
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

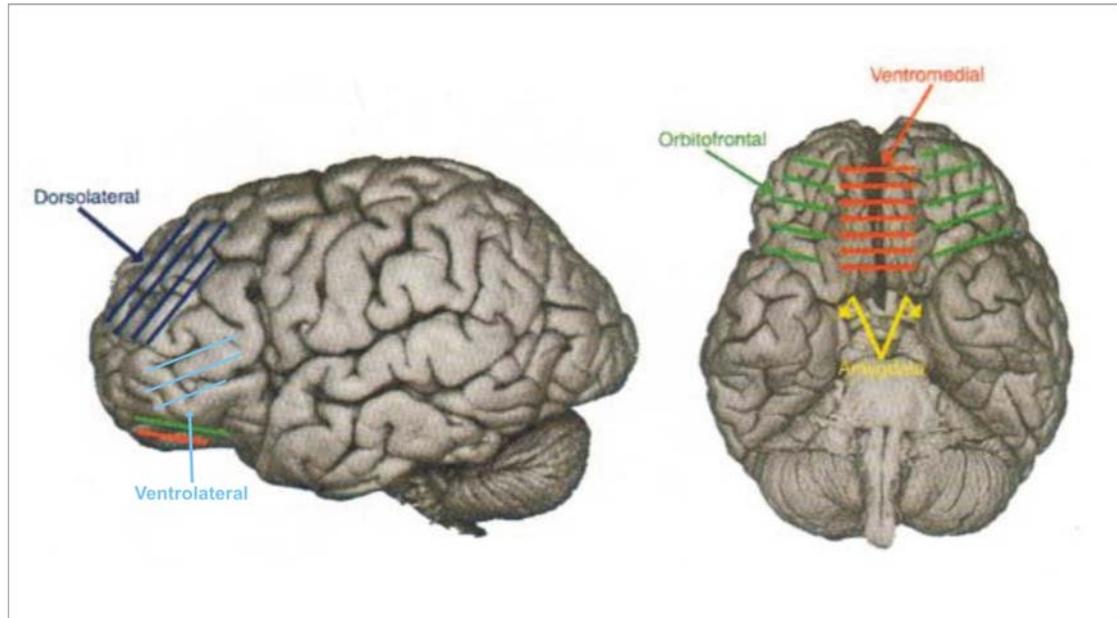


Figure 1-1 Regions of the human prefrontal cortex. Figure reproduced from Davidson & Irwin (1999).

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.

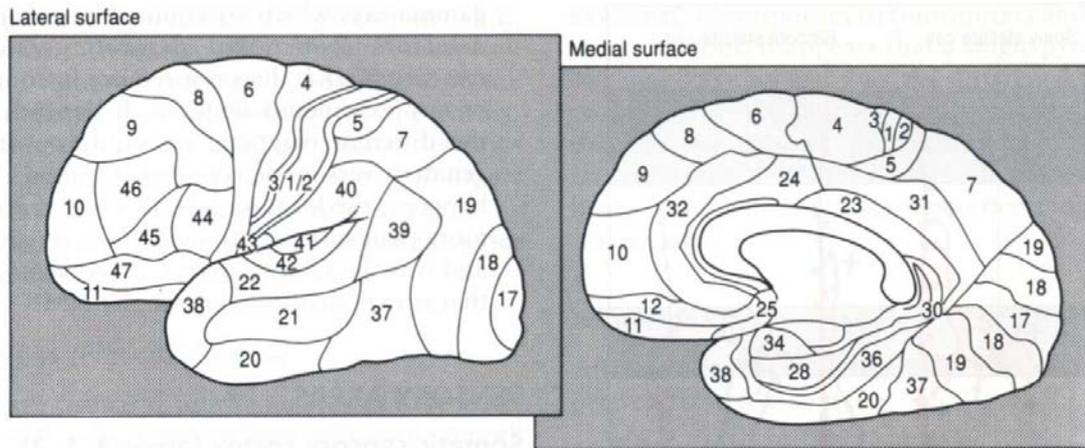


Figure 1-2 Cytoarchitectural areas of Brodmann. Figure reproduced from FitzGerald, (1996)

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, Druzgal 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-*O*-methyltransferase (COMT). COMT is thought to be of importance in the metabolism of released dopamine in the PFC, where dopamine transporters are 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 within the brain (Cooper et al., 2003).

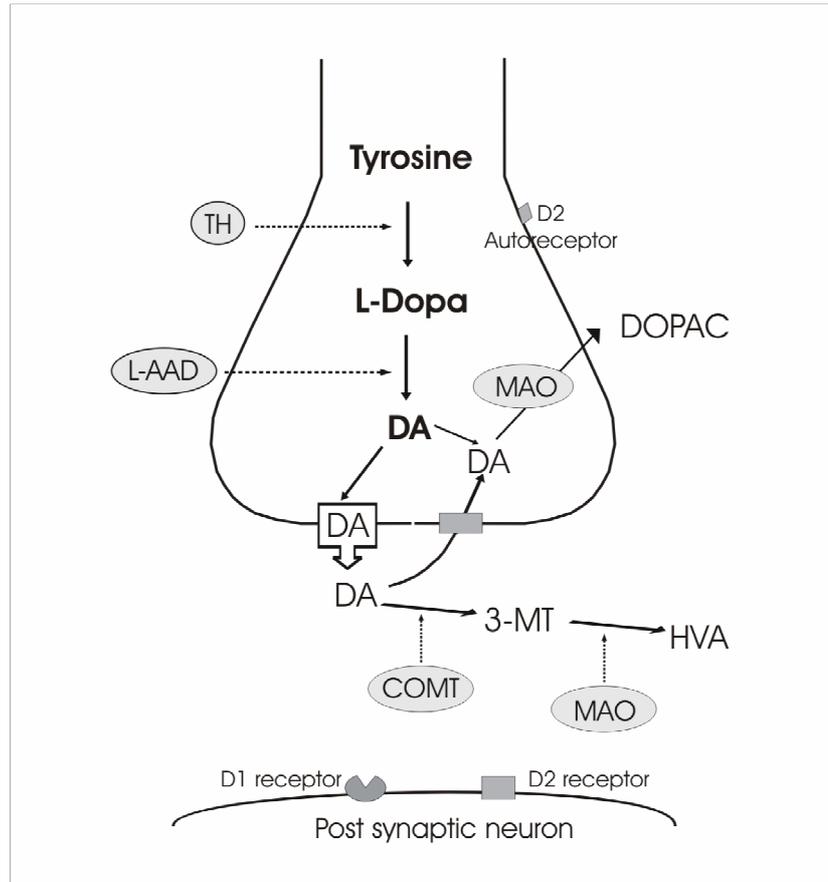


Figure 1-3 Schematic diagram of a typical (striatal) dopamine nerve terminal illustrating the synthesis, release and metabolism of dopamine.

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).

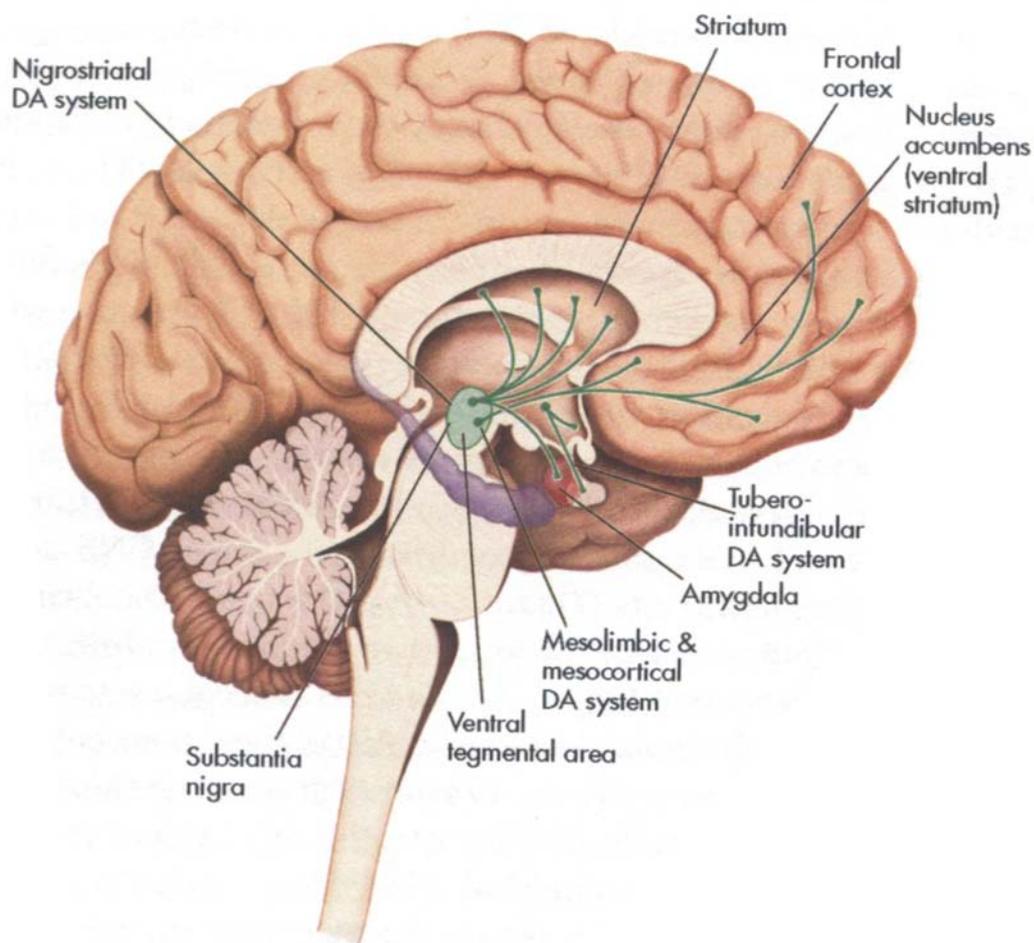


Figure 1-4 The major brain dopaminergic projections. Figure reproduced from Szabo et al. (2000)

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 D₁ 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 iontophored 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)

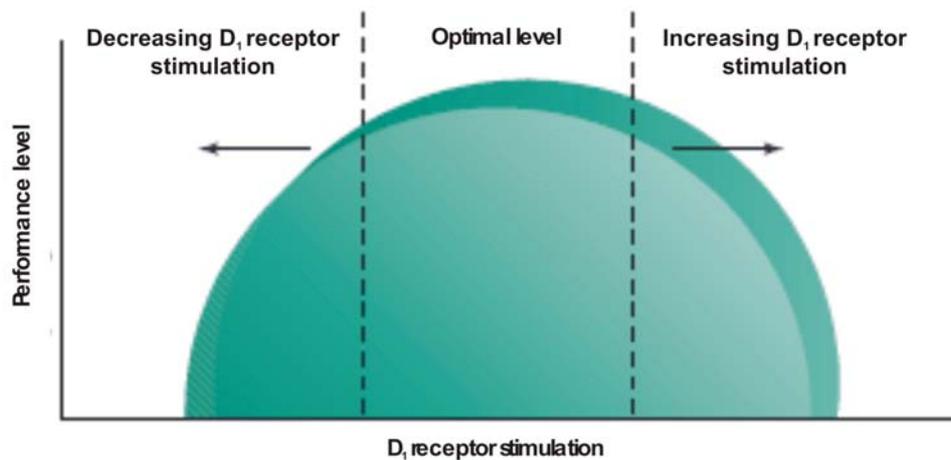


Figure 2-1 Schematic representation of the inverted U shaped function as it may relate to delayed-response performance in the monkey.

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 sites (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

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

Key: BROM = bromocriptine, PERG = pergolide, MPH = 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 D₂ 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 D₁/D₂ receptors, while bromocriptine at low concentrations and early in its time course has predominantly presynaptic inhibitory effects on cortical activity of D₂ 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 D₁ 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 α_2 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 α_2 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

[11C]raclopride binding potential (BP), a measure of dopamine D₂/D₃ receptor availability.

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

Author	Sample	Tasks	Change in performance
Harmer et al. 2001	N= 12	SWM Self-ordered strategic search, SWM delayed-recognition	Performance ↓
Harrison et al. 2004	N=13	SWM delayed-recognition N-SWM digit sequence	SWM: Performance ↓ N-SWM: No effect
Lythe et al. 2005	N=12	SWM delayed-recognition	No effect
McLean et al. 2004	N=49 between-subjects design	SWM Self-ordered strategic search, SWM spatial span	No effects
Roiser et al. 2004	N=20	SWM Self-ordered strategic search	No effects
Mehta et al. 2005	N=14	SWM delayed-recall	No group performance effect. Greater dopamine depletion ↓

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 α_2 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 α_2 adrenergic receptors, but impairs PFC function through actions at α_1 adrenergic receptors (for reviews, see Arnsten, 1997, Arnsten and Li, 2005)

Findings from human studies have supported a role for the α_2 adrenoceptor in working memory. Studies using the α_2 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 2.5 μ g/kg producing a greater deficit in performance than 1.5 μ g/kg. Similarly, Jakala (1999) observed doses of 0.5 μ g/kg and 5 μ g/kg of clonidine to impair SWM (on the self-ordered SWM task), with no effects were observed following a dose of 2 μ g/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 α_2 agonists act pre-synaptically, while higher doses of α_2 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 29 μ g/kg dose of guanfacine (also an α_2 adrenoceptor agonist) improved SWM performance. These authors suggesting that the greater selectivity of guanfacine for α_2 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 modafinil 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 α_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 α_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 α_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, Rasmussen 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 accumbens (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

glutamatergic and dopaminergic systems, it appears probable that these neurotransmitters exert an influence on working memory in humans.

2.5 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 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, potentially through effects within the striatum or through activation of other dopamine receptor sites (i.e. D₃/D₄ receptors) (Arnsten 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 D₁/D₂ 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 D₁/D₂ 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 H₂¹⁵O 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 D₁ 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.

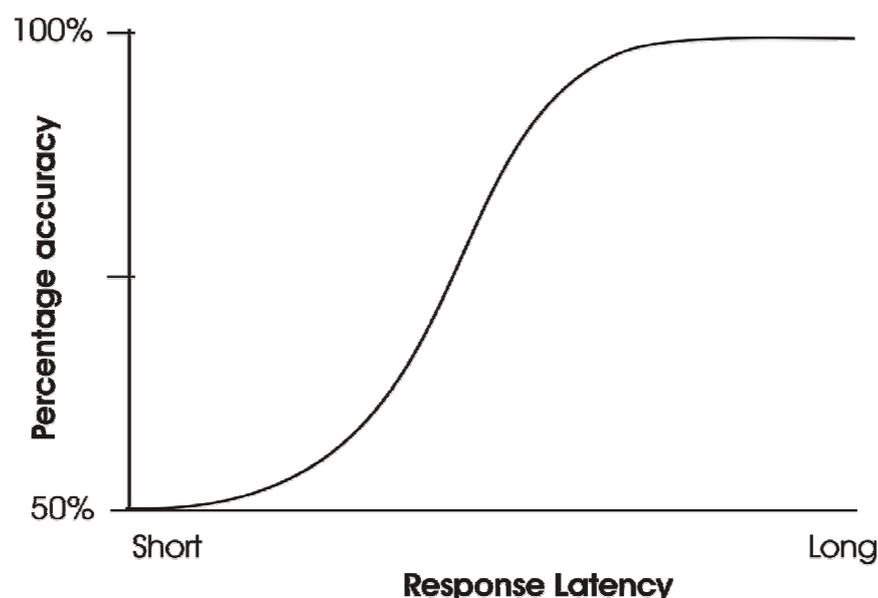


Figure 3-1 Speed-accuracy trade-off curve

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 [$Y=2 \times \arcsin \sqrt{p}$, 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: D₁ receptor K_i value = 447nM and D₂ 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_{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, known 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

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

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 continuous and rapid enough to prevent the ERP from returning to baseline.

Thus SSPT involves eliciting SSVEPs from a continuous 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 5k Ω), 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: $(\text{change in phase} / 2 \times \pi) \times (1000 / 13)$. 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).

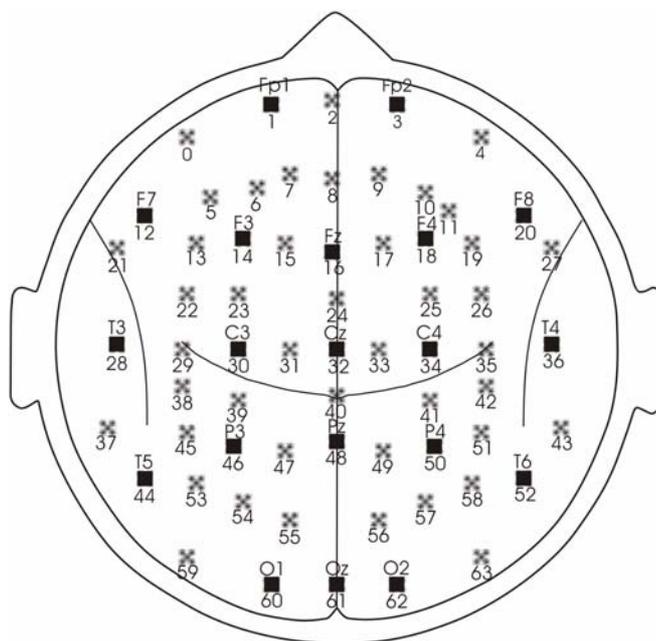


Figure 3-2 Position of 64 scalp electrodes used in SSPT recordings in this thesis

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 H₂¹⁵O 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 previously (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 PET¹. 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.

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

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

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). Drinks 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 ($T+P/\sum LNAA$) 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 ($\mu\text{mol/l}$) and change in percentage from baseline to 5 hours post-drink.

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

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 ($Y = 2 \times \arcsin \sqrt{p}$, 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 [$F(2,34) = 0.63$, $p > 0.1$].

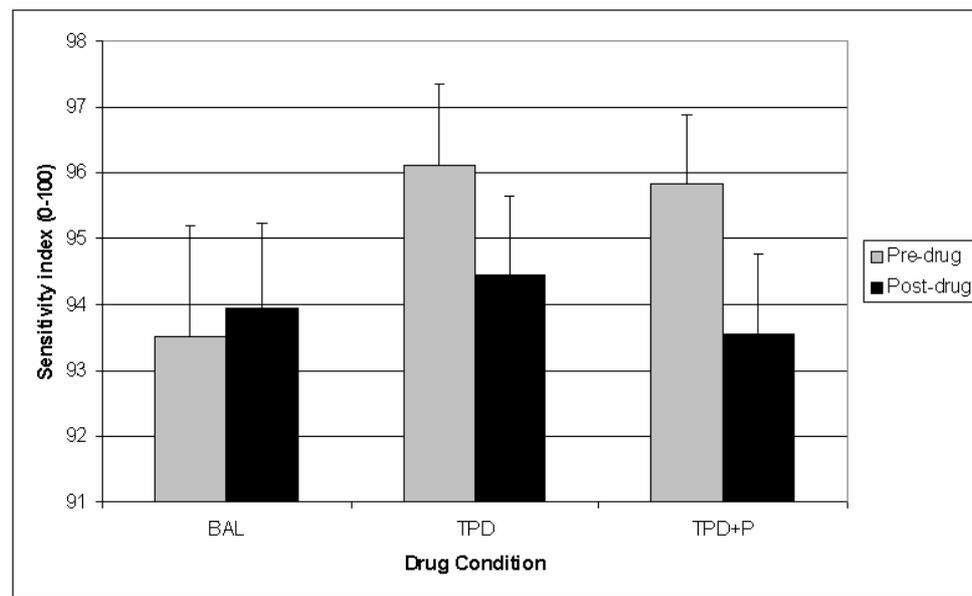


Figure 4-1 Sensitivity Index for the SWM delayed-recognition task at baseline and post-treatment, for each treatment condition. Error bars represent standard error of the mean (SEM).

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.

Tasks	Balanced		TPD		TPD+P	
	Pre	Post	Pre	Post	Pre	Post
Reaction time	254.6 (4.7)	257.3 (5.3)	261.0 (5.1)	265.8 (4.6)	254.4 (5.6)	265.9 (4.3)
CFF	37.3 (1.2)	37.0 (1.4)	38.3 (1.3)	37.2 (1.6)	38.3 (1.6)	35.1 (1.3)
VAS: Alertness	26.7 (3.9)	27.0 (4.5)	24.4 (3.5)	31.3 (4.2)	24.6 (4.1)	26.7 (3.9)
VAS: Tranquillity	30.4 (4.9)	26.5 (3.7)	23.9 (4.2)	22.8 (3.0)	22.8 (2.8)	25.1 (4.6)

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, D_1/D_2 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 ($TP/\Sigma LNAA$) 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 [^{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, 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 pergolides 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.