al. 1998). Similarly, the D2 receptor antagonists sulpiride and haloperidol have been observed to impair working memory performance in some studies (Luciana and Collins 1997; Mehta et al. 1999, 2004), but other studies have failed to show an effect (Mehta et al. 2003, 2005a). The combined D1/D2 receptor agonist pergolide has also demonstrated inconsistent effects, with evidence that it may enhance working memory performance (Muller et al. 1998), may have a beneficial effects on performance in only some individuals, dependent on working memory capacity (Kimberg and D’Esposito 2003), or have no effect on performance (Bartholomeusz et al. 2003; Roesch-Ely et al. 2005). While it has been suggested that baseline working memory capacity could mediate the effect of dopamine agonists on working memory performance, the nature of these effects has also differed between studies (Kimberg and D’Esposito 2003; Kimberg et al. 1997; Mehta et al. 2000).

Several recent studies have examined the effects of global depletion of dopamine on working memory performance. As dopamine (and noradrenaline) rely on an available source of their amino acid precursors tyrosine and phenylalanine for synthesis within the brain, restricting these amino acids has provided a novel technique for experimentally depleting dopamine levels and probing the effects on working memory performance. The mechanism by which acute amino acid depletion is believed to decrease catecholamine synthesis is twofold: firstly, by stimulating protein synthesis, it results in lowered plasma tyrosine levels and secondly, by increasing competition between tyrosine and other amino acids for transport across the blood–brain barrier, tyrosine levels are further decreased (Oldendorf and Szabo 1976; Partridge 1977). Acute tyrosine/phenylalanine depletion (TPD) has been shown to be a useful method of depleting dopamine synthesis in rats (Jaskiw and Bongiovanni 2004; McTavish et al. 1999a,b) and dopamine release in humans (Montgomery et al. 2003), and neuroendocrine findings further indicate decreased dopamine function in humans (Harrer et al. 2001). Findings have suggested that TPD is preferential for dopamine, with little effect on noradrenaline (McTavish et al. 1999a, b). Using this technique, Harrer et al. (2001) were the first to demonstrate impaired spatial working memory performance on a spatial working memory array task and spatial working memory delayed-recognition task. These findings were supported by a subsequent study by Harrison et al. (2004), who observed TPD-related deficits on a variation of the Sternberg working memory task (a spatial delayed-recognition task). Harrison et al. also showed a modality-specific selectivity for TPD, with no deficits observed on a non-spatial working memory delayed-recognition task, consistent with findings that suggest that spatial memory tasks may be more sensitive than non-spatial tasks to dopaminergic medication used in PD (Cools et al. 2002; Kulisevsky et al. 1996; Lange et al. 1992; Postle et al. 1997a,b). However, not all studies have shown spatial working memory deficits after TPD. Recently, McLean et al. (2004), using a task identical to Harmer et al. (2001), failed to observe an impairment in working memory. Similarly, Mehta et al. (2005b) observed that working memory deficits were not observed at a group level after TPD, although there was some suggestion of impairments in participants with greater changes in striatal dopamine levels (as indicated by changes in [11C]raclopride binding), and Roiser et al. (2005) failed to find any effects on performance. It has been suggested that task differences between studies may be a source of the inconsistency in findings (i.e. Mehta et al. 2005b), in line with the suggestion that differences in task demands may be involved in the inconsistency in dopamine agonist findings in humans (i.e. Mehta et al. 2001, 2003; Luciana and Collins 1997).

This study aimed to further investigate the role of dopamine in working memory performance in humans. Our first aim was to examine the effects of TPD on a number of working memory tasks. Due to the inconsistent findings of TPD-related effects on working memory, two of the tasks used in this study were identical to those used by Harrison et al. (2004): a test of spatial working memory delayed recognition (a variation of the Sternberg task previously shown to be modulated by TPD) and a test of non-spatial working memory delayed recognition (a verbal working memory task involving sub-vocal rehearsal and scanning of numbers). Furthermore, the spatial working memory n-back task was also included (1-, 2- and 3-back version) due to the consistent finding of performance impairments in patients with schizophrenia (Abi-Dargham et al. 2002; Callicott et al. 2003; Meyer-Lindenberg et al. 2001), which has recently been correlated to D1 receptor availability in the PFC (Abi-Dargham et al. 2002). We also intended to extend on previous literature and investigate the behavioural effect of stimulating the D1/D2 receptors under conditions of acute tyrosine depletion. We hypothesised that dopamine depletion may preferentially impair spatial working memory over non-spatial working memory, and this impairment may be attenuated or “reversed” after stim-
ulation of D₁/D₂ receptors. Due to the possible interaction of baseline working memory capacity on the modulatory effects of dopamine on working memory, we examined whether any differences exist between participants with high and low baseline levels.

**Methods**

**Participants and design**

Twenty-three healthy men were recruited for the study through advertisements within the local universities and general community. One participant withdrew from the study due to faintness after an initial blood sample, and four participants withdrew from the study due to nausea and/or vomiting after consumption of the amino acids. The resulting sample comprised 18 men (mean age 22.9±6.4 years). All participants were healthy, right-handed, and non-smokers. Exclusion criteria included a history of neurological or psychiatric disorders (including history of depression or anxiety disorders in first-degree relatives), chronic physical illness, medication and/or drug use, or excessive alcohol consumption. Medical and psychiatric suitability to participate in the study was ascertained after an initial screening by telephone (including administration of the PrimeMD, Pfizer 1996) and a consequent semi-structured clinical assessment by a psychiatrist. The study was conducted using a double-blind, balanced-drink (placebo) control, repeated measures design over three separate sessions: (1) 104.4 g balanced control condition (BAL condition), (2) an equivalent mixture deficient in tyrosine and phenylalanine (TPD condition) and (3) TPD mixture + pergolide (0.1 mg) condition (TPD+P condition). Each session was separated by a minimum 5-day washout period, with order of condition randomised using a quasi-Latin-square design. The study was approved by the Swinburne University Human Research Ethics Committee. All participants gave written informed consent.

**Procedure**

All participants attended a pre-study practice day, during which they completed the cognitive battery four times (separated into two sessions, a minimum of 2 h apart), based on evidence that performance stabilizes after four training sessions (Wesnes and Pincock 2002).

On each testing day, participants arrived at the laboratory at 0815 hours, having consumed a low-protein diet (less than 25 g) in the preceding 24 h and fasting from 7.00 P.M. Participants were also asked to refrain from alcohol the day before testing, and to arrive well rested. Each participant was contacted by phone to encourage compliance with the pre-experimental protocol. After arrival, the participant sat quietly for 15 min before commencing baseline testing (described below). At approximately 0900 hours (time 0) the amino acid drink and capsules were administered (details below), and at +3 h post-drink (approximately 1200 hours) an oral dose of pergolide (or placebo capsule) was administered. Post-drug testing commenced at +5 h post-drink to coincide with the peak behavioural effects of TPD (Harmer et al. 2001; Harrison et al. 2004) and pergolide (Markham and Benfield 1997).

To reduce the potential side effects of pergolide, two doses of the peripheral dopamine receptor antagonist domperidone were administered during each testing session (at +30 min and +2 h post-drink; 10 mg per administration/20 mg total). At +2.5 h post-drink, a carrot was also provided to reduce hunger. Subjective side effect questionnaires were administered at +1 and +3 h post-drink. Two 20-ml venous blood samples were taken per session for analysis of plasma amino acid concentrations; the first preceding baseline testing (i.e. before time 0) and the second preceding post-drink testing (at +4 h, 45 min). Testing concluded 5.5 h post-drink, and participants were provided with a high-protein snack before departing.

**Amino acid suspension**

The composition of the balanced amino acid mixtures was based on the original 100 g balanced suspension developed by Young et al. (1985) (in g): L-alanine 5.5, L-arginine 4.9, L-cysteine 2.7, glycine 3.2, L-histidine 3.2, L-isoleu cine 8.9, L-leucine 13.5, L-lysine monohydrochloride 11, L-methionine 3, L-proline 12.2, L-serine 6.9, L-threonine 6.9, L-valine 8.9, L-tryptophan 2.3, L-tyrosine 6.9 and L-phenylalanine 5.7 (with both L-tyrosine and L-phenylalanine excluded for TPD). Drinks were prepared within a few minutes of oral administration by mixing the powdered amino acids with 180 ml orange juice. Due to the unpleasant taste, L-cysteine, L-methionine and L-arginine were encapsulated in gelatin capsules and administered separately. Participants were instructed to swallow the suspension in as short a time as possible. A nose plug was provided during ingestion to reduce olfactory cues.

**Cognitive tasks**

The n-back task was developed at the Brain Sciences Institute using Pipscript software (Brain Sciences Institute, Victoria, Australia). All remaining tasks were taken from the Cognitive Drug Research (CDR Ltd, Goring-on-Thames, UK; http://www.cdr.org.uk) computerized assessment suite, regarded for its validity as a measure of memory and attention and its proven sensitivity in studies of acute tyrosine depletion (Harrison et al. 2004). While the n-back task was administered post-drug only, the CDR tasks were administered both at baseline (pre-drug) and post-drug (+5 h), for consistency with Harrison et al. (2004).

All cognitive tasks were presented via computer and all responses were made using an external button box (yes/no) or critical flicker fusion (CFF) tube. The button box was hand-held with thumbs resting upon the respective button. Participants were instructed to respond “as quickly as pos-
sible but with accuracy as their priority” on all tasks. Participants were seated approximately 1 m from the computer monitor in a dimly lit room (consistent between sessions) and were requested to sit upright throughout the task.

For the CDR tasks, accuracy was recorded as (1) percentage accuracy in correctly identifying the original stimuli and (2) percentage accuracy in correctly identifying the new stimuli. The main accuracy measure, the sensitivity index, was calculated using these two values, and is a measure of overall task efficiency ranging between 0 (chance level accuracy) and 100 (perfect recognition of all stimuli) (Frey and Colliver 1973). Specifically, the sensitivity index assesses the participants’ ability to discriminate between original and novel stimuli, with a high sensitivity index representing both accuracy in correctly identifying the original stimulus and avoidance of falsely identifying novel/distracter stimuli. The main accuracy measure for the n-back task was overall accuracy; however, for consistency, the sensitivity index and accuracy of original and new stimuli were also calculated. Latency in milliseconds was recorded for all tasks. The duration of the test battery was approximately 35–40 min.

The spatial n-back task

This task is a measure of spatial working memory with a sustained attention component. Each task involved the presentation of a series of white dots on a black background (with a central white fixation cross). Each dot was presented for 500 ms, with interstimulus intervals of 3,000 ms. Participants were required to indicate whether each dot was in the same location as the dot “n-back” (either 1-back, 2-back or 3-back, depending on task instructions). The visuospatial control task involved an equivalent task presentation, and participants alternated responses between the left and right response button. Order of n-back task administration was quasi-random.

Spatial delayed recognition

This is a modified version of the Sternberg (1966) maintenance task for working memory, which requires both intact spatial recognition and the manipulation of stored information to discriminate “original” from “novel” spatial cues. The image of a house front displaying nine windows was presented for 10 s, with four of the nine lights turned on (“original” stimuli). Participants were shown 36 consecutive presentations of the house front, with an interstimulus interval of 1 s, with only a single window lit up. Participants were required to identify whether the light was in a matching original position by pressing yes, or in a novel location to one of the four original lights by pressing no as quickly as possible.

Non-spatial delayed recognition

Participants were presented with three different digit sequences (five-span). The digit sequences were easily verbalised and this task involved aspects of both verbal and numeric working memory. Following each sequence, 30 probe digits were presented consecutively and participants indicated whether they had seen these numbers in the five-span by responding with the yes or no buttons as quickly as possible.

Reaction time

This task involved 50 trials of responding to the word “yes” by pressing the appropriate response button as quickly as possible (interstimulus interval ranged between 1 and 3.5 s).

Critical flicker fusion

This task is a traditional psychophysical threshold measure of alertness and attention and was used as a measure of drug-induced sedation (Hindmarch and Parrott 1977). Participants held the CFF unit and fixed their gaze on two red lights at the base. The flicker ranged from 25 to 65 Hz in alternating ascending and descending mode, with three trials “up” and “down”. Participants responded when they perceived the light to either start or stop flickering by pressing “yes” on the button box or to discriminate between the two lights by indicating which one was flickering.

Side effect questionnaire

Participants rated how they felt on an 11-point symptom checklist in response to 11 questions (e.g. “I feel nausea”, “I have stomach pains”, “my heart is beating faster than normal”), on a range from 1 (not at all) to 5 (very much so). A minimum score of 11 indicated that participants felt no symptoms, with a score of 55 indicating extreme negative symptoms.

Amino acid analysis

Concentrations of free amino acids tyrosine (TYR), phenylalanine (PHE), tryptophan (TRYP), valine (VAL), leucine (LEU), and isoleucine (ILE) in plasma were determined using precolumn derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), followed by separation of the derivatives and quantification by reversed-phase high-performance liquid chromatography (RP-HPLC). VAL, LEU and ILE levels were analysed to calculate the ratio of plasma TYR or PHE to other large neutral amino acids (LNAAs). Before derivatisation, plasma samples (100 μL) were diluted 1:1 with internal standard solution and deproteinised by ultrafiltration through a
membrane with a 10-kDa nominal molecular weight cut-off (Ultrafree MC with PL-10 membrane, Millipore, Bedford, MA, USA). The filtrate (100 ml) was then subjected to AQC derivatisation and HPLC analysis using the Waters AccTag AA analysis system (Waters Corporation, Milford, MA, USA) (Cohen 2001).

Statistical analysis

Data were analysed using SPSS (SPSS Inc., Chicago, IL, USA). All data were analysed using repeated measures analysis of variance (ANOVA) if appropriate, but non-parametric statistics were used for data that violated the assumption of normal distribution.

Baseline-dependent analysis was conducted by separating participants into high- and low-baseline working memory groups, based on their median scores in each of the six baseline sensitivity index measures on the CDR working memory tasks (i.e. three treatment conditions, spatial and non-spatial working memory). Participants who scored either above or below the median in at least four of the six baseline sessions were classed as either “high” or “low”, respectively. Additional ANOVAs were conducted on each working memory measure, with the baseline working memory capacity variable introduced as a between-subjects factor.

Correlations were conducted between the change in performance from treatment and placebo conditions (i.e. TPD–BAL and TPD+P–BAL) and the corresponding changes in side effect ratings for all significant cognitive measures in order to examine the possible contribution of side effects to any drug-induced performance impairments.

**Results**

**Plasma amino acid levels**

Complete plasma samples were analysed for ten participants. Separate 3 (treatment condition) by 2 (time) ANOVAs were conducted for each amino acid and revealed significant interactions between treatment conditions and time for tyrosine [$F(1.21,10.97) = 40.39, p<0.001$, with Greenhouse–Geisser correction] and phenylalanine [$F(1.08,9.71) = 42.77, p<0.001$, with Greenhouse–Geisser correction]. Planned contrasts showed that concentrations of both tyrosine and phenylalanine decreased significantly after the TPD and TPD+P condition compared to the BAL condition (all $p<0.001$). The ratio of plasma tyrosine and phenylalanine to large neutral amino acids (T+P/∑LNAA) varied significantly between treatment conditions, as revealed by a 3 (treatment condition) by 2 (time) repeated measures ANOVA [$F(2,18) = 47.60, p<0.001$]. Planned contrasts revealed a significantly greater decrease in the ratio after both the TPD and TPD+P conditions compared to the BAL condition (both $p<0.001$) (see Table 1).

**Spatial n-back task**

One participant was removed from the analysis due to outlying responses on multiple sessions. For the remaining 17 participants, one-way repeated measures ANOVAs were conducted on the accuracy and latency measures for each n-back level. Latency data are presented in Fig. 1.

For the 1-back task, ANOVA revealed a significant difference between treatment conditions on the measures of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean and standard error (SEM) concentrations of amino acids (μmol/l) and change in percentage from baseline to 5 h post-drink</th>
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<td>TYR+PHE/∑LNAAAs</td>
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n=10

BAL: Balanced condition, TPD: tyrosine/phenylalanine depletion, TPD+P: tyrosine/phenylalanine depletion and pergolide

* $p<0.001$, significant change from baseline to 5 h post-drink;

† $p<0.001$, significant difference between treatments
overall accuracy \([F(2,32)=4.07, p<0.05]\) and sensitivity index (SI) \([F(2,32)=3.88, p<0.05]\), with trends for the accuracy of the original stimuli measure \([F(2,32)=2.83, p=0.08]\) and new stimulus measure \([F(2,32)=3.07, p=0.06]\). Planned contrasts showed that accuracy after TPD+P was significantly worse than after the BAL condition [overall accuracy: \(F(2,32)=7.56, p<0.05\); SI: \(F(2,32)=7.58, p<0.05\)], whereas there was no significant difference between BAL and TPD treatment conditions \((p>0.1)\). For the 2- and 3-back tasks, there were no significant differences between treatment conditions on any accuracy measures \((all \ p>0.1)\).

Analysis of the latency data revealed a significant difference between treatment conditions in the 3-back task \([F(2,32)=5.37, p<0.05]\), with a trend towards significance in the 1-back \([F(2,32)=2.66, p=0.086]\) and 2-back tasks \([F(2,32)=3.00, p=0.08]\). Planned contrasts revealed a significant increase in latency after the TPD+P condition compared to the BAL condition for all n-back levels \([1\text{-back}: F(1,16)=4.82, p<0.05; 2\text{-back}: F(1,16)=6.61, p<0.05; 3\text{-back}: F(1,16)=8.25, p<0.05]\), with no significant difference in latency between the TPD and BAL conditions in any of the n-back levels \((all \ p>0.1)\).

Spatial delayed-recognition task

All accuracy data were heavily skewed and nonparametric statistics were employed for analysis. Figure 2 shows the sensitivity index data for pre- and post-drug, which indicates that participants performed more poorly after TPD and TPD+P treatments. Binomial statistics reveal that there was a greater proportion of participants who performed more poorly after TPD+P than after the BAL condition \((p<0.05)\). There was no significant difference in performance between the TPD and BAL conditions or the TPD +P and TPD conditions \((both \ p>0.05)\). However, this effect appears subtle, as confirmed by a Friedman’s test of performance, which revealed no overall significant difference between all three treatment conditions for the sensitivity index \(\chi^2=1.075, p>0.1\), accuracy of identifying the
original stimulus ($\chi^2=1.107$, $p>0.1$), or accuracy of identifying the new stimulus ($\chi^2=0.326$, $p>0.1$).

Non-spatial working memory

The non-spatial working memory sensitivity index data were suitable for parametric analysis. Non-parametric tests were run on the measures of accuracy of identifying the original and the new stimulus due to data skewing. These analyses revealed no significant interactions between treatment condition and performance (all $p>0.1$).

Baseline-dependence analysis

Participants who scored either above or below the median in at least four of the six baseline sessions were classed as either “high” or “low”, respectively. This resulted in a group of six “high” baseline participants, a group of six “low” baseline participants, with six participants excluded from the analysis, as they did not differentiate substantially between high and low baseline scores. There were no significant interactions between the baseline working memory factor and performance on any measure (all $p>0.1$).

Reaction time

There was a main effect of reaction time, with reaction time increasing after all treatment conditions [$F(2,34)=7.00$, $p<0.05$]. However, there was no interaction between treatment condition and time [$F(2,34)=1.16$, $p>0.1$].

Critical flicker fusion

A 3 (treatment condition) by 2 (time) repeated measures ANOVA revealed no significant interaction between treatment conditions and time on the CFF measure [$F(2,34)=0.94$, $p>0.1$].

Subjective side effects and correlations with cognition

Four participants withdrew from the study due to the side effect of nausea, and their cognitive results were not analysed. However, in the remaining sample ($n=18$) there was no difference between the treatment conditions in subjective side effects [$F(2,34)=0.10$, $p>0.1$]. The nausea scale was also examined separately, and similarly, there was no significant difference between treatment conditions ($p>0.1$).

The changes in side effect rating between treatment and placebo (i.e. TPD–BAL and TPD+P–BAL) were correlated with corresponding changes in working memory performance between treatment and placebo (TPD–BAL and TPD+P–BAL) for all significant (and trend) working memory effects described above. There were no significant correlations between any of the cognitive measures and side effect scores (all $p>0.05$).

Discussion

To our knowledge, this is the largest study to date to examine the effects of TPD on working memory performance and the first study to examine the effects of stimulation of $D_1/D_2$ receptors under conditions of acute dopamine depletion in humans. Our findings suggest that (1) acute TPD did not impair performance on any of the spatial working memory tasks (or the non-spatial working memory task) and (2) that $D_1/D_2$ receptor stimulation in a dopamine-depleted state produced a subtle impairment in spatial working memory performance in humans.

The current study was not able to replicate previous findings of TPD-induced working memory deficits (Harmer et al. 2001, Harrison et al. 2004) on a number of working memory tasks with differing task demands, but is consistent with a number of recent studies (McLean et al. 2004; Mehta et al. 2005b; Roiser et al. 2005), which also failed to observe TPD-related working memory deficits. The lack of effect was not due to insufficient plasma tyrosine/phenylalanine depletion, as our plasma analysis revealed comparable depletion levels to that observed in both the Harrison et al. (2004) and Harmer et al. (2001) studies. Although we did observe a more conservative control/placebo than previously reported (indeed, by nature the TPD protocol produces a conservative control, in as much as the ratio of tyrosine/phenylalanine to large neutral amino acid (TP/ΣLNAA) is reduced in the control as well as the TPD condition), there was no indication of working memory impairment in the placebo condition making it unlikely that the conservative control accounted for the lack of effect seen. It is probable that variation in central dopamine depletion accounted, at least in part, for the lack of working memory impairments. Indeed, Mehta et al. (2005a,b) recently reported no TPD-related effects on working memory at a group level, but observed a correlation between central dopamine depletion (as indexed by striatal $[^{11}C]$raclopride binding changes) and performance change. Specifically, impairments in performance were only observed for participants with a high dopamine-depletion level, but virtually no change (and/or subtle improvement) was observed in participants with minimal dopamine-depletion levels. Interestingly, there was no relationship between plasma tyrosine/phenylalanine depletion and either striatal dopamine levels or performance (Mehta et al. 2005a,b).

It is doubtful that the lack of the TPD-related effects resulted from either the type of tasks used or of the study being underpowered. To our knowledge, this is the largest study to date investigating the effects of TPD on working memory, with 18 subjects. Importantly, Harrison et al. (2004) demonstrated TPD-related impairments (approximately 6% impairment) on an identical spatial working memory delayed-recognition task in a sample size of 12 participants. A possible source of difference between the Harrison et al. study and the current study is gender, as
Harrison et al. employed a female sample. Although Harmer et al. (2001) reported no interaction of gender with cognitive performance in their findings of TPD-related working memory impairments, the size of the groups (7 males, 5 females) does limit the generalizability of this finding and it remains possible that males were more resistant than females to the effects of dopamine depletion on this delayed-recognition, spatial working memory task. Furthermore, whereas the current study is the first to investigate TPD effects on the spatial n-back task, previous studies using the spatial n-back task have shown consistent performance impairments in smaller samples of patients with schizophrenia (Abi-Dargham et al. 2002; Callicott et al. 2003; Meyer-Lindenberg et al. 2001), with impairments in performance recently linked to D1 receptors within the PFC (Abi-Dargham et al. 2002).

Our second hypothesis, that D1/D2 receptor stimulation under conditions of dopamine depletion would have a positive effect on working memory performance by reversing TPD-induced impairments, was not supported. In fact, contrary to expectation, D1/D2 receptor stimulation under condition of tyrosine depletion caused a subtle impairment in spatial working memory performance. Although the exact mechanisms responsible for this finding are unclear, there are a number of possible reasons for this effect. Firstly, in light of the fact that TPD did not initially impair working memory, it may not be surprising that pergolide did not improve performance. Previous studies have been contradictory as to the effects of pergolide on working memory performance; while some studies have shown an improvement (Muller et al. 1998), other studies failed to observe an effect (Bartholomeusz et al. 2003; Roesch-Ely et al. 2005). It has been suggested that the lack of effect may be due to ceiling performance effects in already high-performing subjects (Bartholomeusz et al. 2003). In line with this suggestion, as TPD did not initially impair working memory performance in the current study and participants were performing at high accuracy levels, it is possible that on average participants’ performance was already at ceiling level and hence not improved by D1/D2 receptor stimulation.

Secondly, pergolide would be expected to act differently within a dopamine-depleted state. It has been suggested that phasic release of dopamine within a dopamine-depleted system may overstimulate D1 or D2 receptors due to sensitization (Abi-Dargham and Moore 2003; Grace 1991, 1993). The postsynaptic effects of D1 receptors are complex and can be considered as either excitatory or inhibitory, depending on the functional status of the neuron (Yang et al. 1999), and a sensitized D1 system may shift to a more GABAergic pathway. Thus, without the presence of normal endogenous dopamine levels, pergolide may have overstimulated the D1 system and resulted in increased inhibition (Abi-Dargham and Moore 2003). Indeed, the importance of optimal stimulation of D1 receptor in spatial working memory performance is well demonstrated in the non-human primate, with either insufficient or excessive D1 receptor stimulation leading to performance impairment (Williams and Goldman-Rakic 1993, 1995). In addition, low doses of D2 agonists have previously been shown to preferentially stimulate D2 autoreceptors (Di Chiara et al. 1977; Tissari et al. 1983), and it is possible that pergolide’s action at the D2 autoreceptor may have also influenced performance. Indeed, low doses of pergolide can augment motor deficits associated with Parkinson’s disease (Kellett and Steiger 1999), and recent evidence suggests that L-Dopa administration augmented, rather than reversed, TPD-related decreases in cocaine-induced drug craving (Leyton et al. 2004). The complexity of functional effects of dopamine augmentation within dopamine-depleted states is exemplified by the inconsistent effects of dopaminergic medication on cognition in Parkinson’s disease. Dopaminergic medications have been observed to improve, have no effect, or even impair performance on a range of cognitive tasks, and the effects may be dependent on both the basal levels of dopamine within the underlying corticostriatal circuitry and the nature of the task (Cools et al. 2001, 2003; Fern-Pollak et al. 2004; Gotham et al. 1988).

It is unlikely that side effects underlie the detrimental effects of pergolide on performance. First, although four participants did withdraw from the study due to nausea, the remaining participants showed no significant differences on the side effects measure. To more thoroughly examine the possibility that subjective side effects influenced performance, the change in side effect ratings was also correlated with change in performance for each significant performance measure and yielded no significant correlations. As the main working memory performance measure to be impaired was latency, it could also be suggested that the working memory effect observed was actually secondary to sedation. However, there was also no interaction between treatment conditions and performance on the critical flicker fusion task, a well-regarded psychophysical threshold measure of drug-induced sedation (Hindmarch and Parrott 1977). Furthermore, the simple reaction time measure used in this study showed that although the procedure itself caused an increase in reaction time (i.e. reaction time increased after all treatments), there was no interaction with treatment condition, making it unlikely that the increase in latency observed during the n-back task was purely sedation induced.

There was no evidence that the baseline working memory performance of participants influenced the effects of either TPD or TPD and pergolide on working memory performance; however, it remains likely that individual differences within the current sample may have influenced results. Although baseline working memory has previously been related to performance changes after dopamine manipulation (Kimberg and D’Esposito 2003; Kimberg et al. 1997; Mehta et al. 2000), these effects have been inconsistent and sometimes contradictory and may be dependent on factors such as concentrations of drug and time of cognitive testing (in respect to kinetic effects of the drug) (for further discussion, see Kimberg and D’Esposito 2003). It is likely that individual differences may be more reliably linked to functional polymorphism (val-met) in the catechol-O-methyltransferase (COMT) gene (of which baseline-dependent behaviour may be a reflection), based on
evidence that COMT genotype has been observed to influence working memory performance changes in healthy volunteers after amphetamine administration (Mattay et al. 2003) and n-back task performance in patients with schizophrenia after olanzapine treatment (Bertolino et al. 2004).

In summary, our findings suggest that acute TPD does not induce impairments in spatial working memory on tasks previously shown to be susceptible to dopaminergic manipulations. However, the main finding of this study was that stimulation of D1/D2 receptors under dopamine-depleted conditions causes a subtle impairment in spatial working memory performance. Although the findings of a D1/D2 receptor-related impairment (under TPD conditions) highlight the complexity of augmenting dopaminergic transmission within a dopamine-depleted state, the lack of TPD-related effects on a range of tasks, taken together with other recent reports of no working memory effects after TPD (McLean et al. 2004; Mehta et al. 2005; Roiser et al. 2005), questions the reliability of TPD as an experimental method to probe the dopaminergic system and working memory performance in humans.

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APPENDIX 6: Poster presented at the Human Brain Mapping meeting in Hungary (June 2004) and Collegium Internationale Neuropsychopharmacologicum meeting in France (June 2004).
Effects of acute tyrosine depletion on blood flow during a spatial working memory n-back task: A PET investigation

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INTRODUCTION

It is well established that the prefrontal cortex (PFC) and posterior parietal cortex underlies spatial working memory performance. Studies in monkeys, humans, and animals with Parkinson’s disease and Schizophrenia (a condition that impairs spatial working memory specifically) support this view.

Dopamine circuits can be experimentally depleted in monkeys and rodents, and dopamine depletion is dependent on the immediate term of the dopamine (D1/1/2) receptor and the cholinergic/noradrenergic system. Administration of an anticholinergic drug such as haloperidol results in changes in the dopamine (D1/1/2) receptor system.

Some evidence suggests that TP53 can impair spatial working memory (SWB). How does this relate to the brain function of TP53 directly?

METHOD

Participants and Design

- 10 healthy males (mean age: 26.4 ± 3.5 years) were recruited into the study following a standardized vision and robustness (D41) condition and once following an anticholinergic drug and dopamine depletion (D42) condition.
- Order of administration was counterbalanced and the study followed a randomized, repeated measures design.

Amino acid mixture

- The D41 amino acid mixture contained sarcosine (15g), leucine (27.5g), lysine (17.5g), methionine (5g), valine (17.5g), isoleucine (15g), tyrosine (2.5g), threonine (17.5g), and phenylalanine (17.5g). The D42 mixture was identical to the D41 mixture but with threonine (17.5g) and phenylalanine (17.5g) replaced by L-tyrosine (17.5g) and L-phenylalanine (17.5g), respectively.

Procedures

- Participants followed a low protein diet for 24 hours prior to testing, fasting from 8pm.
- Amino acids were administered at approximately 100mg/kg/m2 to a medium-renal remnant rats (n=15). Each rats received a single bolus injection of the amino acid mixture.
- Rats were controlled to have blood pressure using the Rainin TTT-141/12/141 12-channel 90-channel monitors.

Spatial n-back task

- Subjects were placed in a box for each trial, and the box dimensions were measured.
- A target (a 1x1 cm square) was presented for 1 second, followed by a blank screen for 1 second, and then another target (a 1x1 cm square) was presented for 1 second.

Data Analysis

- Analysis was conducted using PPPR (Postprocessing, Preprocessing, and Removal) using the spatial clusters of the relevant regions as reference, and the task-related deficits were obtained.

RESULTS

Behavioral Effects

A significant parametric memory task effect was observed for both reaction time (F(12, 47) = 6.483, p = 0.001) and accuracy (F(12, 47) = 4.376, p = 0.001). Despite this, there was no interaction between task, task difficulty, and drug condition (reaction time: F(12, 47) = 1.317, p = 0.251; accuracy: F(12, 47) = 1.814, p = 0.086).

Task activations

Correlates with previous research task related increases in CBF with dopamine depletion. These increases were larger in the niacin-depleted group than in the control group.

TPD related cBf changes

TPD resulted in widespread increases in CBF. In the control group, there were significant increases in CBF in the anterior cingulate gyrus (BA 32) and the posterior cingulate gyrus (BA 23).

Interaction between task and drug

Interaction analysis revealed that the drug did not significantly augment or attenuate task related CBF (even though lowering the threshold to look at a type II error).

DISCUSSION

This study is a basic neuroscience investigation in order to understand the influence of this task on the frontal cortex, with interactions to the PFC and the prefrontal cortex and the hippocampus.

TPD-related changes in CBV were observed in the anterior cingulate gyrus and the posterior cingulate gyrus. These increases were larger in the niacin-depleted group than in the control group.

Weighted TCCR functioning in the niacin-depleted group was superior, both in terms of reaction time and accuracy. This result is consistent with previous findings.

Overall, these findings add weight to the hypothesis that the drug did not significantly augment or attenuate task-related CBF (even though lowering the threshold to look at a type II error).

REFERENCES


APPENDIX 7: Poster presented at the Australasian Society for Psychiatric Research (ASPR) meeting in Canberra, Australia (December 2002).
A Neurophysiological Study of Human Spatial Working Memory

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Introduction

Working memory reflects a number of distinct brain systems and is an important component of complex cognitive processes. It is thought to be involved in a variety of tasks that require the retention and manipulation of information in the face of distractions, such as maintaining a phone number in mind while engaging in a conversation or remembering the ingredients for a recipe while cooking.

Method

Subjects

39 male subjects (mean age ± standard deviation: 22 ± 4.1 years) participated in the study. All subjects were healthy, right-handed, and had no history of medication or any neurological disease.

Procedure

The study involved a visual working memory task, in which participants were required to remember a series of numbers that were presented on a screen. The numbers were presented in a random order, and participants were instructed to remember the sequence of numbers for a period of time. The task was designed to test the participants' ability to maintain information in working memory and to manipulate it as necessary, such as by comparing or combining the numbers.

SSVEP Data

SSVEP (Spatio-Spectral Visual Event-related Potential) data were collected using a 64-channel EEG system. The electrodes were placed on the scalp according to the International 10-20 System, with reference electrodes on the earlobes. The data were acquired at a sampling rate of 1 kHz and filtered between 0.5 Hz and 50 Hz. The data were analyzed using a custom-built software package that applied fast Fourier transforms (FFT) to the data to identify the SSVEP frequencies.

Results

SSVEP Data

The figure below shows the SSVEP amplitudes for each of the 64 channels. The SSVEP amplitudes were calculated as the mean amplitude over the 2-second period following the presentation of each stimulus. The data were further analyzed to determine the latency and reproducibility of the SSVEP responses.

Behavioural Data

Behavioural data were collected to assess the participants' performance on the working memory task. The data were analyzed using a custom-built software package that applied fast Fourier transforms (FFT) to the data to identify the SSVEP frequencies.

Discussion

The results of this study suggest that the SSVEP responses are sensitive to the task demands. The amplitude of the SSVEP response was found to be correlated with the performance on the working memory task. The latency of the SSVEP response was found to be consistent across the different conditions of the task.

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References


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APPENDIX 8: Publications arising from the current thesis
Publications arising from current thesis

Papers arising from work contained in this thesis:


Published abstracts:
