Cognitive Impairment: Quantification and Possibilities for Pharmacological Treatment

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For Oliver
Acknowledgements

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ABSTRACT

Cognitive impairments are a common feature of neurological and neuropsychiatric disorders, as well as of substance-abuse disorders. The impairments seen in these disorders can be caused by disruptions to common neural substrates, and therefore pharmacological agents can be repositioned from use in neuropsychiatric to neurological disorders, and vice versa. Together, these disorders have been estimated to comprise 13% of the global burden of disease. Indeed, an individual’s ability to successfully perform everyday activities can be limited by deficits in crucial cognitive functions such as attention, response inhibition, planning and working memory. Frontal-striatal networks in the brain have been shown to underlie these vital functions, which are modulated by neurotransmitters including acetylcholine, dopamine, and noradrenaline. Importantly, these functions are susceptible to pharmacological intervention with drugs such as physostigmine, modafinil, and atomoxetine.

In order to explore the nature of a variety of forms of cognitive impairment, which were diverse in severity from mild to more severe, studies were carried out on amateur boxers and sleep-deprived doctors, as well as on patients with subarachnoid haemorrhage (SAH) and on patients with Parkinson’s disease (PD). Quantification of cognitive impairment is the crucial first step in determining which neural networks are involved, and thus which pharmacological agents would be suitable candidates for treatment.

A longitudinal study was carried out using a comprehensive battery of well-validated cognitive tasks, in order to quantify the change in cognitive ability in healthy individuals who participated in amateur boxing. Subtle cognitive impairments, which were related to structural changes, were documented.

Using existing understanding of pharmacological agents, novel treatments for cognitive impairments were explored in relation to sleep-deprived doctors, as well as to PD and SAH patients. A novel treatment for specific cognitive problems in PD was investigated: atomoxetine, a noradrenaline reuptake inhibitor. A double-blind placebo-controlled study revealed that atomoxetine may be a candidate for treatment of response inhibition impairments seen in PD. This finding is important as noradrenergic treatments are not currently used in PD, despite degeneration in the locus coeruleus, the main cortical source of noradrenaline. Another novel treatment explored was modafinil, a drug that has also been shown to modulate the noradrenergic system, as well as the dopaminergic system. Modafinil is currently licensed for use in narcolepsy and shift work sleep disorder. It was found that modafinil remediates task set-switching impairments and reduces impulsivity in sleep-deprived doctors. Furthermore, it was shown that modafinil might be a potential treatment for cognitive impairments found in neurological patients with SAH. In contrast to this, physostigmine, a cholinesterase inhibitor, did not seem to alter the cognitive symptoms investigated.

To summarise, this thesis aims to quantify cognitive impairment in a range of groups, and to explore the potential use of existing pharmacological agents that could be repurposed to treat cognitive impairments in novel ways.
DECLARATION

This work was carried out in the Department of Psychiatry, University of Cambridge under the supervision of Prof. Barbara Sahakian and Prof. John Pickard. This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except as specified below and in the Acknowledgements. This dissertation has not been submitted in whole or in part for any other degree or qualification at any other university, and does not exceed the limit of length as specified by the Faculty Board of Clinical Medicine.

Chapter 3: The diffusion tensor imaging data was processed by Dr Virginia Newcombe.

Chapter 5: The blood samples were analysed by Dr Ralf Regenthal.
PUBLICATIONS THAT HAVE ARISEN FROM WORK CONTAINED IN THIS THESIS


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ABBREVIATIONS

5-CSRT  5 Choice serial reaction time
5-HT  Serotonin
ACh  Acetylcholine
ACoA  Anterior communicating artery
AD  Alzheimer’s disease
ADC  Apparent diffusion coefficient
ADHD  Attention deficit hyperactivity disorder
ANCOVA  Analysis of covariance
ANOVA  Analysis of variance
BDI  Beck depression index
BOLD  Blood oxygen level dependent
CANTAB  Cambridge neuropsychological test automated battery
CGT  Cambridge gamble task
COMT  Catechol–O–methyl transferase
COWAT  Controlled oral word association test
CSF  Cerebrospinal fluid
CT  Computerised tomography
CUABC  Cambridge University amateur boxing club
DA  Dopamine
dAT  Dopamine transporter
DBS  Deep brain stimulation
DDS  Dopamine dysregulation syndrome
DRT  Dopamine replacement therapy
DTI  Diffusion tensor imaging
ESS  Epworth sleepiness scale
FA  Fractional anisotropy
fMRI  Functional magnetic resonance imaging
GCS  Glasgow coma scale
GFAP  Glial fibrillary acidic protein
ICB  Impulsive/compulsive behaviour
IED  Intra-extra dimensional set shift
IFG  Inferior frontal gyrus
IST  Information sampling task
LC  Locus coeruleus
LEU  Levodopa equivalent unit
LIFG  Left inferior frontal gyrus
LpreSMA  Left pre-supplementary motor area
MCA  Middle cerebral artery
MHRA  Medicines and healthcare products regulatory agency
MMSE  Mini mental state examination
MNI  Montreal neurological institute
MPRAGE  Magnetisation-prepared rapid acquisition with gradient echo
MRI  Magnetic resonance imaging
MWM  Morris water maze
NA  Noradrenaline
NART  National adult reading test
NFL  Neurofilament protein
NMDA  N-methyl-D-aspartate
OCD  Obsessive-compulsive disorder
OTS  One touch stockings of Cambridge
PAL  Paired associates learning
PCoA  Posterior communicating artery
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>PFC</td>
<td>Pre-frontal cortex</td>
</tr>
<tr>
<td>PICA</td>
<td>Posterior inferior cerebellar artery</td>
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<tr>
<td>REM</td>
<td>Rapid eye movement</td>
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<tr>
<td>rIFG</td>
<td>Right inferior frontal gyrus</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>RpreSMA</td>
<td>Right presupplementary motor area</td>
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<td>RTI</td>
<td>Reaction time task</td>
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<td>RVIP</td>
<td>Rapid visual information processing</td>
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<td>SAH</td>
<td>Subarachnoid haemorrhage</td>
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<tr>
<td>SAP</td>
<td>IgG-Saporin</td>
</tr>
<tr>
<td>SNpc</td>
<td>Substantia nigra pars compacta</td>
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<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
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<tr>
<td>SSP</td>
<td>Spatial span</td>
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<td>SSRT</td>
<td>Stop signal reaction time</td>
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<td>SST</td>
<td>Stop signal task</td>
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<td>STN</td>
<td>Subthalamic nucleus</td>
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<td>TBI</td>
<td>Traumatic brain injury</td>
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<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s disease rating scale</td>
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<td>VAS</td>
<td>Visual analogue scales</td>
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<tr>
<td>WCST</td>
<td>Wisconsin card sort task</td>
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<td>WTAR</td>
<td>Wechsler test of adult reading</td>
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1. Introduction

1.1. Cognitive Impairment

Cognition is the ultimate function of the brain (Robbins 2011). Impairments in cognition are a common feature of all neuropsychiatric, neurological and substance abuse disorders, and together these diseases constitute 13% of the worldwide disease burden, a larger percentage than cancer or heart disease (World Health Organisation The Global Burden of Disease: 2004 Update, WHO 2008). Cognitive impairments occur across a range of processes such as memory, attention, and learning, and across executive functions, such as planning and problem solving. They can lead to a decrease in quality of life and to a loss of independence, and impact on the individual, their family, the healthcare system, and society as a whole.

The annual cost of mental ill-health in England alone is about £6 billion for economic costs, rising to £77 billion when wider impacts are included, such as reduction of quality of life (The Sainsbury Centre for Mental Health, 2003). The incidence of dementia, and other diseases related to old age, is predicted to increase due to the ageing population, representing an expenditure ‘time-bomb’ with costs to the UK economy reaching more than £50 billion over the next 30 years, unless more effective treatments for cognitive impairment are developed and made widely available (Comas-Herrera, Wittenberg et al. 2007). Furthermore, cognitive impairment can also occur in ‘healthy’ individuals under certain conditions, such as sleep deprivation and malnutrition, which has both a functional and economic impact (Colten and Altevogt 2006). Therefore, the cognitive and mental well-being of populations is key for economic and social prosperity (Beddington, Cooper et al. 2008), so developing and maintaining these is of vital importance. Cognitive and emotional resources can be influenced in both positive and negative ways over the lifespan (Figure 1.1), for example social stimulation, medication and physical activity can all influence cognition and emotional well-being in a positive way. The concepts of cognition and well-being are intimately related. For example, older individuals who report higher levels of well-being also have better cognitive function, even when adjustment has been made for other possible explanatory factors, such as socio-demographic variables, health, and lifestyle (Llewellyn, Lang et al. 2008).
Figure 1.1 Cognitive and Emotional Resources Over the Lifespan

Cognitive and emotional resources can be influenced both positively and negatively over the lifespan. From Beddington et al (2008), with permission.
Understanding the nature of cognitive impairment in disorders is the first step towards treating them. Crucially, identifying neurocognitive endophenotypes that are seen across a number of disorders, and are derived from measures of brain function rather than just behaviour, may be key for highlighting new therapeutic avenues (Robbins, Gillan et al. 2012). Thus, understanding cognitive impairment in all ages and conditions is important. However this thesis focuses on mild head injury, subarachnoid haemorrhage (SAH), Parkinson’s disease (PD) and sleep deprivation, which are disorders that have varying degrees of pathology. The first main aim of this thesis was to quantify cognitive impairment in these populations.

1.2. Pharmacological interventions

Increased understanding of the molecular, cellular, and neuropsychological basis for cognitive impairment can lead to new pharmacological treatments. Alongside other interventions such as physical activity, mental activity, social stimulation, and diet, pharmacological agents represent an important mechanism with which to remediate cognitive impairment. For example, a report commissioned by the Alzheimer’s Research Trust in Cambridge, UK, estimated that a treatment that would reduce severe cognitive impairment in the elderly by just 1%, would cancel out all the estimated increases in long-term care costs due to the ageing population (Comas-Herrera, Wittenberg et al. 2007).

Despite this, there is currently a crisis in drug development for mental illness and neurology, with drug companies withdrawing from the field or redirecting their investments. This has led researchers to call for a new approach to drug discovery: to focus on the treatment of specific cognitive impairments that have a neurobiological basis, which would have a clinical effect across a number of patient groups, as opposed to a disorder based approach (Insel and Sahakian 2012; Robbins, Gillan et al. 2012). Using this method, drugs which are shown to enhance a neurocognitive function, for example impulsivity, can be repurposed across a range of disorders where this symptom occurs. This idea is not new, but advances in genetics, neuroimaging and cognitive neuroscience now facilitate this approach.

Therefore, the second broad aim of this thesis was to utilise knowledge of the brain’s psychopharmacological mechanisms, and the known effects of pharmacological agents on biochemistry and cognition, in order to determine the potential of these drugs to be used for conditions for which they are not currently prescribed. Specifically, the pharmacological agents modafinil, atomoxetine, and physostigmine were investigated. This work aimed both to extend the clinical use of these agents as well as to increase the understanding of their mechanisms of action.

This chapter provides a brief overview of the conditions studied, the neurochemical agents used, and the pharmacology of the implicated neurotransmitter systems. Specific details related to the individual studies are provided in the experimental Chapters 3-6, each of which is presented with a full introduction and discussion of the relevant results.
1.3. **Conditions Investigated in this Thesis**

1.3.1. **Head Injury**

Traumatic Brain Injury (TBI) affects people of all ages, with peak incidence in the 15-24 years age group. TBI represents the most common cause of death and disability in young people (Ghajar 2000). Because of this, TBI represents a high long-term burden. Brain injury is very heterogeneous, and therefore this presents challenges both to the quantification of cognitive impairments and to the development of treatments.

Traditionally, the study of lesions in patients and animals has been the paradigm of cognitive neuroscience, with the lesion model laying the empirical foundation for learning about brain organisation. The advent of neuroimaging techniques such as computerised tomography (CT) and magnetic resonance imaging (MRI) allowed for the localisation of brain injury in vivo, and, along with the development of cognitive psychology, enabled the localisation of brain function. This was based on the assumption that the cognitive difficulties experienced by neurological patients are an approximation in terms of the functioning of the normal system, with certain isolable subsystems or transmission routes operating in an impaired fashion (Shallice 1988). Key to this methodology are double dissociations, whereby a patient (or group of patients with overlapping lesion sites) performs poorly on one task, but within the normal range on another, and a second patient (or group of patients with overlapping lesions in a distinct area) shows the opposite pattern. The wide variety of focal lesion locations seen after brain injury is reflected in the vast range of cognitive impairments seen. However, because certain areas of the brain are particularly vulnerable when acceleration-deceleration forces are applied, certain brain injury profiles are common, including orbitofrontal, anterior and inferior temporal contusion, and diffuse axonal injury (Weber and Maas 2007). Reflecting this, the cognitive impairments seen are often diffuse with more prominent deficits seen in attention, memory, and executive functions including cognitive flexibility, planning and impulsivity (McAllister 1992). In association with these cognitive deficits, behavioural changes are often observed after TBI, particularly the exacerbation of pre-morbid impulse control problems.

Some cognitive impairments observed in head injury patients do not correlate with the site of focal injuries, indicating subtle diffuse damage, or damage to integrated networks. Possible mechanisms for diffuse damage include the shearing of white matter tracts, ischemia, or raised intracranial pressure. It has been suggested that the cholinergic system is particularly vulnerable to damage after TBI, perhaps due to the unique role choline plays in metabolic processes whereby it is not only used to form the neurotransmitter acetylcholine, but also in cell membrane synthesis. Therefore, when choline is depleted after the excitotoxic release of neurotransmitters which occurs post head injury, neurons may use the choline bound in the cell membrane to create Acetylcholine (ACh). There are a number of evidence sources to suggest that cholinergic disruption occurs in association with secondary injuries post TBI. For example, animal studies demonstrate that reductions in ACh receptors are not detectable three hours post-injury, although they are present twenty-four hours post-injury (DeAngelis, Hayes et al. 1994). The anatomical pattern of degeneration post-head injury in animal models has also been shown to evolve over time, with
only hippocampal abnormalities being detectable first, followed later by thalamus and basal forebrain abnormalities (Leonard, Grady et al. 1997; Chen, Pickard et al. 2003).

In support of the cholinergic hypothesis, there is consistent evidence that impairments in memory, sustained, selective, and divided attention, and delayed reaction times are seen across head injury patients following severe, moderate and mild injury (Arcia and Gualtieri 1994; Whyte, Polansky et al. 1995; Arciniegas, Adler et al. 1999; Hellawell, Taylor et al. 1999; Chan 2000; Polo, Newton et al. 2002; Salmond, Chatfield et al. 2005). ACh is crucial for these cognitive functions (see Section 1.5.3). Indeed, damage to the basal nucleus of Meynert has been reported following fatal head injury in humans (Murdoch, Nicoll et al. 2002), and patients with mild TBI have been shown to have abnormal resting state thalamus networks using functional MRI (Tang, Ge et al. 2011).

Overall, TBI can cause both focal as well as diffuse damage. It can be difficult to determine which systems are vulnerable to damage after mild cases, although the cholinergic hypothesis goes some way to doing this. In Chapter 3 the association between mild head injury sustained in amateur boxing and cognition and brain structure was explored.

1.3.2. Subarachnoid Haemorrhage

SAH is the bleeding into the skull and the meninges that usually follows the rupture of an aneurysm or an arteriovenous malformation. The most frequent sites of aneurysm are the anterior communicating artery (ACoA), middle cerebral artery (MCA) and posterior communicating arteries (PCoA) (Figure 1.2). SAH can occur spontaneously, or as a result of TBI. SAH accounts for roughly one in every twenty strokes, and in most populations the incidence is 6-7 per 100,000 people per year (Ingall, Asplund et al. 2000). Although incidence increases with age, half the patients are younger than 55 years at the time of SAH (Linn, Rinkel et al. 1996). Cognitive impairments are commonly reported after SAH. The exact cognitive profile of patients is yet to be fully defined.

Patients with SAH have historically been observed to suffer from poor neuropsychological and functional outcome, but in more recent years, due to advances in surgery and perioperative care, the cognitive symptoms seen appear to be less severe in nature. However, cognitive deficits which impact on quality of life are still common following SAH, even in patients who make a good recovery in terms of self-care (Hackett and Anderson 2000). Cognitive impairment is seen in the domains of memory, learning, attention, executive function, decision making, and risk adjustment (Mavaddat, Sahakian et al. 1999; Mavaddat, Krikpatrick et al. 2000; Salmond, DeVito et al. 2006; Passier, Visser-Meily et al. 2010; Latimer, Wilson et al. 2012; Sheldon, Macdonald et al. 2012).

The underlying pathology which causes cognitive impairment in SAH is probably a combination of diffuse brain injury and focal damage. Diffuse damage can occur as a result of the neurotoxic effects of widespread subarachnoid blood from the initial haemorrhage, and raised intracranial pressure, alongside focal injury through infarction or haematoma. Compromised blood supplies to regions supported by the vessel may
also cause neuropathological changes. Although the interconnected networks of arteries limit the extent of
the damage to tissue caused by compromised blood supply, such damage might explain some of the
differences seen between different aneurism locations. The end result is focal and scattered brain injury

It has been proposed that, in the same way as for TBI, the cholinergic system is susceptible to damage
following SAH. Support for the cholinergic hypothesis of cognitive impairment has been provided by a
study that reported an association between score on the Mini-Mental State Examination (MMSE) in SAH
and the tropicamide drop test (Nozaki, Sakai et al. 2002), which is classically used as a test for Alzheimer’s
disease (AD), which shows exaggerated pupil dilation in response to a cholinergic antagonist, tropicamide.
Furthermore, Bendel, Koivisto et al. (2006) reported loss of hippocampal volume, using volumetric MRI, in
patients with good or moderate clinical outcome one year after SAH, which correlated with performance
on tests of visual memory, attention, mental flexibility, and psychomotor speed. These findings have
indicated that cholinesterase inhibitors may be suitable candidates to remediate cognitive impairments
seen in SAH. One such drug, rivastigmine, has been associated with improvements on the Cognitive
Subscale of the Alzheimer Disease Assessment Scale, Rivermead Behavioural Memory Test, and Frontal
Assessment Battery in SAH. These findings are, however, limited due to the design of the study, which was
open-label and not placebo-controlled (Wong, Wong et al. 2009).

In summary, although cognitive impairment is commonly reported in SAH, the pattern of deficits is not well
established, and there are no current systematic treatments available to patients for the deficits seen.
Chapter 4 aimed to address this by quantifying cognitive impairments in SAH, and also by exploring the
possible use of two pharmacological agents in this population.
Figure 1.2: The Major Arteries of the Brain

(A) Ventral view. The enlargement of the boxed area shows the circle of Willis. Lateral (B) and midsagittal (C) views showing anterior, middle, and posterior cerebral arteries. (D) Frontal section showing the course of the middle cerebral artery. From Purves (2001).
1.3.3. Parkinson’s disease

PD is a common age-related neurodegenerative disorder first described by James Parkinson in 1817. Today, the diagnosis of PD is supported by positron emission tomography (PET) or single photon emission computed tomography (SPECT), and, as there is currently no definitive test for PD, clinical observations must be used. The characteristic clinical features of PD are motor in nature and include resting tremor, rigidity, bradykinesia, and gait impairment with postural instability. The hallmark pathology of PD is a degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), accompanied by the appearance of Lewy Bodies in some surviving neurons in this location. The neuronal loss is progressive, and there is a significant, lengthy pre-symptomatic phase, illustrated by the fact that clinical signs do not become apparent until at least 50% of the neurons are lost (Marsden 1990). Since the early 1970s, PD has been treated with the dopaminergic precursor levodopa, routinely administered in combination with the decarboxylase inhibitor carbidopa (Yahr, Duvoisin et al. 1969). Dopaminergic medication is the standard care for PD, and has benefited millions of patients throughout the world. Virtually all patients improve in terms of motor features of the disease, quality of life, independence, and mortality.

Dopaminergic therapy remedies some of the cognitive symptoms seen in PD. For example, a restorative effect is seen on cognitive tasks that tap into the frontostriatal pathways such as planning on the Tower of London task, working memory, and switching between well-learned tasks. However, dopaminergic therapy has a deleterious effect on some aspects of cognition, particularly during the early stages of PD. Specifically, impulsive responding and impaired reversal learning is reported in patients receiving dopaminergic treatment, but not in untreated patients (Swainson, Rogers et al. 2000; Cools, Barker et al. 2001). In the earlier stages of the disease process, degeneration of dopaminergic neurons tends to be limited to the putamen and the dorsal caudate nucleus, and only later does this degeneration progress to the more ventral parts of the striatum and the mesocorticolimbic dopamine system. The overdosing hypothesis proposes that the dopaminergic medication administered normalises dopamine (DA) levels in the depleted dorsal striatum, but detrimentally overdoses the relatively intact ventral striatum, and its connections to the ventral prefrontal cortex (PFC); this results in medication induced cognitive impairments (Cools, Frank et al. 2009). This has been elegantly illustrated by a double dissociation, whereby PD patients ‘On’ their medications show increased impulsive responding, but remediated task-set switching, whereas the same patients ‘Off’ their medications show impairment in task-set switching, but are not impulsive (Cools, Barker et al. 2003). It is thought that DA overdose of the ventral striatum is linked to the development of Impulsive/Compulsive Behaviours (ICBs) that are sometimes seen in PD.

ICBs are a diverse group of behaviours which include compulsive eating, hypersexuality, kleptomania, pathological gambling, compulsive shopping, and punding (repetitive mindless behaviour, such as collecting). However, the extent to which ICBs reflect the same underlying pathophysiology remains unclear. The emergence of ICBs in relation to DA agonists is not unique to PD, as patients prescribed DA
agonists for restless leg syndrome and multiple system atrophy also develop these behaviours (Klos, Bower et al. 2005; McKeon, Josephs et al. 2007; Tippmann-Peikert, Park et al. 2007). To date, the DOMINION study is the most comprehensive evaluation of ICBs in PD involving 3,090 patients from 46 sites in North America. The study reported a six-month prevalence of 13.6% (Voon, Sohr et al. 2011). Patients with ICBs show elevated impulsivity on measures of delay discounting (Housden, O'Sullivan et al. 2010; Voon, Reynolds et al. 2010). Compulsive drug use, termed dopamine dysregulation syndrome (DDS), has been linked to ICBs such as punding (Evans, Katzenschläger et al. 2004). It has been suggested that DDS is best explained by Robinson’s and Berridge’s incentive sensitisation theory (Lawrence, Evans et al. 2003), whereby a progressive increase in the rewarding effects of dopaminergic drugs over time becomes compulsively wanted rather than liked.

More recently, deep brain stimulation (DBS), particularly in the subthalamic nucleus (STN), has been established as an effective treatment for motor symptoms in PD, and has also been shown to improve quality of life in patients (Deuschl, Schade-Brottin et al. 2006). However, post-operatively some patients experience the treatable and often transient side effects of weight gain, dyskinesias, speech dysfunction, muscle contraction and visual disturbances (Deuschl, Herzog et al. 2006). Cognitive outcome of DBS patients suggests that there is relatively little cognitive morbidity in well-selected patients. However declines in word fluency, verbal memory, visuospatial memory, processing speed, associative learning, and executive function, including response inhibition, have been found across a number of studies (Voon, Kubu et al. 2006). Furthermore, there seems to be an increased risk of suicide following DBS, with risk factors being post-operative depression, being single, or a history of impulse control disorder or compulsive medication use (Voon, Krack et al. 2008). Interestingly, the STN is part of the circuitry that plays a prominent role in response inhibition. Stimulation of the STN in PD patients has been shown to ameliorate their deficits on tasks that require response inhibition (van den Wildenberg, van Boxtel et al. 2006). However, these results have not been found consistently (Jahanshahi, Ardouin et al. 2000; Hershey, Revilla et al. 2004; Ballanger, van Eimeren et al. 2009).

As the classic motor symptoms of PD have become better treated with dopaminergic therapies, the non-dopaminergic features have become more problematic. Indeed, in addition to degeneration in dopaminergic neurons, glutamatergic, GABAergic, noradrenergic, serotonergic, histaminergic, and cholinergic cell types are also vulnerable (Jellinger 1991; Lang and Obeso 2004; Langston 2006; Ahlskog 2007). Braak’s staging hypothesis posits that the pathological process of PD starts in the predisposed areas of vulnerability of the olfactory bulb, anterior olfactory nucleus, and dorsal motor nucleus of the vagal nerve. The disease then progresses in a non-random way, irrespective of the neurotransmitter synthesised within that neuron. Braak has proposed six stages of brain pathology in sporadic PD, three of which occur in the presymptomatic phase (Braak and Del Tredici 2008). Therefore, cognitive impairment seen in PD that is not manipulated (either improved or worsened) by dopaminergic therapy could reflect degeneration in other neurotransmitter systems. For example, there is evidence to suggest that degeneration of the basal forebrain cholinergic nuclei and ascending cholinergic pathways leads to cognitive impairment in PD.
patients without dementia (Shimada, Hirano et al. 2009; Bohnen, Muller et al. 2010; Bohnen and Albin 2011). Furthermore, the degree of deficit seen in task set switching has been shown to be related to disease severity, specifically the Hoehn and Yahr stage, rather than dopaminergic status (Kehagia, Murray et al. 2010). DA depletion in the caudate nucleus has no effect on extra-dimensional set shifting in the marmoset (Collins, Wilkinson et al. 2000) and likewise switching attention between different perceptual aspects of a stimulus is insensitive to dopaminergic manipulation in PD (Lange, Robbins et al. 1992; Lewis, Slabosz et al. 2005; Kehagia, Murray et al. 2010). There is increasing evidence that noradrenaline (NA) is involved in set-switching (Kehagia, Murray et al. 2010), and therefore the degeneration in the locus coeruleus (LC), which is the main cortical source of NA, could account for the impairment seen.

Chapter 5 addresses these issues by exploring the use of a noradrenergic agent, atomoxetine, to treat the symptoms of impulsivity in PD.

1.3.4. SLEEP DEPRIVATION

Sleep disturbances are a common problem across neuropsychiatric disorders, including PD, AD, multiple sclerosis and mood disorders (Benca 1996; Peterson and Benca 2006). Poor sleep quality in patient populations is often caused by disease pathology, as well as side-effects of medication, and can cause and exacerbate disease-related cognitive impairment. In healthy populations sleep deprivation can be used to model the cognitive impairment seen in patient populations such as those with AD, as well as in the healthy ageing population, as inadequate sleep impairs human performance on a wide variety of cognitive and behavioural tasks (Harrison, Horne et al. 2000). It has been suggested that this model could be used to test the effectiveness of new candidate drugs in clinical trials. Sleep deprivation can also occur in healthy populations as a consequence of everyday life by factors such as shift work, military deployment, and having young children. This can lead to increased accident rates and decreased efficiency at work (Rajaratnam, Barger et al. 2011; Roth 2012). Sleep loss is now considered a major public safety and health concern, as a reduction in sleep of as little as two hours for a few nights can cause reductions in cardiovascular, immune, and endocrine functions.

There are two processes that determine the propensity to sleep: the circadian process, which influences the timing of sleep and wakefulness, and the homeostatic process, which increases sleep-need based on the duration of wakefulness. Increases in homeostatic sleep needs are associated with subjective sleepiness and impaired cognitive function, as well as with neurochemical changes. One way in which arousal is modulated is through the arousal spectrum, which is linked to the actions of NA, serotonin, histamine, DA, and ACh, which as a group have been called the ascending reticular activating system (Vincent 2000). This ascending neurotransmitter system can be thought of as a cortico-striatal-thalamic-cortical loop which regulates arousal in part by controlling the size of a thalamic filter, whereby sensory input is filtered out for normal sleep or sensory input is allowed to the cortex for normal wakefulness. The role that each neurotransmitter plays in the ascending reticular activating system will now be considered. During wakefulness, NA levels increase, provoking muscle tone and enhancing synaptic plasticity.
Conversely, the reduction of NA in sleep is associated with a decrease in muscle tone, such as seen in rapid eye movement (REM) sleep. Activity of the noradrenergic neurons of the LC declines during sleep, and they are virtually silent during episodes of REM sleep (Berridge and Abercrombie 1999). NA α1-receptors excite wakefulness-promoting neurons such as the brain stem serotonergic raphe, basal forebrain, and thalamic neurons (Brown, Basheer et al. 2012). In contrast, α2-receptors inhibit sleep-promoting neurons (Thakkar, Strecker et al. 1998). Serotonergic neurons discharge fastest in states of relaxed wakefulness, slightly slower during active wakefulness, and are essentially silent during REM sleep (Jacobs and Fornal 1991).

High levels of serotonin, for example as caused by antidepressant drugs, decrease REM sleep (Vazquez-Palacios, Hernandez-Gonzalez et al. 2010). Tonic levels of DA do not vary across wakefulness and the different kinds of sleep, but these neurons exhibit more bursts of phasic activity in REM sleep (Dahan, Astier et al. 2007). The activity of histaminergic neurons promotes wakefulness (Strecker, Nalwalk et al. 2002; Thakkar 2011), and drugs that enhance histaminergic neurotransmission increase arousal and improve attention (Van Ruitenbeek, Vermeeren et al. 2010). The cell bodies of histaminergic neurons are only found in the tuberomammillary nucleus of the posterior hypothalamus, and these project to wakefulness-promoting brain regions. Histamine levels have been found to remain elevated during a 6 hour period of sleep deprivation (Strecker, Nalwalk et al. 2002). ACh facilitates the cortical activation of both wakefulness and REM sleep (Brown, Basheer et al. 2012). Overall, in this way the five neurotransmitters of the ascending reticular activating system work in concert to regulate cortical arousal on a smooth continuum. In contrast to this, there is another set of circuits in the tuberomammillary nucleus of the hypothalamus that regulates sleep and wake discontinuously, like an on-off switch.

There are two key neurotransmitters that regulate the sleep-wake switch, histamine from the tuberomammillary nucleus, and gamma-Aminobutyric acid (GABA) located in the ventrolateral preoptic area. Sleep is maintained by the GABA neurons, which inhibit wake-promoting nuclei during sleep. During wakefulness, this inhibition is turned off, facilitating activation within the tuberomammillary nucleus of the hypothalamus and in turn the cortex. It has been suggested that sleepiness during the day is caused by activation in the ventrolateral preoptic area, thus releasing GABA (Stahl 2008).

A wide range of cognitive functions are impaired following sleep deprivation, reflecting the range of neurotransmitter systems affected. In particular, fMRI studies of sleep deprivation have consistently shown reduced parietal and lateral occipital activation during a variety of cognitive tasks, suggesting a common mechanism underlying performance decline (Habeck, Rakitin et al. 2004; Chee, Chuah et al. 2006; Chee and Chuah 2007; Tomasi, Wang et al. 2009). It has been suggested that failure of attention may underlie decline in cognition (Chee and Chuah 2007). Executive functions also seem vulnerable following sleep deprivation. For example, impairments on attentional set-shifting tasks have been found in both sleep-deprived rats and humans (Heuer, Kleinsorge et al. 2004; McCoy, Tartar et al. 2007). Sleep-deprived humans show perseveration errors, which are characterised by the inability to shift attention away from a perceptual dimension which has previously commanded attention (Gottselig, Adam et al. 2006). These perseveration errors are similar to those seen in patients with lesions to the PFC (Owen, Roberts et al. 2006).
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Lack of sleep is also associated with deterioration in planning, an executive function that draws heavily on the resources of the PFC (Dagher, Owen et al. 1999). Unsurprisingly, capacity, speed, and accuracy on working memory tasks are all affected by deprivation of sleep (Hagewoud, Havekes et al. 2010; Drummond, Anderson et al. 2012). Sleep deprivation also impairs the ability to make judgments about the passage of time, a phenomenon associated with impulsivity (Wittmann and Paulus 2008). Indeed, it has been shown that those who experience prolonged wakefulness are more impulsive on delayed discounting tasks (Reynolds and Schiffbauer 2004).

Overall, the investigation of sleep deprivation is important for understanding sleep-related cognitive impairments in both patient and healthy populations. The neurobiology regulating sleep is complicated and not yet fully understood. Therefore, in Chapter 6 the atypical stimulant modafinil was used as a potential cognitive enhancer in doctors following twenty four hours without sleep.

1.4. Pharmacological agents used in this thesis

1.4.1. Modafinil

Modafinil is an atypical stimulant that has been has been approved by the Food and Drug Administration for the treatment of excessive sleepiness associated with shift work sleep disorder. Modafinil is a proven wake-promoting agent, originally licensed for use in narcolepsy patients. It represents a valuable treatment option due to its low abuse potential (Jasinski 2000). Because of its low abuse potential, together with its stimulant-like properties, modafinil has been proposed as a candidate treatment for cocaine (Dackis, Kampman et al. 2005; Anderson, Reid et al. 2009) and methamphetamine dependence (Anderson, Li et al. 2011; Holtz, Lozama et al. 2011). The findings in humans, however, have so far been mixed. Although there is still uncertainty as to modafinil’s exact mechanism of action, it has been shown to have robust effects on several different chemical systems in the brain, including catecholamines, serotonin, glutamate, GABA and hypocretins.

1.4.1.1. Dopamine

Studies have suggested an interaction between modafinil and the dopaminergic system. For example, modafinil pre-treatment reduces cocaine self-administration in humans in a laboratory setting (Hart, Haney et al. 2008).

In humans, at clinically relevant doses, modafinil blocks DA transporters by 50% and increases extracellular levels of DA in the caudate, putamen and nucleus accumbens (Volkow, Fowler et al. 2009). The binding is weak and incomplete, causing a slow rise in tonic levels of DA that is sustained over a relatively long period of time. An imaging study in monkeys showed occupation of DA transporters in the striatum after intravenously administered modafinil, along with occupancy of NA transporters (Madras, Xie et al. 2006). Furthermore, microdialysis studies have shown increases in extracellular DA in the nucleus accumbens (Murillo-Rodriguez, Haro et al. 2007) and in the PFC (de Saint Hilaire, Orosco et al. 2001), although the
increase in the nucleus accumbens has been shown to be weak, compared to amphetamine, and is also
accompanied by a reduction of GABA release (Ferraro, Antonelli et al. 1997).

Mice lacking DA transporters (Wisor, Nishino et al. 2001), and also those lacking D1 and D2 receptors (Qu,
Huang et al. 2008), do not respond to the wake-promoting effects of modafinil, although it should be kept
in mind that mice lacking DAT transporters also have elevated levels of D1 and D2 receptors, which could
have confounded the results from this study. A recent knockout study in rats has shown that the dopamine
D1 receptor is required for the majority of modafinil-induced effects on exploration in mice (Young,
Kooistra et al. 2011). The data from this study was also supported by a pre-treatment experiment in which
modafinil-induced increases in activity and hole-poking were attenuated by a D1 antagonist (SCH23390).
The effects on the specific receptors could be downstream to Dopamine transporter (DAT) inhibition, as
similar effects have been shown with a selective DAT inhibitor (GBR 12909). Modafinil increases motivation
in a similar way to a DAT inhibitor, and this effect is reduced in mice with only 50% D1R expression,
compared to wild-type mice (Young and Geyer 2010).

1.4.1.2. Noradrenaline

There is converging evidence for the role of noradrenaline (NA) in modafinil’s actions. Animal studies have
shown occupancy of NA transporters in the thalamus by modafinil (Madras, Xie et al. 2006), and elevated
NA levels in the PFC, as well as in the medial hypothalamus (de Saint Hilaire, Orosco et al. 2001). In
humans, an fMRI study has revealed decreased activity in the LC, suggesting that modafinil modulates the
ascending noradrenergic system, the main source of cortical NA, resulting in adaptive shifts in cortical gain
and thus optimizing information processing (Minzenberg, Watrous et al. 2008).

Pharmacological studies have shown that intact α1-receptors are necessary for modafinil effects. In non-
human species, the α1-adrenergic receptor antagonist prazosin attenuates the arousal and activity
increases induced by modafinil (Duteil, Rambert et al. 1990; Hermant, Rambert et al. 1991; Lin, Roussel et
al. 1992; Mitchell, Bogenpohl et al. 2008). It should be kept in mind that prazosin has also shown an affinity
for α-2B and α-2C adrenoceptors (Bylund 1992). This converges with evidence from knockout experiments
showing that genetic ablation of α1-adrenoceptors markedly attenuates behavioural activation caused by
modafinil (Stone, Cotecchia et al. 2002). In humans, the co-administration of prazosin in healthy volunteers
blocks the beneficial effects of modafinil seen on the more difficult levels of the Tower of London test of
frontal lobe function (Winder-Rhodes, Chamberlain et al. 2010).

There is uncertainty as to whether modafinil exclusively activates neurons in the LC without affecting NA
neurons found outside the LC involved in cardiovascular and salivary regulation, as some studies have
found sympatholytic effects (Heitmann, Cassel et al. 1999; Hou, Freeman et al. 2005), whereas others have
reported hypertension and increased heart rate, as well as elevations in plasma NA and adrenaline,
indicative of elevated adrenomedullary discharge (Makris, Rush et al. 2004; Muller, Steffenhagen et al.
2004; Taneja, Diedrich et al. 2005).
It should be noted that there is evidence to suggest that DA may also activate certain α1-receptors of the LC (Lin, Quartermain et al. 2008), so the NA effects could be down-stream to DAT binding in modafinil. It is also a possibility that modafinil may have a dual noradrenergic-dopaminergic mechanism of action, in the same way as more typical stimulants, such as methylphenidate (Mitchell, Bogenpohl et al. 2008).

1.4.1.3. OTHER NEUROTRANSMITTER SYSTEMS

The GABAergic system appears to be systematically down-regulated by modafinil (in the hippocampus, hypothalamus, thalamus, striatum, globus pallidus), whereas other neurotransmitters seem to be up-regulated by the drug (Scoriels, Jones et al. 2012). After administration of modafinil, increases in glutamate are seen in the cortex, the hippocampus, the hypothalamus, the thalamus, substantia nigra, and LC, but not the striatum (Touret, Sallanon-Moulin et al. 1994; Ferraro, Antonelli et al. 1997; Ferraro, Antonelli et al. 1999; Xiao, Fu et al. 2004; Dawson, Thompson et al. 2012). Serotonin (5-HT) levels also increase in the cortex and amygdala following administration of modafinil, and under the combined influenced of modafinil and fluoxetine (the selective 5-HT reuptake inhibitor), the dorsal raphe serotonergic system is activated (Ferraro, Fuxe et al. 2005). There may be temporal changes in modafinil’s neuromodulatory effect. For example, a microdialysis study in rats showed an initial increase in extracellular 5-HT, DA and NA in the first 60 minutes following modafinil administration. 5-HT levels remained high in the PFC three hours after drug administration, whereas in the hypothalamus only NA levels were enhanced, while DA and 5-HT levels remained low (de Saint Hilaire, Orosco et al. 2001).

1.4.1.4. EFFECTS ON COGNITION

In healthy, non-sleep-deprived populations, modafinil improves cognition on tests of spatial planning, response inhibition (stop signal task (SST)), visual recognition, short term memory, and working memory (Turner, Robbins et al. 2003; Winder-Rhodes, Chamberlain et al. 2010; Muller, Rowe et al. 2013). Other single dose studies have also shown improvements on attentional and logical reasoning tasks (Baranski, Pigeau et al. 2004). However, these improvements have not always been replicated in healthy, non-sleep-deprived humans (Randall, Shneerson et al. 2003; Winder-Rhodes, Chamberlain et al. 2010). Interestingly, modafinil has also been shown to improve task enjoyment, or motivation in healthy, non-sleep-deprived adults (Muller, Rowe et al. 2013). In schizophrenia patients, modafinil has been shown to improve set-shifting performance (Goetghebeur and Dias 2009). However, this effect is not seen in individuals with a first episode of psychosis, although modafinil improves their verbal working memory, spatial working memory, use of strategy on a memory task, and analysis of emotional face expressions (Scoriels, Barnett et al. 2011; Scoriels, Barnett et al. 2012). In Attention Deficit Hyperactivity Disorder (ADHD), modafinil improves spatial planning and response inhibition (Turner, Clark et al. 2004). Due to its arousal properties and ‘cognitive enhancing’ effects in healthy humans, there has been an increase in its off-label use, and thus media interest in this compound. For a discussion of the ethical issues surrounding off-label use of pharmacological agents see Chapter 7.
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Modafinil is not currently approved for reversing sleep-related cognitive deficits in healthy adults. However, it is already used for a related condition, shift work sleep disorder. Furthermore, modafinil is approved for performance-maintenance within certain branches of the military. Modafinil’s effects on healthy adults deprived of sleep for 41 hours have been shown to be dose-dependent. For example, a study using 100, 200, or 400mg of modafinil found that 100mg of modafinil restored performance on a psychomotor vigilance test to 75% of baseline, whereas 200mg restored performance to approximately 90% of baseline, with 400mg restoring it to 100% of baseline (Wesensten, Belenky et al. 2002). In a 23-day study where night shifts were simulated, 400mg of modafinil was shown to be better than 200mg at improving performance on digit recall and digit symbol substitution (Hart, Haney et al. 2006). Following loss of a night’s sleep, modafinil has been shown to improve performance on attention and memory tests, such as choice reaction time, paired-associates memory, and n-back memory tasks (Bonnet, Balkin et al. 2005; Wesensten 2006; Minzenberg and Carter 2008). Modafinil has also been shown to improve executive function under conditions of sleep deprivation. 200mg of modafinil has been shown to improve fluency, flexibility, and originality on the Torrents’ test of creative thinking-verbal, decreased perseverative responding on the Wisconsin Card Sort Task (WCST), and decreased errors on a category test which assesses response inhibition, during a simulation of shift-work sleep disorder (Walsh, Randazzo et al. 2004). Following 60 hours of sleep loss, 200mg improves performance on the Stroop task (Batejat and Lagarde 1999). Performance is also improved on the Tower of London test of planning following 400mg of modafinil, but there was a slowed average time taken between moves (Killgore, Kahn-Greene et al. 2009). This finding converges with the increased accuracy, but increased latency in non-sleep deprived healthy adults (Turner, Robbins et al. 2003). Preservative responding and errors are also decreased on the WCST. However, in relatively high doses (300mg), modafinil also causes over-estimation of performance, and may negatively impact certain aspects of communication (Pigeau, Naitoh et al. 1995; Gurtman, Broadbear et al. 2008).

1.4.2. Atomoxetine

Atomoxetine is a relatively selective NA reuptake inhibitor, which antagonises the presynaptic transporter with very little affinity for NA receptors. In animals, atomoxetine increases cortical DA and NA when given systematically, but importantly it does not seem to effect subcortical DA (Bymaster, Katner et al. 2002), offering a clinical advantage to stimulant drugs which have abuse potential (Volkow 2006).

Atomoxetine has been shown to have consistent effects on impulsivity. In rats, atomoxetine reduces impulsivity on the 5-choice serial reaction time task (5-CSRT) (Robinson, Eagle et al. 2008), and also dose-dependently improves response inhibition on the SST without affecting the Go reaction time (Robinson, Eagle et al. 2008). This converges with findings in humans, with atomoxetine decreasing stop signal reaction time (SSRT) in healthy adults and adults with ADHD, with no effect on Go reaction time (Chamberlain, Del Campo et al. 2007; Chamberlain, Hampshire et al. 2009). Because of these properties, atomoxetine has been approved by the Food and Drug Administration for the treatment of ADHD.
(Faraone, Biederman et al. 2005), representing the first non-stimulant-based medication that has proven efficacy in ADHD.

The mechanism by which atomoxetine improves inhibitory control on the SST has been investigated using fMRI, and it was demonstrated that atomoxetine increases Blood Oxygen Level Dependent (BOLD) responses in the right inferior parietal/superior temporal junction, an area which has been shown to subserve response inhibition on the task. The magnitude of the BOLD response seen correlated significantly with the plasma levels of atomoxetine during successful inhibition only, such that higher levels were associated with greater brain activation (Chamberlain, Hampshire et al. 2009).

As well as impulsivity, atomoxetine has been shown to remediate cognitive impairment after experimental TBI. For example, low doses of atomoxetine, but not high doses, administered immediately after experimental traumatic head injury in rats results in improved performance on the Morris Water Maze (MWM) (Reid and Hamm 2008). However, atomoxetine has not been shown to have an effect on inattention in early Huntington’s disease (Beglinger, Adams et al. 2009), or on a brief assessment of cognition in schizophrenia (Friedman, Carpenter et al. 2008).

1.4.3. Physostigmine

Physostigmine is an acetylcholinesterase inhibitor that is approved by regulatory agencies in Europe and the US Food and Drug Administration as an agent to reverse the anticholinergic effects of clinical or toxic overdoses. Initial studies using physostigmine showed that it can improve memory in healthy individuals (Davis, Mohs et al. 1978), and well as patients with dementia (Davis, Mohs et al. 1979).

In animal studies, physostigmine improves memory in aged primates (Bartus and Dean 1988), and in primates with scopolamine-induced amnesia (Bartus 1978). Physostigmine reduces activation in the right inferior frontal cortex in healthy adults during a working memory task, as well as improving performance on the task, suggesting that the decrease in activity was related to enhanced processing efficiency (Furey, Pietrini et al. 1997). Cholinergic enhancement with physostigmine also causes selective increases in perceptual processing during a working memory test (Furey, Pietrini et al. 2000).

The development of physostigmine as an agent to remediate cognitive impairment has been limited by its short plasma half-life (approximately 30 mins), and a Cochrane review of physostigmine studies in AD up to 2001 concluded that the net evidence of the effectiveness of physostigmine for the treatment of symptoms of AD is limited, and adverse effects are high (Coelho and Birks 2001). However, a new extended-release formulation of physostigmine has been developed which shows efficacy in AD (van Dyck, Newhouse et al. 2000).
1.5. **NEUROTRANSMITTER SYSTEMS**

The monoaminergic and cholinergic neurotransmitter systems innervate the PFC, and play a central role in cognitive function (Robbins 2000). Manipulation of catecholaminergic levels can result in impairments when levels of NA and DA are reduced, whilst improvements result from agonists of these agents. Impairments have, however, been linked to high catecholaminergic levels, for example at high doses of agent agonists, following the inverted-U-shaped function (Dreher and Burnod 2002). The neurotransmitter systems closely linked to the drugs administered in this thesis will now be discussed.

1.5.1. **Noradrenaline**

Noradrenaline (NA) was first discovered in 1946. NA is synthesised from DA by dopamine β-hydroxylase, requiring ascorbate as a cofactor. NA is released from nerve terminals via action potential discharge. This triggers calcium-dependent release of the transmitter into the synaptic cleft, which in turn provokes a response from the post-synaptic receptors (Stahl 2008). Following its release, NA is mostly recaptured by the presynaptic NA reuptake transporter. It is then metabolised by monoamine oxidase or catechol-O-methyltransferase, or repacked into vesicles for storage and subsequent release. This mechanism is the same as for DA. Drugs that manipulate NA levels can either block the reuptake of NA, for example atomoxetine, or antagonise/agonise adrenergic receptors, for example guanfacine and Beta blockers.

NA postsynaptic receptors are broadly classified into β1, α1, and α2, and there is one presynaptic autoreceptor, the α2-receptor. NA has varying affinities for the different adrenergic receptors in the PFC: it has the highest affinity for α2-receptors, then lower affinity for α1-receptors, and the lowest affinity for β-receptors. The receptor subtype engaged seems to determine the effect that NA has on cognition, as illustrated by studies of working memory (Birnbaum, Gobeske et al. 1999). The binding of NA to α2-adrenoceptors has a beneficial effect on the PFC function, and α2a agonists such as clonidine, guanfacine, medetomidine, improving the working memory and attention functions of the PFC (Arnsten, Cai et al. 1988; Jakala, Riekkinen et al. 1999). In contrast to this, high levels of NA release, for example during stress, impair working memory via α1-adrenoceptors. Stress-induced working memory deficits can be prevented by blocking α1 adrenoceptors in the PFC (Birnbaum, Gobeske et al. 1999), and conversely PFC dysfunction can be induced by infusing an α1-adrenergic agonist into the PFC (Arnsten and Li 2005).

The NA-containing neurons within the LC provide most of the NA present within the central nervous system. These cells have a tonic level of activity which is regulated by a variety of neurotransmitter inputs. The ascending projections of the LC include the PFC, basal forebrain, thalamus, hypothalamus, amygdala, and hippocampus. The descending NA projections extend down the spinal cord, and regulate pain pathways. The projections terminate in most of the same places where serotonin pathways terminate. However, there are few NA projections to the striatum and to the nucleus accumbens. There is a reciprocal relationship between the PFC and the LC, as the PFC provides one of the few intelligent inputs back to LC...
neurons (Arnsten and Goldman-Rakic 1984; Jodo, Chiang et al. 1998). In primates, the somatosensory cortex in the parietal lobe receives the densest NA innervation, and higher cortical areas, such as the PFC, have a moderate NA input (Lewis and Morrison 1989).

The cells in the LC degenerate in a number of conditions, including PD, AD, and depression. In contrast, cells in the LC can become overactive under conditions of stress, and thus contribute to many stress-related cognitive disorders, such as Post-Traumatic Stress Disorder. In animals, normal ageing is associated with a small degree of LC cell loss, which strongly correlates with the degree of memory impairment (Leslie, Loughlin et al. 1985). Studies of aged monkeys show that the orexin/hypocretin input from the hypothalamus reduces by 50%. Transplantation of NA neurons in old rodents protects them from age-related loss of memory (Collier, Gash et al. 1988).

NA cells of the LC degenerate to a greater degree in PD. Depletion of NA in the PFC may contribute to the array of PFC deficits seen in these patients, as neuropathological studies show that the loss of the rostral LC cells correlates with cognitive decline (Chan-Palay and Asan 1989). Cognitive deficits seen include working memory deficits, decreased planning, and impaired set-shifting (Owen, James et al. 1992). Furthermore, the medications given to PD patients often worsen their cognitive impairments. The DA agonists given to restore movement increase both dopaminergic and NA transmission. However, these doses are often too high for the caudate and PFC, as these sites have a more modest depletion (Cools, Barker et al. 2003).

The LC is integrally involved in the regulation of arousal, including the sleep and waking cycle. LC neurons have a circadian rhythm and the fluctuation in activity requires an intact dorsomedial hypothalamus (Gonzalez and Aston-Jones 2006). Light-deprivation produces a loss of NA in the PFC and both light-deprivation and lesions to the LC decrease the amplitude of the circadian rhythm in the sleep-wake cycle (Gonzalez and Aston-Jones 2006). NA modulates alertness through a variety of actions, many of which involve stimulation of β-receptors, which in turn suppresses rhythmic activity in the thalamus and shifts neurons to a single-spike firing state, enabling the transmission of information (McCormick, Pape et al. 1991). This effect can be modulated by α-receptors, for example stimulation of α1-receptors in the thalamus provokes an alert state whereas stimulation of postsynaptic α2-receptors produces sedation (Buzsaki, Kennedy et al. 1991). During sleep deprivation, NA systems along with cholinergic and other monoaminergic systems are activated. Sleep deprivation in rodents decreases the number of β-receptors in the brain, increases NA synthesis and tyrosine hydroxylase gene expression in LC neurons, thus increasing levels of tyrosine hydroxylase (Porkka-Heiskanen, Smith et al. 1995). These changes could be due to sleep deprivation or the methods associated with it. They may also represent a compensatory mechanism to the decreased arousal associated with sleep deprivation.

NA manipulation in healthy humans influences certain cognitive processes, including tests of sustained attention, working memory, and impulsivity (Arnsten, Steere et al. 1996; Chamberlain, Hampshire et al. 2009). The effect of selective NA drugs on working memory has been hypothesised to be due to increased
attentional mechanisms, thus reducing distractibility. Drugs which increase extracellular levels of NA, such as methylphenidate, atomoxetine and modafinil, have all been shown to improve response inhibition on the SST (Aron, Dowson et al. 2003; Overtoom, Verbanen et al. 2003; Turner, Robbins et al. 2003; Turner, Clark et al. 2004; Chamberlain, Del Campo et al. 2007). Animal studies have confirmed the role of NA in response inhibition, with systematic dosing with atomoxetine, methylphenidate, and modafinil all improving response inhibition on a stop-signal analogue, and atomoxetine reducing impulsive responding on the 5-CSRT task (Blondeau and Della-Hagedorn 2007; Eagle, Tufft et al. 2007; Robinson, Eagle et al. 2008). Furthermore, the α2- adrenergic agonist, guanfacine, selectively impairs stopping in rats (Bari, Mar et al. 2011).

1.5.2. **DOPAMINE**

DA is synthesised in the same way as NA, by the conversion of tyrosine to dopa, followed by the decarboxylation of dopa to DA. DA in dopaminergic neurons cannot be converted to NA, as they lack the enzyme DA β-hydroxylase. The release and reuptake of DA occurs in the same way as for NA. Stimulant drugs, such as modafinil, act as indirect agonists by increasing the concentration of DA and NA by blocking the presynaptic reuptake transporter.

There are two broad types of DA receptor: D1-type (D1 and D5 receptors) and D2-type (D2, D3, D4 receptors). It is thought that DA increases signal-to-noise processing. For example, in the PFC, postsynaptic D1 receptors enhance signal-to-noise processing partially as a product of boosting N-Methyl-D-aspartate (NMDA) receptors, which preserves neuronal activity, and GABA-A receptor currents, which inhibits incoming glutamate traffic.

The mesencephalic DA pathways have been mapped into three circuits, the mesostriatal (originates in the substantia nigra and the ventral tegmental area), mesolimbic, and mesocortical projections (originates in the ventral tegmental area and innervates the PFC and insular cortices). However, there is cross talk between these pathways, and overlapping roles across the circuits. The mesostriatal pathway accounts for about 75% of the DA in the brain. The cell bodies lie in the substantia nigra and the axons terminate in the corpus striatum. It is this nigrostriatal pathway which degenerates in PD, leading to akinesia and motor rigidity. The mesolimbic pathway cell bodies line various groups in the midbrain, and their fibres project via the medial forebrain bundle, to parts of the limbic system, particularly the nucleus accumbens. The effect of stimulant drugs, such as amphetamine, on instrumental responding, seems to depend on DA release in the ventral striatum. Therefore, this system is closely associated with conditioned reinforcement and incentive salience (Berridge 2007). The mesocortical system is associated with the cognitive functions of the PFC, such as working memory and attentional set-shifting.

Manipulation of the mesocortical DA system has effects on working memory processes in a triphasic manner (Robbins 2000). In rats, D1 antagonists produce impairments in working memory tasks, whereas low-dose agonists improve performance, but high-dose agonists produce deficits on the task (Zahrt, Taylor...
et al. 1997). This is illustrative of the inverted-U-shaped function, first described by Yerkes and Dodson, whereby performance efficiency varies as a function of activation, and optimal levels of activation differ depending on the task.

In humans, there are no selective D1 receptors available for research, meaning that several hypotheses stemming from animal studies remain untested. The selective DA D2 receptor agonist bromocriptine improves performance on working memory in humans who are low performers, and impairs working memory in high performers, whilst also impairing reversal learning, thus illustrating that optimal levels of DA activity will not be optimal for others (Kimberg, D’Esposito et al. 1997; Mehta, Swainson et al. 2001). Bromocriptine also enhances task-switching performance in individuals who score high on impulsivity, but impairs performance in low impulsive individuals (Cools, Sheridan et al. 2007). Methylphenidate has received considerable research attention because of its success as an ADHD treatment. Like other stimulant drugs, it enhances presynaptic DA function, but also affects other monoamine neurotransmitter systems. On the Cambridge Neuropsychological Test Automated Battery (CANTAB) spatial working memory task, methylphenidate improves performance in healthy volunteers, whilst reducing regional cerebral blood flow in the frontoparietal circuitry, perhaps reflecting an enhanced signal-to-noise ratio (Mehta, Owen et al. 2000).

PFC DA function is also modulated in an inverted-U-shaped function by the catechol–O–methyl transferase (COMT) polymorphism. The COMT gene contains a functional polymorphism (val and met) that determines high and low activity of this enzyme. Humans with val/val alleles have more rapid inactivation of the released PFC DA than those with the met/met genotype. Individuals with the val/met are intermediate between the homozygote individuals. Val/val individuals benefit most from the enhancing effect of amphetamine, whereas met/met individuals tend to perform worse when on the drug (Mattay, Goldberg et al. 2003). The COMT phenotype also modulates response to dopaminergic medication in PD, with met/met individuals showing the greatest degree of cognitive deficit in tests of planning and recognition memory (Foltynie, Goldberg et al. 2004; Williams-Gray, Hampshire et al. 2007). However, this effect is only found early on in the course of the disease, suggesting that the relatively intact ventral striatal loops are being overdosed in these patients (Williams-Gray, Evans et al. 2009).

1.5.3. Acetylcholine

ACh was among the first neurotransmitters to be identified. It is synthesised within cholinergic neurons by the enzyme choline acetyltransferase from the compounds choline and acetyl-CoA. Once released into the synapse, ACh is converted into the inactive metabolites choline and acetate by cholinesterase enzymes. Cholinesterase enzyme activity can be suppressed by cholinesterase inhibitors, which has clinical significance in the treatment of AD.

The basal forebrain is a set of nuclei which together make up the most prominent site of cholinergic cell bodies, and include the basal nucleus, the medial septal nucleus and the diagonal band. These cells project
Introduction

to the cortex, hippocampus, and amygdala. The basal nucleus of Meynert is the main source of cortical cholinergic innovation. The other source of ascending cholinergic innervation is the brain stem, which follows the same route as the monoaminergic pathways.

Despite projecting to the entire cerebral cortex, the primate basal nucleus receives cortical projections only from limbic and paralimbic brain regions. This enables the basal nucleus to enhance selectively the release of cortical ACh throughout the cortex in response to events that are of limbic relevance. For example, neurons of the basal nucleus in monkeys are selectively sensitive to novel and motivational events (Wilson and Rolls 1990). In the cerebral cortex, there are two main types of ACh receptors: muscarinic (M1, M2, M3, M4, M5) and nicotinic (α2 - α7, β2 – β4).

The cholinergic systems innervating the PFC have a vital role in the cognitive functions of attention and memory. Impairment of the cholinergic pathways causes attentional deficits (Lawrence and Sahakian 1995; Perry and Hodges 1999). Behavioural studies utilising the immunotoxin 192 IgG-Saporin (SAP) have provided consistent evidence of the role of ACh in attention. Infusions of SAP into the basal nucleus severely impair sustained attention (McGaughy, Kaiser et al. 1996). Multiple cortical infusions of SAP and also smaller doses into the basal nucleus produce qualitatively similar but less severe impairment in sustained attention (McGaughy, Kaiser et al. 1996; McGaughy, Decker et al. 1999). The degree of attentional impairment seen in these studies correlates with the extent of deafferentation. In vivo microdialysis in primates performing various tests of attention has repeatedly demonstrated an increase in ACh efflux in the area of the frontoparietal or medial prefrontal cortex (Passetti, Dalley et al. 2000; Dalley, McGaughy et al. 2001). On the 5-CSRT task, the animal equivalent of the CANTAB reaction time test, infusions of high doses of SAP into the basal nucleus of Meynert produce decreases in ACh in the medial PFC (McGaughy, Dalley et al. 2002). The compromised levels of ACh correlated with cortical cholinergic deafferentation and attentional impairments. There is correlational evidence to suggest that these animal models translate to humans: deficits in the cholinergic system are associated with impairments in attention, such as in AD, PD, and Lewy Body dementia. Furthermore, administration of the acetylcholinesterase inhibitors improves attentional function, for example administration of tacrine to AD patients improves the attentional impairments seen in AD on the human analogue of the 5-CSRT task (Eagger, Levy et al. 1991). Cholinesterase inhibitors have also been shown to improve vigilance and attention in patients with chronic TBI (Tenovuo 2005).

Initial evidence for the involvement of ACh in memory came from the fact that cholinergic markers in the cerebral cortex were reduced in people who die with AD, and this decrease correlates with cortical pathology and the degree of cognitive impairment (Perry, Tomlinson et al. 1978). However, it is difficult to differentiate the role of ACh in attention from its role in encoding. Human memory studies demonstrate that the blockade of muscarinic ACh receptors by administration of scopolamine interferes with the encoding of new verbal information, while having little effect on retrieval of previously stored information (Hasselmo 1995; Hasselmo and Wyble 1997). Scopolamine primarily affects episodic memory whilst
sparing semantic and procedural memory (Broks, Preston et al. 1988). Scopolamine has also been shown to impair encoding in monkeys, but shows little effect when administered whilst recognising objects encoded without scopolamine. The encoding effects seen were focused in the parahippocampal regions, as impairments were only seen after infusions in this area (Tang and Aigner 1996).

1.6. **AIMS AND HYPOTHESES OF THIS THESIS**

This thesis firstly aimed to quantify cognitive impairment in a range of disorders, namely head injury, SAH, PD, and sleep deprivation. These disorders cover a range of ages and severity, from young adults with mild pathology in the head injury group, to older adults with more severe pathology in the PD group. The cognitive impairments explored covered both reversible (sleep deprivation) and degenerative (PD) states, and also acute (SAH, head injury) and chronic conditions (PD). In particular, neurocognitive endophenotypes which are closely associated to brain function, such as impulsivity, were focused on.

In three of the studies, pharmacological agents (modafinil, physostigmine, and atomoxetine) were used in novel ways in order to explore their effects on cognitive functioning in these groups. Therefore, the second aim of this thesis was to assess the potential of these agents to be repurposed for new clinical use, based on their known pharmacological effects. It was hypothesised that modafinil, physostigmine, and atomoxetine would have distinguishable cognitive effects due to their different pharmacological profiles. However, it was expected that modafinil and atomoxetine would both affect cognitive domains that are neuromodulated by the noradrenergic system. Details of specific hypothesis related to the separate studies are provided in the introduction to each experimental chapter.
2. **General Materials and Methods**

2.1. **Ethical Approval**

The Cambridge Local Research Ethics Committee approved all the studies, and all patients and volunteers gave written, informed consent. Specific details relating to the individual experiments can be found in the appropriate chapters.

2.2. **Overview of the Experimental Designs Used**

Each of the experiments in this thesis was conducted using one of the following designs: parallel between-subjects design, crossover within-subjects design, or a within-subjects longitudinal design. A parallel design is preferable in that it removes the problem of practice effects, but was not used in situations where patient heterogeneity was considered to be an issue. In such situations, a crossover design was used instead. A within-subjects longitudinal design was also utilised in order to determine change in cognition over time.

2.2.1. **Parallel Between-Subjects Design**

This design was used in Chapter 6. In parallel designs, participants are randomly allocated to either an ‘active’ (for example a drug) or to a placebo condition. The placebo is given as an inactive tablet that is made to look identical to the active drug. A double-blind procedure is used: participants and researchers are blind to the allocation of drug and placebo.

A parallel design removes the problem of practice effects, which can complicate the interpretation of findings. This design is possible where a large, relatively homogenous pool of participants is available, such as was the case with the healthy volunteers in Chapter 6. As a baseline assessment is not carried out, it is important for the groups to be well matched. In Chapter 6, the participants were matched for age, level of education, verbal IQ, and gender.
2.2.2. **Crossover within-subjects design**

This design was used in Chapters 4 and 5. In a crossover design experiment, participants receive both the active drug, and a placebo encapsulated to look identical to the drug. The drug and placebo are administered on separate occasions, in a pseudo-random order, to ensure that practice effects are counterbalanced, and therefore theoretically removed from the final analysis. The advantage of this design is that each participant acts as their own control. This design is useful when the heterogeneity of patients makes it very difficult to create two matched groups, as is the case in Chapters 4 and 5.

2.2.3. **Longitudinal within-subjects design**

A longitudinal design enables conclusions to be drawn about individual change over time, as in Chapter 3. It separates out changes within individuals from cohort effects, or differences between participants at baseline. Each subject serves as their own control, so the error of between-subject variation is excluded.

The challenges of longitudinal designs include attrition of participants, resulting in missing data. Analysis may also be made difficult due to co-dependency of variables in the data, and time-varying covariates.

2.2.4. **Statistical analysis**

Statistical analysis was carried out using Statistical Package for the Social Sciences, PASW Statistics version 18 (SPSS; Inc., Chicago, IL, USA). Raw data were always examined to establish whether or not they met the assumptions of parametric analysis. The Kolmogorov-Smirnov test was used to establish whether the data were normally distributed, and Levene’s test was used to assess heterogeneity of variance. Where the assumptions were violated, the data were transformed using either logarithmic transformations \(x = \log_{10}y\), square root \(x = \sqrt{x}\), or arcsine transformations \(x = 2\sin^{-1}\sqrt{y}\) as described by Cardinal and Aitken (2006).

Data satisfying the requirements of parametric analysis were analysed using analysis of variance (ANOVA), t-tests, repeated measures ANOVA, or, in the case of gender distribution, using \(\chi^2\) tests. Non-parametric tests were employed when the assumptions of parametric analysis were not met, as is made clear when relevant. It is important to note that, for the sake of clarity, untransformed values are presented in the tables and figures.

In Chapter 3, a one-way repeated measures ANOVA was used to compare stop signal reaction time (SSRT) at time 1 (prior to the start of boxing training), time 2 (twenty four hours after their first fight) and time 3 (one year after the first fight). In order to assess the relationship between changes on SSRT and change in the imaging variables, a non-parametric spearman’s rho correlation was used, because of the small sample size.
In Chapter 4, one-way repeated measures ANCOVAs were used to compare performance on the cognitive tasks on drug (either modafinil or physostigmine) to placebo. In both Chapters 4 and 5, multiple regression analysis was utilised in order to explore the relationship between change in performance, and a number of predictor variables. Multiple regression allows one to ask the question about how well a set of variables (e.g. in Chapter 4: baseline performance, order of drug administration; Chapter 5: drug plasma concentration, disease severity, dopaminergic medication, order of drug administration) is able to predict change in performance on the cognitive tasks that are of interest, and allows one to assess which variable is the best predictor of the outcome. The variables were examined to ensure they did not violate the assumptions of multicollinearity (using the variance inflation factor), normality, linearity, and homoscedasticity. For all analyses, a $p$ value of $<0.05$ was considered significant, while $0.05 < p < 0.1$ was considered a trend towards significance.

The analyses in this thesis were not corrected for multiple comparisons, and so any findings should be considered to be exploratory. Therefore, in order to aid interpretation, actual $p$ values are given.

2.3. Neuropsychological Assessment

Neuropsychological assessment comprised both computerised and non-computerised tests. The majority of the computerised tests were taken from the Cambridge Neuropsychological Test Automated Battery (CANTAB). This battery assesses a range of cognitive functions, including: visual memory, working memory, verbal memory, planning, attention, decision making, executive function, and response control. All the tests have a high level of validation, and have been used to assess cognition in over 100 disorders. The tests allow comparative assessment of cognitive performance across different neuropsychiatric, neurological and substance abuse groups, as well as healthy volunteers, allowing the processes underlying particular forms of cognitive function to be investigated and determined. Importantly for the experiments in this thesis, the CANTAB tests have a high sensitivity to cognitive change resulting from neurochemical manipulations, and have been extensively used to investigate the effects of pharmaceutical agents on cognition. These tests also have the added advantage of being closely comparable to tests used in neural models of cognition in non-human primates and rodents.

In what follows, the cognitive tests used in this thesis and their structural and neurochemical sensitivities are described. There are also summaries of the individual tests used in the relevant chapters.

2.3.1. Non-computerised Tests

2.3.1.1. IQ Estimation – National Adult Reading Test (NART) and Wechsler Test of Adult Reading (WTAR)

Adult reading tests were used to estimate premorbid intelligence and in order to ensure that different groups were matched for IQ. The National Adult Reading Test (NART) and Wechsler Test of Adult Reading (WTAR) are both word reading tests in which fifty words, printed in order of difficulty, are read out by the
participant. All of the words are spelt ‘irregularly’ according to common rules of grapheme to phoneme translations. The tests therefore assess previous learning of the word, rather than the ability to apply standard pronunciation rules. The advantage of these tests is that they are quick and easy to administer, which is especially important in clinical settings. The NART is widely used and the association between NART scores and premorbid IQ has been shown to be fairly good (Hart, Smith et al. 1986; Beardsall and Brayne 1990; Willshire, Kinsella et al. 1991). The WTAR is part of with the Wechsler Adult Intelligence Scale and Wechsler Memory Scale and performance on the WTAR is correlated with these more detailed IQ assessments.

2.3.1.2. MINI-MENTAL STATE EXAMINATION (MMSE)

The MMSE is a 30-point questionnaire that screens for global cognitive impairment, assesses the severity of cognitive impairment, and can be used to document cognitive changes occurring over time (Folstein, Robins et al. 1983). The MMSE comprises questions relevant to: orientation to time; orientation to place; registration (repeating named objects); attention and calculation (counting backwards from 100 in 7’s); recall (of objects named in the registration section); language (naming a pencil and a watch, writing down a sentence); repetition (of a phrase); complex commands (following written and verbal instructions); figure copying (copying a figure of two overlapping pentagons). A score in the range of 30-24 on the MMSE is considered to be in the normal range, and anything lower than 24 is usually interpreted as impairment. The MMSE has been shown to have high levels of sensitivity for moderate-to-severe cognitive impairment; however, it does have lower levels of sensitivity for mild degrees of impairment (Tombaugh and McIntyre 1992).

2.3.1.3. SUBJECTIVE MEASURES – VISUAL ANALOGUE SCALE (VAS)

In all of the experiments where drugs were administered (Chapters 4, 5 and 6), participants were asked at various points to complete visual analogue scales (VAS) (Bond and Lader 1976). This was done to provide a record of the subjective experience of the testing session, and was used to complement the objective neuropsychological measures of drug manipulation. At each time point, participants were asked to rate their feelings in relation to sixteen dimensions. The following measures were used: alert-drowsy; calm-excited; strong-feeble; muzzy-clear-headed; well-coordinated-clumsy; lethargic-energetic; contented-discontented; troubled-tranquil; mentally slow-quick witted; tense-relaxed; incompetent-proficient; happy-sad; antagonistic-amicable; interested-bored; withdrawn-gregarious; and attentive-dreamy. The dimensions were presented as 100mm lines, an example of which is found below. At each end of the line was written a different extreme of the emotion (for example, ‘alert’ and ‘drowsy’), with the participant marking on the line the point that described how they felt.

ALERT  ___________________________________________ DROWSY
2.3.1.4. **Digit span**

This task is part of the Wechsler Adult Intelligence Scale (Wechsler and Matarazzo 1972) and is a common test for measuring span of immediate verbal recall (Lezak 1995). The task consists of participants’ repeating sequences of digits that are read out by the tester at the rate of one digit per second. Initially, the participant is asked to repeat the sequences forwards, before in a second phase having to repeat the sequences backwards. For every correct sequence, a point is awarded, meaning a maximum of two points is awarded for each sequence length. If neither sequence of a particular length is repeated correctly, the participant is moved onto the second phase of the test, or, if already on the second phase, the test is terminated. Scores are on the forwards and backwards tests are added together in order to give an overall score. The highest number of digits recalled correctly, or the actual span, is also recorded. The forwards digit test is often linked to the efficiency of attention, while the backwards digit test requires working memory (Lezak 1995).

2.3.2. **Computerised tests of visual memory**

2.3.2.1. **Paired associates learning (PAL)**

In this test of visuospatial memory, boxes displayed on a screen open in a randomised order. One or more of them contains an abstract pattern (Figure 2.1). The patterns are then displayed in the middle of the screen, one at a time, and the participant must touch the box where the pattern was originally located. If the participant makes an error, no feedback is given, but the test is recommenced, with the boxes opening again prior to this restart in order to remind the participant of the location of the patterns. Up to ten attempts are allowed at each stage. The difficulty level increases through the test until eight boxes all contain a pattern. The test is adaptive so that if a trial is not completed despite multiple attempts, the test automatically terminates, and the errors score that is calculated includes an adjustment for errors at those stages that were not completed.

![Figure 2.1](image)

**Figure 2.1** Screen shot of the Paired Associates Learning task

One of the periphery boxes opening up to reveal a pattern inside. After the pattern found in each of the boxes has been displayed, participants are presented with patterns one at a time in the middle of the screen, and have to touch the box that previously contained that pattern.
Materials and Methods

This form of episodic memory is particularly dependant on integrity of the entorhinal and transentorhinal cortex and hippocampal areas (Owen, Sahakian et al. 1995). These areas are the first to be affected by Alzheimer’s pathology, and therefore this task is sensitive to early and differential diagnosis of Alzheimer’s disease (AD) (Blackwell, Sahakian et al. 2004). A recent imaging study in healthy adults has shown that bilateral hippocampal activation increases during the encoding stage of the PAL task as task load increases (de Rover, Pironti et al. 2011). Furthermore, the performance of AD patients in CANTAB PAL has been shown to be significantly improved following oral administration of a cholinesterase inhibitor (Greig, Sambamurti et al. 2005).

PAL is also impaired in first-episode psychosis, chronic schizophrenics, and chronic drug users (Wood, Proffitt et al. 2002; Barnett, Sahakian et al. 2005; Ersche, Clark et al. 2006). A touch screen automated object-in-place PAL task has been recently adapted for rats and has been shown to be sensitive to manipulation of glutamatergic systems within the hippocampus (Talpos, Winters et al. 2009).

2.3.3. Computerised tests of working memory

2.3.3.1. Reverse spatial span (SSP)

In this test of visuospatial short-term memory capacity, participants are presented with nine white boxes, some of which briefly change colour in a variable sequence (Figure 2.2). A tone sounds to indicate the end of the sequence, and then participants must touch the boxes which changed colour in the opposite order to that in which they were displayed by the computer. On the first trial only two boxes change colour, but this number increases by one on successful completion of each sequence, until the hardest level is reached where nine boxes change colour. The test is terminated if the participant fails in three attempts to touch the boxes in the requisite order at any given sequence length. Spatial span, which is the highest level at which the participant manages to recall the locations of all the boxes within three attempts, and the number of errors made are the main measures of this test.

**Figure 2.2 Screen shot of the reverse spatial span task**

The boxes change colour one at a time and participants then have to replicate the sequence in the reverse order to that in which it was displayed by the computer, by touching the boxes.
Materials and Methods

Research using SSP has tended to use the forward version of the task, where the boxes have to be touched in the same order in which the computer displays them. The reverse version is more challenging, and places higher demands on working memory.

On the forward version, the D2 receptor agonist bromocriptine (Mehta, Swainson et al. 2001) has been shown to improve performance, whilst it has been shown to be impaired in disorders such as schizophrenia (Pantelis, Barnes et al. 1997) and Parkinson’s disease (PD) when patients are ‘off’ their dopaminergic medication (Lange, Robbins et al. 1992). Neuromodulation using either tyrosine depletion (McLean, Rubinsztein et al. 2004), or the dopamine agonist sulpiride (Mehta, Sahakian et al. 1999) does not affect performance on the task.

In healthy young adults, modafinil does not improve span length or errors on the forwards version of the SSP task (Turner, Robbins et al. 2003). However, modafinil does improve SSP performance in adults with Attention Deficit Hyperactivity Disorder (ADHD) (Turner, Clark et al. 2004). Furthermore, dopamine-noradrenaline reuptake inhibitor methylphenidate improves performance of healthy young adults on SSP, but only on the first instance that they encounter the task (Elliott, Sahakian et al. 1997). Sleep-deprived adults have not been shown to be impaired at SSP compared to rested controls, and there is no effect of the acetylcholinesterase inhibitor donepezil on SSP performance in sleep-deprived adults (Dodds, Bullmore et al. 2011). Donepezil also does not have an effect on SSP performance in older adults (Beglinger, Tangphao-Daniels et al. 2005). Again, these studies have used the forward, rather than the reversed, version of the task.

2.3.4. Computerised tests of attention

2.3.4.1. Rapid visual information processing (RVIP)

In this test of sustained attention, a white box appears in the centre of the computer screen, inside which digits, from 2 to 9, appear in a pseudo-random order, at the rate of 100 digits per minute (Figure 2.3). Participants have to detect target sequences of digits (for example, 2-4-6, 3-5-7, and 4-6-8) and to indicate when they do so using a press pad. The sequences that participants are asked to identify stay on the screen throughout the task, although they are encouraged to focus on the central box. This means that this task requires working memory, as well as sustained attention. The probability of a hit (the frequency of targets correctly detected), the probability of a false alarm (the number of responses when no sequence was displayed), and response latency are all recorded. The main measures for this task are thus target sensitivity (RVIP A’) and response bias (RVIP B”), which are calculated in the following fashion from the proportion of correct and incorrect responses. $P_h$ is the probability of a correct hit, and $P_{fa}$ is the probability of a false alarm:

$$A’ = 0.5 + \frac{P_h - P_{fa} + (P_h - P_{fa})^2}{4P_h(1-P_{fa})}$$
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\[ B^* = \frac{P_h(1 - P_h) - P_{\alpha}(1 - P_{\alpha})}{P_h(1 - P_h) + P_{\alpha}(1 - P_{\alpha})} \]

A positron emission tomography (PET) study has indicated that the inferior frontal gyrus, parietal cortex, fusiform gyrus and right rostral frontal gyrus subserve this task (Coull, Frith et al. 1996). Performance on the RVIP task is not impaired by the administration of the D2-antagonist sulpiride (Mehta, Sahakian et al. 1999), or by tyrosine depletion (McLean, Rubinsztei n et al. 2004), indicating that dopamine is not central to this task. Performance on this task is impaired after the administration of the noradrenergic α2 receptor agonist clonidine (Coull, Middleton et al. 1995), and administration of the α2 antagonist idazoxan improves performance in patients with dementia of the frontal type (Coull, Sahakian et al. 1996). The RVIP task is also sensitive to pharmacological manipulation of the cholinergic system using scopolamine and nicotine, in both healthy adults and patients with dementia of the Alzheimer’s type (Wesnes and Warburton 1984; Sahakian, Jones et al. 1989).

There are mixed finding for the effect of the drug modafinil on the RVIP task. A study in healthy volunteers showed an improvement in target detection on 200mg of modafinil, but not 100mg (Randall, Viswanath et al. 2005). However, an equivalent study did not find any effect (Turner, Robbins et al. 2003), and a further study that utilised a dose of 300mg also did not find an effect (Winder-Rhodes, Chamberlain et al. 2010). Modafinil does not improve performance on the RVIP task in trichotillomania patients (Chamberlain, Grant et al. 2010), but it does improve target sensitivity in ADHD patients when they do the task for the first time (Turner, Clark et al. 2004).

**Figure 2.3** Screen shot of the rapid visual information processing task

Participants have to detect 3-digit sequences when they occur amongst random digits presented in the central box at a rate of 100 digits per minute.
2.3.4.2. Reaction time (RTI)

The RTI is a task of attention that measures simple and choice reaction time, movement time and vigilance during simple and 5-choice reaction time trials. The participant must hold down a button until a yellow spot appears in a white circle on the screen, and then touch the yellow spot as quickly as they can. The spot appears in a single location during the simple reaction time phase (Figure 2.4) and in one of five locations in the 5-choice reaction time phase.

![Figure 2.4 Screen shot of the reaction time task](image)

Participants hold down a button until a yellow spot appears inside the white circle, which cues them to respond by touching the circle on the screen. Initially there is only one white circle on the screen, so only one location to attend to. In the subsequent stage there are five white circles, and the yellow spot can appear in any one of these.

The CANTAB RTI is a direct analogue of the rodent 5-choice serial reaction time test (5-CSRT), one of the most well-studied animal behaviour paradigms. In the rat, 5-CSRT shows sensitivity to discrete lesion sites in the prefrontal cortex, to cholinergic lesions in basal forebrain, and sensitivity to several classes of compound. Choice reaction time accuracy has been shown to be impaired by saporin-induced lesions of the basal nucleus of Meynert (McGaughy, Dalley et al. 2002). Such deficits have been shown to be reversible using cholinergic agents such as physostigmine (Muir, Dunnett et al. 1992).

In humans, the CANTAB RTI test shows differential pharmacological sensitivity; for example clonidine, but not guanfacine, impairs five-choice reaction time performance in young healthy volunteers (Jakala, Riekkinen et al. 1999). Patients with mild AD show improved accuracy and latency on the 5-CSRT following administration of the cholinesterase inhibitor tacrine (Sahakian and Coull 1993).

2.3.5. Computerised tests of decision making and response control

2.3.5.1. Cambridge gamble task (CGT)

The CGT is a test of decision-making ability, which measures the speed of decision making, the quality of decisions, and risk adjustment. On each trial, the participant is presented with a row of ten boxes across the top of the screen, some of which are red and some of which are blue. The ratio of red to blue boxes
varies from trial to trial allowing the examination of decision-making behaviour over a range of different probabilities. On each trial, the participant is told that a yellow token has been hidden inside one of the coloured boxes, and that they must guess whether it will be hidden in a red box or a blue box (Figure 2.5).

After a training phase, participants are offered bets on their choice of colour being correct. In this gambling phase, participants start with a number of points, displayed on the screen, and can select a proportion of these points, displayed in either rising or falling order, in a second box on the screen, to gamble on their confidence in this judgement. A stake box on the screen displays the current amount of the bet. The participant must try to accumulate as many points as possible. There are two conditions in the CGT, the ascending first (where stakes are displayed in ascending order for two stages, then in descending order for two stages) and descending first (where stakes are displayed in descending order for two stages, then in ascending order for two stages). The main outcome measures are quality of decision making (proportion of trials on which the participant chose to gamble on the most likely outcome), deliberation time to select their choice of red or blue, risk taking (percentage bets made on their choice being correct), risk adjustment (measures the tendency to bet a higher proportion of points on trials where the colour chosen is in the large majority, as compared to when a smaller majority of boxes are the chosen colour), delay aversion (the difference in amount bet between the ascend and descend conditions), and overall proportion bet (average proportion of the current points total that is bet).

![Figure 2.5 Screen Shot of the Cambridge Gamble Task](image)

Participants have to decide whether they think a yellow token is hidden in the ‘red’ or ‘blue’ boxes presented at the top of the screen, by touching the relevant box at the bottom of the screen. Once they have made their choice, they are presented with ascending or descending ‘bets’ in a central right-hand box (not displayed here) and instructed to try to increase their total points by placing a bet on their choice being correct.

The CGT allows for the fractionation of different components of decision making across a range of well-defined and clearly indicated contingencies (Rogers et al., 1999). It measures decision making under risk (i.e. with explicit probabilities) rather than under ambiguity, and it dissociates risk taking from impulsivity, because in the ascending bet condition participants who want to make risky bets have to wait patiently (Manes, Sahakian et al. 2002). The design of the CGT also minimises demands for stimulus-reinforcement learning, reversal learning, and working memory (Clark et al., 2008).
CGT performance is mediated by the orbitofrontal cortex (Rogers, Owen et al. 1999). Increased betting behaviour on the CGT has been reported in frontotemporal dementia (Rahman, Sahakian et al. 1999), subarachnoid haemorrhage of the anterior communicating artery (Mavaddat, Kirkpatrick et al. 2000), and damage to orbitofrontal/ventrolateral and insular but not dorsolateral prefrontal cortices (Manes, Sahakian et al. 2002; Clark, Bechara et al. 2008). Also, Lawrence, Luty et al. (2009) showed that non-treatment-seeking subjects with pathological gambling were intact in terms of deliberation times versus controls, but were more likely to go bankrupt, and gambled more points regardless of box ratio. Children with ADHD bet less rationally, are more delay averse and display less optimal risk adjustment compared to controls (DeVito, Blackwell et al. 2008). CGT is sensitive to dopamine manipulation. PD patients are more delay averse when ‘On’ their dopaminergic medications, as compared to ‘Off’ medications (Cools, Barker et al. 2003). Patients recovered from depression exhibit a reduced propensity to gamble following acute tyrosine depletion (Roiser, McLean et al. 2005).

Modafinil does not seem to have an effect on CGT performance in adults with ADHD (Turner, Clark et al. 2004). However, healthy young adults on modafinil take longer to make a decision compared to those on placebo (Turner, Robbins et al. 2003). After administration of methylphenidate, children with ADHD (DeVito, Blackwell et al. 2008), and dementia patients of the frontotemporal type, bet more conservatively on the CGT (Rahman, Robbins et al. 2006).

2.3.5.2. INFORMATION SAMPLING TASK (IST)

The IST (Clark, Robbins et al. 2006) examines reflection impulsivity, the tendency to gather and evaluate information prior to making a decision (Kagan 1966). The participant is presented with a 5x5 array of grey boxes, which can be opened one at a time by touching the screen, to reveal the underlying distribution of two colours (Figure 2.6). On each trial, participants are asked to decide which of the two colours is in the majority. They are instructed that they can open as many boxes as they wish in order to reach their decision. When they have decided, participants indicate their choice by touching a coloured square at the bottom of the screen. After the participant has indicated their choice, all the remaining grey boxes on the screen reveal their colours and a message is displayed to inform the participant whether or not they were correct. The colours change from trial to trial.

There are two conditions on the IST: the fixed win condition, in which the subject is awarded 100 points for a correct decision regardless of the number of boxes opened, and the decreasing win condition, in which the number of points that can be won for a correct decision starts at 250 and decreases by 10 points for every box touched. In either condition, an incorrect decision costs 100 points. Participants perform ten trials in both the fixed win and decreasing win condition. The key measures for this task are the mean number of boxes opened in each of the two conditions.
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**Figure 2.6 Screen shot of the Information Sampling Task**

Participants are asked to decide which of the two colours is in the majority by touching the grey boxes to reveal their colour. Participants are free to open as many boxes as they like in order to arrive at a decision. In the fixed win condition 100 points is given for a correct decision, regardless of the number of boxes opened. In the decreasing win condition the number of points that can be won starts at 250, but this is reduced by 10 points for every box opened.

Former and current users of amphetamine and opiates (Clark, Robbins et al. 2006), and current users of cannabis (Clark, Roiser et al. 2009), sample less information compared to controls; they respond when there is a lower probability of getting the problem right. Furthermore, adolescent current users of cannabis show increased reflection impulsivity that is related to a greater exposure to the drug and an earlier age of onset of use (Solowij, Jones et al. 2012). However, past and present ecstasy users do not differ from healthy abstinent controls in their performance on this task (Clark, Roiser et al. 2009).

Patients with obsessive-compulsive disorder (OCD) do not adapt their sampling behaviour according to the reinforcement contingencies, unlike controls who sample less in the decreasing condition (Chamberlain, Fineberg et al. 2007). Tryptophan depletion in healthy volunteers increases the amount of information sampled in the decreasing win, but not the fixed win condition, suggesting that it is serotonin that promotes the avoidance of immediate aversive outcomes (Crockett, Clark et al. 2012).

### 2.3.5.3. Stop signal task (SST)

The SST measures proponent response inhibition, which can be defined as the inhibition of a pre-planned physical response. Participants are required to respond rapidly to an arrow that is displayed in the centre of the screen, by pressing the left button on a button box when the arrow points left, and pressing the right button when the arrow points right. These Go trials make up 75% of the trials. On the remaining trials, a 500 Hz bleep is sounded, to signal that the participant must try to inhibit their proponent response to the displayed Go stimulus (i.e. the arrow) (Figure 2.7).
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**Figure 2.7 Schematic of the Stop Signal Task**

Arrows are displayed in the centre of the screen, to which participants are required to respond as quickly as possible by pressing the left or right button on a press pad, according to the direction in which the arrow is pointing. In a minority of cases, a ‘beep’ sounds which signals to the participant that they should withhold their response to the arrow for that trial.

The key component of the SST is the stop signal reaction time (SSRT), which is the estimated time required between the presentation of the Stop signal and the Go signal for the participant to successfully inhibit their response. Logan, Cowan et al. (1984) propose that the Stop and Go processes are independent of one another, and that a ‘race’ occurs between the two processes for completion. If the Go response wins, a response occurs, and if the Stop response wins, a response is inhibited. The model assumes that the placement of the Stop signal will bias the race; if the Stop signal occurs early in the trial it will usually be inhibited, and if the Stop signal occurs late in the trial the response will rarely be inhibited. The SSRT is estimated using a staircase tracking algorithm whereby the initial position of the Stop signal is adjusted closer to the mean Go reaction time following a correct Stop trial, but away from the mean Go reaction time following an incorrect Stop trial, resulting in it stopping at the point at which 50% of the Stop trials are performed correctly. The SSRT is an index of impulsivity, and has been shown to be impaired in several neuropsychiatric conditions linked to problems suppressing inappropriate impulsive behaviour (Boonstra, Oosterlaan et al. 2005; Lijffijt, Kenemans et al. 2005; Monterosso, Aron et al. 2005; Chamberlain, Fineberg et al. 2006; Chamberlain, Fineberg et al. 2007; Penades, Catalan et al. 2007).

Performance on the SST is subserved by a neural network, including the inferior frontal gyrus, the basal ganglia (caudate-putamen), and the presupplementary motor area (Aron, Behrens et al. 2007; Madsen, Baare et al. 2010). In particular, lesion and fMRI studies have shown that successful response inhibition is dependent on the inferior frontal gyrus, particularly the right hemisphere (Rubia, Smith et al. 2005).

The successful response inhibition on the SST has been shown to be sensitive to modulation of noradrenaline (NA). The NA reuptake inhibitor atomoxetine improves response inhibition in patients with ADHD (Chamberlain, Del Campo et al. 2007), healthy adults (Chamberlain, Muller et al. 2006), and rats...
Modafinil also improves response inhibition in healthy adults in a dose-dependent manner (Turner, Robbins et al. 2003), and ADHD patients (Turner, Clark et al. 2004), perhaps through its action on NA. Importantly, modafinil has no effects on the Go process of the SST (Turner, Clark et al. 2004; Eagle, Tufft et al. 2007), unlike conventional psychostimulants (Bedard, Ickowicz et al. 2003; Lijffijt, Kenemans et al. 2006).

2.3.6. **Computerised tests of executive function and planning**

2.3.6.1. **Intra-extra dimensional set shift (IED)**

In this test, which is based on the Wisconsin Card Sort Test (WCST), participants’ ability to shift attention from one category of stimuli to another or to ‘shift cognitive set’ is assessed. The test stimuli are made up of two dimensions: pink shapes and white lines. The pink shapes are the simple stimuli, whereas later in the task compound stimuli are introduced which are made up of both pink shapes and white lines (Figure 2.8). The participant starts by seeing two simple pink shapes within any of the four boxes on the screen, and must learn which one is correct by using the visual and auditory feedback. After six correct responses, the test moves onto the next stage. The stages are as follows:

1. Simple discrimination of simple stimuli (pink shapes)
2. Simple discrimination reversal: the other pink shape exemplar becomes correct
3. Compound discrimination: a second dimension is added (white lines). The correct dimension and exemplar remain the same as in the previous stage (Figure 2.8).
4. Compound reversal discrimination: the rule is reversed and the original pink exemplar becomes correct.
5. Intradimensional stage: new compound stimuli are introduced, but participants are still required to attend to the pink shapes, rather than the white lines.
6. Intradimensional reversal: the other pink shape exemplar becomes correct.
7. Extradimensional shift discrimination: new compound stimuli are presented, but this time, the other stimulus dimension (white lines) is now correct, meaning that participants have to override their previous bias.
8. Extradimensional shift reversal: the other white line exemplar becomes correct.

If at any stage the participant fails to reach the criterion after 50 trials, the test terminates. The number of stages passed, trials completed before the criterion, errors made, and response latency are recorded. Reversal and shifting can be evaluated independently, which permits assessment of perseveration to a specific exemplar or stimulus dimension.
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**Figure 2.8** Screen shot of the intra-extra dimensional set shift task

Participants must grasp various rules concerning the shapes (see text for details) in order to progress through the eight stages of the task.

A number of patient groups have impaired performance on this task, including those with bipolar disorder (Clark, Iversen et al. 2002), schizophrenia (Elliott, McKenna et al. 1995), depression (Beats, Sahakian et al. 1996), Huntington’s disease (Lawrence, Hodges et al. 1998) and PD (Owen, Beksinska et al. 1993). An imaging study has shown activations in the prefrontal regions, including the left anterior prefrontal cortex and right dorsolateral cortex, when extradimensional shifting is compared to intradimensional shifting (Rogers, Andrews et al. 2000), whilst reversal learning showed activations in the left caudate nucleus (Rogers, Andrews et al. 2000).

Modafinil has been shown to improve attentional set shifting performance on the IED task in patients with chronic schizophrenia (Turner, Clark et al. 2004), but not healthy young adults (Elliott, McKenna et al. 1995), or adults with ADHD (Turner, Clark et al. 2004). Furthermore, 200mg of modafinil increases the number of errors made on the IED task in middle-aged adults (Randall, Fleck et al. 2004).

### 2.3.6.2. One Touch Stockings of Cambridge (OTS)

The OTS is a spatial planning task based on the Shallice (1982) ‘Tower of London’ task. The participant is shown two sets of three coloured balls, with the numbers 1 to 6 displayed across the bottom of the screen (Figure 2.9). Participants are asked to touch the number along the bottom of the screen that indicates the minimum number of ball moves needed in order to make the balls in the bottom arrangement match the top arrangement. In the case of error, participants continue until they select the correct number. The percentage of correct first choices, the latency of response, and the number of attempts are the main measures of this task. The motor demands are kept low because participants are obliged to plan responses as the balls cannot actually be moved.
This planning task produces activations in a distributed network of cortical areas incorporating the prefrontal, cingulate, premotor, parietal and occipital cortices (Owen, Doyon et al. 1996), and is sensitive to damage to the prefrontal lobes (Owen, Downes et al. 1990). Neuroimaging studies have revealed that performance on the OTS is particularly associated with activation in the dorsolateral prefrontal cortex (Baker et al., 1996; Dagher, Owen, Boecker, & Brooks, 1999; Owen, Evans, & Petrides, 1996).

OTS performance is impaired in patients with PD (Cools, R. A. Barker, Barbara J Sahakian, & Robbins, 2001; Lange, Robbins, Marsden, & James, 1992; Morris et al., 1988; Owen et al., 1992) and is sensitive to dopamine manipulation in PD. Deficits in planning accuracy are remediated by L-Dopa in patients with PD (Lange et al., 1992).

Modafinil improves accuracy on the OTS task in healthy adults, adults with ADHD, and chronic schizophrenia, but this tends to be associated with a slower latency to response (Turner, Robbins et al. 2003; Turner, Clark et al. 2004; Turner, Clark et al. 2004). The effect of modafinil on accuracy, but not response latency, is antagonised by the α1 adrenoceptor antagonist prazosin (Winder-Rhodes, Chamberlain et al. 2010).
3. Cognition in Amateur Boxers

3.1. Introduction

The harmful long-term effects of professional boxing on the brain are well documented, particularly the long-established relationship between professional boxing and dementia pugilistica (Roberts, Allsop et al. 1990; Forstl, Haass et al. 2010). However, it is less clear what, if any, the consequences of amateur boxing are. Protective measures have been put in place in amateur boxing, including: head guards; more heavily cushioned gloves; stopping the bout if the points difference becomes too large (>20); shortened bout lengths to two minutes; the option for a boxer to interrupt the bout; and the option for the ringside doctor to intervene (International Boxing Association, 2012). It has been argued that because of these measures and improved monitoring of athletes, amateur boxing has become one of the safest contact sports (Jako 2002). However, the objective of an amateur boxing match is still to deliver blows to the front or sides of an opponent’s head as well as to the torso in order to score points, and, as a by-product of this, ‘knock-outs’ can occur, whereby a blow causes a subsequent intermittent loss of consciousness. Evidence for the risk of acute and chronic injuries caused by boxing has prompted the British Medical Association to campaign for an outright ban on the sport (British Medical Association, 2003). Despite this, amateur boxing is growing in popularity, making it prudent that the effect of amateur boxing on the brain be investigated.

The brain sits inside the skull surrounded by cerebral spinal fluid. A sudden force applied to the head can cause brain trauma at the site of impact or at the contrecoup side of impact as the brain ricochets against the skull. Equally, the acceleration and deceleration can cause shearing of white matter and a series of secondary events can be triggered, including: damage to the blood-brain barrier; inflammation; raised intracranial pressure; cerebral edema; ischemia; cerebral hypoxia and dysfunction of mitochondria. An Olympic boxer punching a dummy (wearing a head guard) has an average peak force of 2625 N for middleweight boxers and 4345 N for super heavyweights, and an average peak rotational acceleration of
Cognition in Amateur Boxers

6343 rad/s² (Wallikko, Viano et al. 2005). This can result in a translational acceleration of the brain of more than 50 g (Stojsh, Boitano et al. 2010). The frontal lobes are particularly susceptible to damage because they sit at the front of the skull, near its rough bony ridges, with impact against this rough surface resulting in coup and contrecoup injury, and also shearing injuries to white matter (Marquez de la Plata, Garces et al. 2011). Acute axonal and neuronal damage can be indexed by cerebrospinal fluid (CSF) biomarkers. For example, a recent study has demonstrated that 80% of samples of elite Olympic boxers had increased CSF levels of total tau, neurofilament protein (NFL), glial fibrillary acidic protein (GFAP) and S-100B protein within 6 days after a bout. Furthermore, both NFL and GFAP remained elevated after a resting period of 14 days, particularly in those who had fought many bouts previously (Neselius, Brisby et al. 2012).

The potential for widespread and diffuse injuries in amateur boxing is reflected by the wide range of cognitive impairments found in this group, including: delayed recall, working memory, visuospatial skills, reaction time, attention, and executive functions (Heilbronner, Henry et al. 1991; Stewart, Gordon et al. 1994; Matser, Kessels et al. 2000; Moriarity, Collie et al. 2004). However, these impairments have not been found across all boxers and studies (Brooks, Kupshik et al. 1987; Butler, Forsythe et al. 1993; Butler 1994; Moriarity, Collie et al. 2004). Impairments in delayed recall have been documented in amateur boxers even after training sparring when the boxers were wearing headgear (Matser, Kessels et al. 2000). The impairments seen were more pronounced and longer lasting after boxing matches. Relatively little is known about the cognitive impact of cumulative mild head trauma and multiple concussions as a result of amateur boxing. In jockeys, multiple concussions are associated with decrements in response inhibition on the Stroop task and impaired attentional function, with a greater degree of impairment seen in younger athletes (Wall, Williams et al. 2006). Macciocchi, Barth et al. (2001) reported that the neuropsychological consequences of two concussions sustained more than two weeks apart did not differ from those of a single injury, whereas Collins, Grindel et al. (1999) found that multiple concussion was associated with a worse performance on tasks involving complex attention. Young high-school athletes have prolonged memory dysfunction after sports-related concussion, compared to older college-athletes, even though the injuries sustained were more severe in the older group (Field, Collins et al. 2003). The age effect seen in this study may reflect the increased vulnerability of the developing brain to injury.

In young boxers, damage to the frontal lobes may disrupt the normal development of this area, which continues through adolescence and young adulthood. Throughout adolescence there is an increase in white matter and a decrease in grey matter in the frontal and parietal cortices (Pfefferbaum, Mathalon et al. 1994; Reiss, Abrams et al. 1996; Giedd, Blumenthal et al. 1999; Sowell, Thompson et al. 1999; Sowell, Thompson et al. 2001; Sowell, Peterson et al. 2003; Barnea-Goraly, Menon et al. 2005). Most studies agree that this increase in white matter density is steady and linear in nature, and this has consistently been interpreted as reflecting continued axonal myelination during childhood and adolescence (Giedd, Blumenthal et al. 1999; Barnea-Goraly, Menon et al. 2005). Two interesting studies by Sowell et al have indicated that the development of the brain continues beyond adolescence. They showed that the loss of
grey matter in the frontal cortex accelerates during adulthood between the early 20s and up to the age of 30 (Sowell, Thompson et al. 2001). A further study of individuals aged between 7 and 87 years showed that although the increase in white matter in the dorsal prefrontal, parietal, and temporal cortices is most dramatic in early adulthood, the process continues well beyond adolescence, and even up to the age of 60 (Sowell, Peterson et al. 2003).

One of the main limitations of the current studies into the cognitive and neurological effects on boxers is that it is difficult to determine causality and, in addition, they rely on the self-reporting of concussions, which may not be reliable. We therefore aimed to recruit de novo amateur boxers so that we could determine the cognitive and neurological effects of boxing on the brain. Because of the vulnerability of the frontal lobes to damage, we utilised a test that is closely associated with frontal lobe function: the stop signal task (SST). This task is one of the most widely used measures of inhibitory control. Successful response inhibition has been shown to be highly dependent on the frontal lobes, particularly the right Inferior Frontal Gyrus (rIFG), for more details on which see Section 2.3.5.3 (Rubia, Smith et al. 2005; Aron, Behrens et al. 2007; Madsen, Baare et al. 2010). Importantly, this task has well-defined neural networks, the integrity of which has been shown to be associated with task performance (Ersche, Jones et al. 2012).

On the basis of previous research, a region of interest (ROI) was created in order to investigate the relationship between the cognitive domain of response inhibition and brain structure. As it was predicted that the changes seen might be subtle, diffusion tensor imaging (DTI) was used.

DTI allows the measurement of the integrity of white matter tracts in the human brain, thus providing a more sensitive measurement of discrete axonal injury, even in areas where focal damage is not seen on standard structural MRI scans. This technique characterises the diffusion of water molecules in tissue environments that are influenced by the microstructural organization of tissues and their constituent cells. The diffusion tensor can be used to represent the magnitude of water diffusion (quantified by the apparent diffusion coefficient), whether such diffusion is directionally non-uniform (anisotropy), and the orientation of that direction (eigenvectors/eigenvalues). These characteristics make DTI an ideal in vivo tool to assess the loss of white and/or grey matter integrity.

Using this technique, white matter injury has been related to cognitive impairment in traumatic brain injury (TBI). Global white matter pathology has been shown to be related to greater impairments in executive function, memory, and attention (Kraus, Susmaras et al. 2007), psychomotor performance (Niogi, Mukherjee et al. 2008), and evoked motor responses (Yasokawa, Shinoda et al. 2007). Localised DTI abnormalities have also been related to specific tasks. For example, impairments in learning and memory are associated with increased diffusivity in the left posterior cingulated, left hippocampal formation and left temporal and frontal and occipital cortex (Salmond, Menon et al. 2006). However, the best demonstration that localised DTI abnormalities are related to specific cognitive domains comes from pairwise associations between performance on specific tasks and DTI measures in related brain areas, whilst demonstrating the absence of correlation with DTI measures in unrelated brain regions. Such a
double dissociation has been demonstrated by Niogi, Mukherjee et al. (2008) who showed that DTI abnormalities in the uncinate fasciculus correlated with performance on memory tasks and abnormalities in the left corona radiate were related to attentional performance. Importantly, the reverse correlations were not significant, providing evidence for the specificity of regional DTI abnormalities. More recently, Newcombe, Outtrim et al. (2011) elegantly demonstrated that specific cognitive domains of the Cambridge Gamble Task (CGT), namely impulsivity, risk adjustment and rational choice, correlated inversely and specifically with the severity of DTI abnormalities in regions that have been implicated for these cognitive performances. The performance on the specific cognitive domains of the task did not correlate with DTI abnormalities in areas not implicated in their performance.

Therefore, this study aimed to longitudinally follow de novo amateur boxers by assessing them at baseline, after their first competitive bout, and one year later. Each assessment comprised the SST task and a DTI scan, in order to determine whether changes in cognition and white matter density were related. It was hypothesised that changes in stop signal reaction time (SSRT) on the SST would be related to changes in the rIFG, with improving SSRT being related to increases in white matter density, and impairments in performance being related to decreases in white matter. It was predicted that boxing would result in a decrease in white matter over time.

3.2. Materials and methods

3.2.1. Subjects and procedures

Participants were recruited from the Cambridge University Amateur Boxing Club (CUABC). Potential participants were invited to take part in the study at the beginning of the academic year as long as they had just joined the boxing club, they had never boxed before, had not taken part in a contact sport since the age of 18, had not had a past traumatic brain injury, previous history of neurological or psychiatric disease, or any previous neurosurgery. All participants were screened before each visit for: metal implants or risk of metal foreign bodies; neurological or neurosurgical events; any significant change in health. The study was approved by Cambridge Local Research Ethics Committee (Ref: 06/Q0108/161).

Thirty boxers gave their informed consent to take part in the study. All of these boxers received a full medical examination by a member of the neurosurgical team at Addenbrooke’s Hospital, Cambridge, and were stated to be fit to become a member of the Amateur Boxing Association of England. Recruited boxers also took part in a full cognitive assessment and had a structural and DTI scan prior to their first boxing match. The majority of the boxers received this assessment before they started sparring in training. Eleven of the recruited boxers either left CUABC, or were not selected to fight in a competitive bout. Six of the recruited boxers had a post-bout assessment, but then did not return for the final assessment a year later, because of time (N=3) or distance to travel (N=3). One boxer returned for the one year follow-up but was unable to have a scan as he had had an implant fitted in his arm. Therefore, twelve boxers completed the study: their demographic details are displayed in Table 3.1.
Within 24 hours of the boxers’ first amateur boxing bout they received their second scan and cognitive assessment. The time between the first baseline assessment and the after-bout assessment was 51 days on average (see Table 3.1 for individual timing details). The protocol outlined that boxers should then be followed up again one year after their first competitive bout, although in practice there was an average of 440 days between the post-bout and final assessments (Table 3.1).

**Table 3.1 Participant demographics**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Gender</th>
<th>Age (years)</th>
<th>IQ (NART)</th>
<th>YOE (Years)</th>
<th>Time lapse 1</th>
<th>Time lapse 2</th>
<th>Significant events</th>
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<td>26</td>
<td>118</td>
<td>22</td>
<td>5</td>
<td>371</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>21</td>
<td>126</td>
<td>17</td>
<td>52</td>
<td>607</td>
<td>Developed epilepsy between T2 and T3</td>
</tr>
<tr>
<td>D</td>
<td>M</td>
<td>23</td>
<td>118</td>
<td>19</td>
<td>41</td>
<td>418</td>
<td>Chronic subdural haemorrhage between T2 and T3</td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>23</td>
<td>120</td>
<td>19</td>
<td>12</td>
<td>569</td>
<td></td>
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<tr>
<td>F</td>
<td>M</td>
<td>20</td>
<td>120</td>
<td>16</td>
<td>12</td>
<td>401</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>M</td>
<td>25</td>
<td>117</td>
<td>21</td>
<td>5</td>
<td>628</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>M</td>
<td>20</td>
<td>112</td>
<td>16</td>
<td>19</td>
<td>452</td>
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<tr>
<td>I</td>
<td>M</td>
<td>24</td>
<td>119</td>
<td>20</td>
<td>111</td>
<td>357</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>M</td>
<td>21</td>
<td>120</td>
<td>17</td>
<td>99</td>
<td>370</td>
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<tr>
<td>K</td>
<td>F</td>
<td>20</td>
<td>115</td>
<td>16</td>
<td>95</td>
<td>375</td>
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<tr>
<td>L</td>
<td>M</td>
<td>22</td>
<td>118</td>
<td>18</td>
<td>74</td>
<td>373</td>
<td></td>
</tr>
</tbody>
</table>

For gender M=male and F=female; Age was recorded at the first testing session; NART is the predicted verbal IQ from the National Adult Reading Test; YOE is the number of years of full time education completed at the first assessment session (all participants were full-time students at the University of Cambridge); Time lapse 1 is the number of days between the first assessment session and the after-bout assessment; Time lapse 2 is the number of days between the after-bout and final assessments.
3.2.2. **Neuropsychological measures**

Participants were tested using the SST from the CANTAB battery. The test was computerised and ran on a Paceblade touch-screen computer, with responses being registered via a press pad. The SST is a test of response inhibition, involving speeded left or right responses to a Go stimulus, but this response should be withheld on trials where a Stop signal (300Hz tone) is presented. The key variable on this task is the SSRT: an estimation of the time taken to internally suppress a Go response, which is a measure of response inhibition. For a fuller description of the SST, please see Section 2.3.5.3.

3.2.3. **Diffusion tensor imaging**

The DTI data underwent eddy current correction and Fractional Anisotropy (FA), Apparent Diffusion Coefficient (ADC) and eigenvalue map were created using The Oxford Centre for fMRI of the brain (FMRIB) Diffusion Toolbox (http://www.fmrib.ox.ac.uk/fsl/). To aid coregistration the skull and extracranial soft tissue were stripped from the magnetisation-prepared rapid acquisition with gradient echo (MPRAGE) images using the Brain Extraction Tool [16](Smith, 2002). Diffusion maps were coregistered to Montreal Neurological Institute (MNI) space using a two-step approach. First, all patient and control b=0 images were coregistered to each subject’s MPRAGE using the vtkCISG (visualization toolkit Concussion in Sport Group) normalised mutual information algorithm (http://www.vtk.org). This coregistration matrix was applied to the FA and ADC maps. Each subject’s MPRAGE was subsequently coregistered to MNI space. The transformation matrix normalising the MPRAGE image was then applied to the diffusion maps which were in the subject’s MPRAGE space, so all were in MNI space.

In order to help control for Type 1 errors, previous imaging studies were used to select the regions of interest (ROIs) before commencing the imaging analysis. On the basis of previous studies, ROIs for the IFG and the presupplementary motor area were created, using Hammer’s probabilistic atlas (Hammers, Allom et al. 2003). Figure 3.1 displays the rIFG ROI. Other ROIs were created as control areas: anterior corpus callosum, posterior corpus callosum, whole brain.

The mean ADC, FA and eigenvalues for the different ROIs were calculated using in-house software (written by Dr. Guy Williams and Dr. Virginia Newcombe). Axial diffusivity was defined as the major eigenvalue ($\lambda_1$) and radial diffusivity as the average of the two minor eigenvalues ($\frac{(\lambda_2 + \lambda_3)}{2}$). As it was hypothesised that white matter would be particularly vulnerable to damage following mild TBI, the FA was predicted to be a more sensitive measure than ADC. For this reason, and to minimise the number of comparisons, FA was prospectively chosen as the outcome measure for this study. Mean values of FA were imported into SPSS for correlational analysis.
3.3. Results

Group analysis did not reveal any significant change over time in white matter density for any of the ROIs processed (anterior corpus callosum, posterior corpus callosum, whole brain, rIFG, lIFG, left pre-supplementary motor area (LpreSMA), right pre-supplementary motor area (RpreSMA); p>0.05, derived from Wilcoxon signed ranks tests).

When taken together as a group, there were no significant changes in SSRT, either from baseline to post-bout, or from post-bout to one year follow up (p>0.05, derived from Wilcoxon signed ranks tests). However, there was variability in change within the group. Across the three assessment sessions boxers A, D, I, J, K, and L showed a shortening in SSRT (improvement), which was related to decreases in the mean apparent diffusion coefficient calculated for the rIFG (Table 3.2). Boxers C, E, F, G, and H showed increased impulsivity (longer SSRT) on at least one of the time points (Table 3.2).


<table>
<thead>
<tr>
<th>Participant</th>
<th>SSRT t1</th>
<th>SSRT t2</th>
<th>SSRT t3</th>
<th>rIFG FA t1</th>
<th>rIFG FA t2</th>
<th>rIFG FA t3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>216.95</td>
<td>199.83</td>
<td>169.83</td>
<td>.186</td>
<td>.267</td>
<td>.259</td>
</tr>
<tr>
<td>B</td>
<td>174.48</td>
<td>--------</td>
<td>156.45</td>
<td>.281</td>
<td>.305</td>
<td>.272</td>
</tr>
<tr>
<td>C</td>
<td>201.45</td>
<td>153.83</td>
<td>168.95</td>
<td>.280</td>
<td>.281</td>
<td>.276</td>
</tr>
<tr>
<td>D</td>
<td>266.93</td>
<td>230.53</td>
<td>186.18</td>
<td>.264</td>
<td>.286</td>
<td>.290</td>
</tr>
<tr>
<td>E</td>
<td>129.90</td>
<td>165.30</td>
<td>132.63</td>
<td>.276</td>
<td>.271</td>
<td>.279</td>
</tr>
<tr>
<td>F</td>
<td>175.83</td>
<td>143.98</td>
<td>147.28</td>
<td>.271</td>
<td>.279</td>
<td>.269</td>
</tr>
<tr>
<td>G</td>
<td>213.33</td>
<td>238.58</td>
<td>190.40</td>
<td>.292</td>
<td>.280</td>
<td>.295</td>
</tr>
<tr>
<td>H</td>
<td>--------</td>
<td>126.95</td>
<td>138.90</td>
<td>.267</td>
<td>.274</td>
<td>.273</td>
</tr>
<tr>
<td>I</td>
<td>172.73</td>
<td>165.08</td>
<td>133.48</td>
<td>.262</td>
<td>.262</td>
<td>.261</td>
</tr>
<tr>
<td>J</td>
<td>195.28</td>
<td>170.10</td>
<td>159.13</td>
<td>.281</td>
<td>.278</td>
<td>.285</td>
</tr>
<tr>
<td>K</td>
<td>284.23</td>
<td>268.05</td>
<td>210.03</td>
<td>.270</td>
<td>.261</td>
<td>.271</td>
</tr>
<tr>
<td>L</td>
<td>294.73</td>
<td>267.53</td>
<td>245.80</td>
<td>.284</td>
<td>.273</td>
<td>.282</td>
</tr>
</tbody>
</table>

SSRT: Stop signal reaction time; rIFG: Right inferior frontal gyrus; t1: first assessment; t2: second assessment; t3: third assessment.

----- indicates a missing data point.

Change scores were calculated for SSRT, Go RT, Anterior corpus callosum FA, Posterior corpus callosum FA, whole brain FA, rIFG and lIFG. Participant H did not complete the SST at the baseline assessment, and participant B did not complete the SST at the post-bout assessment, because his fight-related injuries (neck) made it uncomfortable for him to do the task. Therefore there were only eleven SSRT change scores entered into each analysis. The change in SSRT between the post-bout and final assessment was negatively correlated with FA in the rIFG ($r^2$=-0.67, $p=0.024$, Figure 3.2). The change in SSRT was not related to FA change in the whole brain, or any of the other brain regions (Table 3.3). Change in SSRT between the baseline and post-bout assessment was not associated with any of the brain regions analysed (Table 3.3).
### Table 3.3 Correlation Coefficients for Functional Anisotropy Values for Each Region of Interest with Change in Stop Signal Reaction Time

<table>
<thead>
<tr>
<th></th>
<th>SSRT T1 vs T2</th>
<th>P-value</th>
<th>SSRT T2 vs T3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior corpus callosum FA</td>
<td>-0.328</td>
<td>0.354</td>
<td>-0.218</td>
<td>0.519</td>
</tr>
<tr>
<td>Posterior corpus callosum FA</td>
<td>-0.491</td>
<td>0.150</td>
<td>0.209</td>
<td>0.537</td>
</tr>
<tr>
<td>Whole Brain FA</td>
<td>-0.362</td>
<td>0.304</td>
<td>-0.023</td>
<td>0.947</td>
</tr>
<tr>
<td>rIFG FA</td>
<td>-0.515</td>
<td>0.128</td>
<td>-0.67</td>
<td>0.024</td>
</tr>
<tr>
<td>lIFG FA</td>
<td>-0.345</td>
<td>0.328</td>
<td>0.323</td>
<td>0.332</td>
</tr>
<tr>
<td>RpreSMA</td>
<td>-0.164</td>
<td>0.651</td>
<td>0.455</td>
<td>0.16</td>
</tr>
<tr>
<td>LpreSMA</td>
<td>-0.036</td>
<td>0.920</td>
<td>0.392</td>
<td>0.233</td>
</tr>
</tbody>
</table>

FA is fractional anisotropy; rIFG is the right Inferior frontal gyrus, and lIFG is the left inferior frontal gyrus; RpreSMA is the right presupplementary motor area, and LpreSMA is the left presupplementary motor area. SSRT is stop signal reaction time. T1 is time one (baseline testing session), T2 is time 2 (post-bout testing session) and T3 is time 3 (one year follow up). P values were derived from two-tailed non-parametric spearman’s rho correlations. Significant correlation is highlighted in bold.

![Figure 3.2 Relationship between change in SSRT and rIFG FA](image)

There was a significant relationship between change in stop signal reaction time (SSRT) and change in the fractional anisotropy (FA) of the right inferior frontal gyrus (rIFG), between the second testing session (after the first competitive bout) and the one year follow up. Individual participants are labelled in the same way as for Table 3.2.
3.4. Discussion

This study demonstrated an association between change in white matter density and cognition. It was predicted that boxing would result in a decrease in white matter density, however, this was not found as an overall group effect for any of the regions examined. However, the secondary hypothesis that FA in the rIFG and SSRT would be related to one another was supported. Specifically, increases in white matter density in the rIFG were associated with SSRT getting shorter, whereas decreases in white matter density in the rIFG were associated with SSRT getting longer. This change was in the direction hypothesised. This relationship was not found for any of the other brain regions examined (lIFG, anterior corpus callosum, posterior corpus callosum, whole brain, RpreSMA, LpreSMA). This finding replicates a previous study, which showed an association between the same ROI and performance on the SST (Ersche, Jones et al. 2012), and concurs with previous studies that have demonstrated that the rIFG is important for response inhibition on the SST (Aron, Fletcher et al. 2003; Rubia, Smith et al. 2005; Aron, Behrens et al. 2007; Chamberlain, Hampshire et al. 2009; Madsen, Baare et al. 2010). Interpreting these findings is difficult due to the limitations of the study: the sample size is very small and there are no controls. Therefore, this study should be viewed as a pilot investigation that should be extended before conclusions can be made. Despite these important caveats, possible explanations for the results are explored below.

The lack of a matched control group makes it impossible to determine if the variations in white matter density and response inhibition across time are due to noise, part of normal development, or caused by boxing-related damage. There is evidence to suggest that myelination continues through adolescence and early adulthood (Bartzokis, Beckson et al. 2001; Sowell, Thompson et al. 2001; Giedd 2004; Blakemore and Choudhury 2006; Qiu, Tan et al. 2008). Therefore, it is likely that the increase in FA in the rIFG that is related to improvement in SSRT is part of the normal developmental process. However, it should be noted that we did not see an overall group increase in white matter density over time in any brain region inspected. This could be because a number of our cohort showed a decrease in white matter density. Loss of white matter in the frontal lobes has been associated with head injury related atrophy, or as a feature of brain ageing in older adults, which does not tend to be seen in those under the age of 40 (Christiansen, Larsson et al. 1994; Bartzokis, Beckson et al. 2001). Five of our participants showed a decrease in white matter density in the rIFG between the first bout assessment and the one year follow-up. Three of these participants (H, C and F; Figure 3.2) also showed an increase in SSRT. It could be that these participants may have had the normal developmental process disrupted, or mild atrophy may have occurred, potentially as a result of amateur boxing.

Interestingly, a relationship was only seen between the post-fight and one year follow-up. This second time-lapse was longer, representing an average of 440 days, as compared to 51 days for the first time-lapse. This may be driven by the fact that increases in myelination are only seen over a longer period of time. It also suggested that decreases in white matter density relating to cognition are not apparent after...
Cognition in Amateur Boxers

one amateur boxing bout, but rather the changes seen represent chronic damage that develops over time. This converges with previous findings that multiple concussions are associated with decrements in cognition, including response inhibition (Collins, Grindel et al. 1999; Wall, Williams et al. 2006). If these findings are replicated, it may suggest that although the safety measures put in place in amateur boxing protect from acute damage, there may be a small amount of repetitive injury build up, resulting in diffuse damage, perhaps in a similar way to professional boxing (Jordan 2000).

Also of note are the two neurological events that occurred in our sample: Boxer C developed epilepsy between the post-fight and year-follow-up assessment, and Boxer D had a chronic subdural haemorrhage that was surgically evacuated between his post-fight and one year follow-up assessment. We are not able to determine whether these significant neurological events were caused by boxing, but in a sample of our size this incidence is above what would be predicted in a population of healthy young adults. Boxer C was one of the three boxers who showed a decrease in white matter density in the rIFG and a longer SSRT. Boxer D showed increases in white matter in the rIFG that were associated with improved performance on the SST. It might be predicted that boxer D would have atrophy in other areas.

If the findings of this study still stand after the proposed extensions, they have important implications. Increased impulsivity as a result of amateur boxing could have real implications in terms of function and quality of life. Indeed, impulsivity is a risk factor for developing substance abuse disorders (Dalley, Fryer et al. 2007; Casey and Jones 2010; Ersche, Turton et al. 2010; Ersche, Jones et al. 2012), and increased impulsivity is thought to be an important underlying factor for impulse control disorders, including behavioural addictions such as pathological gambling (Vitaro, Arseneault et al. 1999; Slutske, Caspi et al. 2005; Lawrence, Luty et al. 2009), and is associated with poor decision making (Clark and Manes 2004; Clark, Dombrovski et al. 2011; Studer and Clark 2011).

The sensitivity of the SST to detect small changes in white matter density indicates that it could be used as a potential marker for damage in mild TBI. Identifying impairments such as increased impulsivity could indicate individuals who would benefit from treatment. This is particularly important as untreated impulsivity in TBI characterises many of the personality and behavioural changes following injury (McAllister 1992), and the disastrous consequence of this is illustrated by the finding that untreated head injury is associated with an increased likelihood of committing violent crime (Leon-Carrion and Ramos 2003). The pharmacological agent methylphenidate, which increases both dopamine and noradrenaline in the prefrontal cortex, has been shown to ameliorate impulsivity in TBI (Pavlovskaya, Hochstein et al. 2007). Perhaps a more suitable choice for treatment, due to its more selective effects on the noradrenergic system, is atomoxetine. Atomoxetine has been shown to improve response inhibition on the SST in healthy adults (Chamberlain, Hampshire et al. 2009), and adults with attention deficit hyperactivity disorder (ADHD) (Chamberlain, Del Campo et al. 2007), and has recently been approved for the purpose of treating ADHD, a condition that is characterised by pathological impulsivity. Furthermore,
atomoxetine increases activation in the rIFG during Stop trials when individuals are trying to inhibit their responses (Chamberlain, Hampshire et al. 2009).

One of the main limitations of this study is the small sample size. One factor that contributed to this was the high drop-out rate, with a large number of participants not going on to fight after their baseline assessment. Boxers were recruited early in term time, when they had just joined the boxing club, in order to complete the baseline assessment before sparring had started. Because of this some of our participants were not subsequently selected to be part of the boxing squad, or stopped boxing training after trying it for a term. High attrition rate is one of the known limitations of a longitudinal design, but this design does allow conclusions about individual change over time. This study would also be strengthened by the addition of a matched control group, in order to determine whether the changes seen are related to amateur boxing, developmental processes, or experimental noise. A control group should be matched in terms of educational level, age, and, importantly for this group, exercise level. The amateur boxers had a vigorous training regime that included aerobic exercise, which is associated with improvements in cognition (Hillman, Erickson et al. 2008). An ideal control group for our cohort would be a university rowing team, as they would be matched in terms of demographics and level of exercise. Importantly, rowing is a non-contact sport, so it will enable the effects of development and exercise to be separated out from head injury. This study might also be limited in the extent to which any findings in our cohort can be generalised to all amateur boxers. Our participants were all aged at least 20 when they first started boxing, and they had all completed at least 16 years of education at the time they were recruited. The amount of training our cohort did was also limited to University term time, which represented two blocks of eight weeks in an academic term, as training was stopped for the final term for exams. This is not representative of the majority of amateur boxing clubs. Therefore, this study should also be extended to include a number of different boxing clubs to enable generalisation.

In summary, it was demonstrated that change in the ability to inhibit a proponent response is related to change in white matter density in the rIFG in a group of amateur boxers. The majority of boxers showed an improvement over time, and a small number showed increased impulsivity and reduced white matter density in the rIFG. Significant neurological events were also documented in two of the boxers over the course of the study. However, it is difficult to draw conclusions until this study is extended to include a larger sample size and matched controls. All of the amateur boxers who took part in this study successfully completed their degrees and went on to either further study or employment.
4. Modafinil and Physostigmine in Subarachnoid Haemorrhage

4.1. Introduction

Although the exact cognitive sequelae of subarachnoid haemorrhage (SAH) has yet to be fully determined, it has been shown that cognitive impairments have a real impact on patients’ functioning and quality of life (Al-Khindi, Macdonald et al. 2010; Haug, Sorteberg et al. 2010; Chahal, Barker-Collo et al. 2011). Therefore it is pertinent that the cognitive deficits are identified and treatments are explored for this group (Rinkel and Algra 2011). SAH often occurs as a result of moderate to severe trauma to the head, but can also appear spontaneously, usually as the result of a ruptured intracranial aneurysm which occurs in 50-85% of cases (Biller, Toffol et al. 1987; Maurice-Williams 1987; Nyquist, Naval et al. 2010). Aneurismal SAH represents about 5% of the yearly incidence of stroke in the UK (Bamford, Sandercock et al. 1990). Intracranial aneurysms are usually found in the medium sized arteries at the base of the brain in the Circle of Willis. Sites most frequently involved in aneurismal rupture are the anterior communicating artery (ACoA; 30-38%), middle cerebral artery (MCA; 13-25%) and posterior communicating artery (PCoA; 25%) (Nyquist, Naval et al. 2010).

The immediate effect of an aneurysm rupture is the accumulation of blood in the subarachnoid space of the brain, leading to a sudden rise in intracranial pressure and also a reduction in the cerebral perfusion pressure, both of which lead to acute onset of symptoms (Brinker, Seifert et al. 1992). The pathophysiology of brain injury after SAH may originate from three phenomena: transient global ischemia, subarachnoid blood clot, and acute hypertension. These lead to secondary effects (astrocytes, brain edema, apoptosis), resulting in both focal and diffuse brain injury. The variation in the location of aneurysm and the diffuse injuries that occur mean that a diverse range of cognitive and behavioural
symptoms are seen following SAH. Studies of patients who make a good neurological recovery (Glasgow Outcome Scale 1 or 2) have shown that up to 60% may suffer from neuropsychological sequelae that may be debilitating and lead to poor functional outcome (Hutter and Gilsbach 1993; Tidwell, Dias et al. 1995; Bjeljac, Keller et al. 2002). There has been some debate over whether cognitive outcome is affected differentially by the intervention methods of clipping or coiling, with no clear advantage for either method being decided upon (Mukerji, Holliman et al. 2010). It is, however, agreed that both groups have cognitive impairments (Santiago-Ramajo, Katati et al. 2010). The cognitive impairments reported by patients include short-term memory loss, impairments in concentration, attention, cognitive flexibility, and language problems such as aphasia and verbal fluency (Bjeljac, Keller et al. 2002; Passier, Visser-Meily et al. 2010; Sheldon, Macdonald et al. 2012; Vieira, Azevedo-Filho et al. 2012). The international subarachnoid aneurysm trial has reported on the cognitive outcomes of 395 patients at their twelve months follow-up visit. Cognitive impairment was found in one third of the patients, across the domains of verbal memory, language, processing speed, non-verbal memory, and executive function (Scott, Eccles et al. 2010).

Classically, patients with aneurysms of the ACoA, which is the site most commonly affected, exhibit behavioural and personality changes as well as memory impairments. This is not surprising given the fact that the collaterals of the ACoA supply blood to the frontal lobes and the basal forebrain (Critchley 2002). Potentially affected regions following MCA aneurysm include the lateral aspect of both hemispheres, the basal ganglia and the posterior part of the anterior limb of the internal capsule (Weir 1998). Following PCoA SAH, the brain regions most likely to be affected are the thalamus, hypothalamus, and posterior limb of the internal capsule (Weir 1998). As well as compromised blood supply to these areas, diffuse damage results in global deficits, some of which seem to be common to SAH patients, independently of aneurysm site.

Animal models of SAH have employed the Morris Water Maze (MWM) paradigm, which assesses learning and short term memory. It has been shown that rats exhibit both motor and spatial learning deficits after SAH by injections of blood into the cistern magna (Takata, Sheng et al. 2008). These motor deficits have not been replicated in other rat models. However, in rats, SAH by endovascular perforation, which causes transient global ischemia, has been shown to cause mild impairment on the MWM (Silasi and Colbourne 2009). More recently, it has been shown that rats with anterior circulation SAH develop delayed deficits in spatial learning on the MWM, which was associated with neuronal injury and death in the hippocampus (Jeon, Ai et al. 2010). Degeneration of the cholinergic basal forebrain neurons is evident after experimental SAH in rats, as well as a decline in the density of hippocampal and neocortical cholinergic terminals, and loss of long-term potentiation in this area (Lohr, Tzouras et al. 2008; Tariq, Ai et al. 2010). Lohr et al. suggested that the likely mechanism for cholinergic degeneration is the direct effect of blood in the basal cisterns resulting in tissue hypoxia.
The evidence from animal studies converges with findings in patients: SAH patients with all lesion locations have been shown to have impairments on verbal fluency tests, language, and verbal memory within 7-15 days of their symptom onset, prior to any surgical intervention (Vieira, Azevedo-Filho et al. 2011). ACoA patients who had all received a favourable outcome according to the Glasgow Outcome Scale (1 or 2), 6-24 months after surgery have impaired semantic fluency, and also make more errors on the Cambridge Neuropsychological Test Automated Battery (CANTAB) spatial working memory and pattern recognition tasks (Mavaddat, Sahakian et al. 1999). The pattern recognition task is highly sensitive to temporal lobe dysfunction (Owen, Sahakian et al. 1995), and patients with mild Alzheimer’s disease (AD) show deficits on this task, most likely reflecting early hippocampal pathology (Sahakian, Morris et al. 1988). The verbal fluency task is predominantly a test requiring a semantic lexical search and is particularly impaired in patients with temporal lobe lesions (Hodges, Patterson et al. 1992), and in mild AD patients (Rosser and Hodges 1994), and activation in the left temporal regions is seen whilst this task is performed (Mummery, Patterson et al. 1996). A recent study of free recall performance in SAH patients found that patients with ruptures in all locations show impairments on recalling lists of words. However, ACoA patients were only impaired at recalling lists that were disorganized, perhaps reflecting a deficit in executive functions (Sheldon, Macdonald et al. 2012). These impairments could be related to damage in the basal forebrain cholinergic structures, as cholinergic dysfunction as measured by pupil dilation response to dilute tropicamide is high in SAH patients with memory impairment (Nozaki, Sakai et al. 2002).

As well as memory impairments, SAH patients also report behavioural changes which are consistent with damage to the frontal lobes. However, SAH patients do not always show impairment on tasks that are considered to tap into executive functions. For example, SAH patients show normal performance on the strategy measure of the CANTAB spatial working memory task, as well as attentional set shifting, and the Tower of London planning task (Mavaddat, Sahakian et al. 1999), performances in all of which have been shown to be impaired in patients with frontal lobe lesions (Owen, Downes et al. 1990; Owen, Roberts et al. 1991; Sahakian and Owen 1992). Furthermore, SAH patients with ACoA aneurysms perform normally on the Wisconsin Card Sort Task (WCST) (Shoqerat, Mayes et al. 1990; Rousseaux, Godefroy et al. 1996). However, classic frontal lobe tests such as these tend to be subserved by the dorsolateral prefrontal cortex, whereas the areas of the cortex supplied by the ACoA, which is the most common site of aneurysm, include the orbital or ventromedial aspects of the frontal lobes. The CANTAB Cambridge Gamble Task (CGT) taps into the orbitofrontal circuitry, and ACoA patients show increased risk taking on the task, by placing higher bets on their chosen colour of box (Mavaddat, Kirkpatrick et al. 2000). ACoA patients also show decision-making deficits on the Iowa Gambling Task, with 70% of patients not learning to adjust their choices to the advantageous decks by the end of the task (Escartin, Junque et al. 2012). Furthermore, patients with SAH secondary to aneurysms in the MCA and PCoA also show deficits on the CGT (Salmond, DeVito et al. 2006). Specifically, they do not adjust their behaviour in the same way as controls according to the expectation of rewards: they place smaller bets on the outcome compared to
controls when the odds are in their favour, but larger bets when they are less likely to win, even though they are just as good as controls at choosing which colour had the highest probability of winning. In this study, MCA and PCoA patients also showed some impulsivity on the CGT. Although the pattern of impairments seen in ACA, and MCA and PCoA patients differs, the performance in all groups suggests abnormalities in frontal networks.

Currently, there are no standard treatments available to remediate the cognitive impairments in SAH patients. Given the cognitive sequelae, drugs which have been shown to affect the cholinergic systems or improve executive functions would be ideal candidates. It would be expected that cholinesterase inhibitors would remediate the memory deficits seen in SAH. Indeed, an open-label pilot study using rivastigmine, which blocks both acetylcholinesterase and butyrylcholinesterase, has been conducted in SAH (Wong, Wong et al. 2009). Rivastigmine is an established treatment for mild-to-moderate AD and has been shown to improve measures of global functioning and cognition in this group. The sixteen patients showed significant improvement on the Alzheimer’s Disease Assessment Scale-cognitive, particularly in global function, on the Rivermead behavioural memory test for prospective memory, and on the frontal assessment battery (Wong, Wong et al. 2009). However, as this was an open-label study it is hard to draw conclusions. There have been no studies to investigate the pharmacological remediation of impairments of executive function in SAH patients.

Therefore, a double-blind, placebo-controlled, crossover study was conducted to look at the effects of a single dose of the cholinesterase inhibitor, physostigmine, on cognitive function, including memory, in SAH patients. Physostigmine has been shown to improve visual recognition memory in Alzheimer’s patients (Thal, Masur et al. 1989). Cholinergic agents have been tried in a small number of patients with traumatic brain injury, and three reported case studies using single doses of intravenous physostigmine have suggested that the drug improves alertness and verbal memory in these patients (Goldberger and Curtis 1982; Weinberg, Auerbach et al. 1987; Eames and Sutton 1995). Memory function was indexed using the CANTAB Paired Associates Learning (PAL) test, a visuospatial episodic memory test, which has been shown to be closely linked to hippocampal function. The PAL test is sensitive and specific to the early and differential diagnosis of AD (Blackwell, Sahakian et al. 2004). Increased load on the task is related to increased activation in the hippocampal formation in healthy controls (de Rover, Pironti et al. 2011).

A potential treatment for executive function impairments in SAH is the atypical stimulant modafinil. Modafinil has been shown to improve executive functions in a variety of patient groups, as well as healthy controls. For example, it reduces impulsivity on the stop signal task (SST) in a dose-dependent manner in healthy controls (Turner, Robbins et al. 2003), and in patients with Attention Deficit Hyperactivity Disorder (ADHD) (Turner, Clark et al. 2004). It also improves planning as indexed by the CANTAB One Touch Stockings of Cambridge (OTS) task in healthy adults (Turner, Robbins et al. 2003; Winder-Rhodes, Chamberlain et al. 2010) and in patients with ADHD (Turner, Clark et al. 2004). Modafinil has also been
shown to improve attentional set shifting in schizophrenic patients (Turner, Clark et al. 2004) and to improve executive performance on a working memory task in first episode psychosis (Scoriels, Barnett et al. 2012). Modafinil was therefore administered as part of the double-blind, placebo-controlled, crossover study, and the CANTAB stop signal reaction time (SSRT) was used as a measure of executive function. The SST measures proponent motor disinhibition, which is a facet of impulsivity shown to be subserved by the right inferior frontal cortex, as part of a network that includes subcortical connections. Although SAH patients report behavioural disinhibition, impulsivity in this patient group has not been investigated outside of the context of decision making and reward, as on the CGT.

The study therefore aimed to investigate whether the pharmacological agents modafinil and physostigmine remediated the cognitive impairments associated with SAH. It was predicted that modafinil would improve executive impairments in SAH patients, and that physostigmine would improve performance on tests of memory and attention.

4.2. Materials and methods

4.2.1. Subjects and procedures

Twenty-eight participants were recruited from the cohort of patients who had been treated at Addenbrooke’s Hospital for acute SAH (see Table 4.1 for clinical details and Table 4.2 for demographic details). Exclusion criteria were: history of alcohol or drug addiction; scoring less than 23 on the Folstein Mini Mental State Examination (MMSE); pre-morbid IQ below 70 (as estimated by the National Adult Reading Test (NART)); history of pre-morbid psychiatric illness; concurrent use of medications contraindicated with modafinil or physostigmine; physical handicap that would prevent the completion of the cognitive tasks; moderate to severe hypertension (blood pressure readings higher than 170mmHg systolic, 90 mmHg diastolic or pulse rate over 90 bmp) or angina or cardiac arrhythmias; asthma; lung disease; gangrene; diabetes; liver problems; any known sensitivity to cholinesterase inhibitors. The study was approved by Cambridgeshire Research Ethics Committee (Ref: 02/212), and written informed consent was given by all participants prior to testing.
<table>
<thead>
<tr>
<th>Participant number</th>
<th>Site of Aneurysm</th>
<th>Clip/Coil</th>
<th>GCS</th>
<th>Years since Bleed</th>
<th>Further structural information (if visible from CT Scan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R ACoA</td>
<td>Clip</td>
<td>15</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MCA</td>
<td>Clip</td>
<td>12</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>R ACoA</td>
<td>Clip</td>
<td>15</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>L VA PICA</td>
<td>Clip</td>
<td>15</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>L ACoA L MCA</td>
<td>Coi</td>
<td>15</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>ACoA</td>
<td>Clip</td>
<td>15</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>BASILAR</td>
<td>Coi</td>
<td>15</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>ACoA</td>
<td></td>
<td>13</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>ICA</td>
<td>Clip</td>
<td>14</td>
<td>3.3</td>
<td>small MCA</td>
</tr>
<tr>
<td>15</td>
<td>R ACoA</td>
<td>Coi</td>
<td>15</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>L ACoA L MCA</td>
<td>Coi</td>
<td>15</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>L PICA</td>
<td>Clip</td>
<td>8</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>L MCA</td>
<td>Clip</td>
<td>15</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>ACoA</td>
<td>Coi</td>
<td>15</td>
<td>0.8</td>
<td>Ventricular dilation</td>
</tr>
<tr>
<td>20</td>
<td>L ACoA</td>
<td>Clip</td>
<td>15</td>
<td>0.6</td>
<td>Low attenuation affecting left caudate and adjacent limb of internal capsule.</td>
</tr>
<tr>
<td>21</td>
<td>R ACoA</td>
<td>Clip</td>
<td>3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>R MCA</td>
<td>Clip</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>ACoA</td>
<td>Coi</td>
<td>15</td>
<td>0.6</td>
<td>Ventricles a little dilated. Low density area in left formal area</td>
</tr>
<tr>
<td>24</td>
<td>ACoA</td>
<td>Coi</td>
<td>15</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>R MCA</td>
<td>Coi</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>R PCoA</td>
<td>Coi</td>
<td>15</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>ACoA</td>
<td>Coi</td>
<td>15</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>ACoA</td>
<td>Coi</td>
<td>14</td>
<td>0.8</td>
<td>Enlarged third and lateral ventricles</td>
</tr>
</tbody>
</table>

GCS is the score on the Glasgow Coma Scale at admission to hospital with bleed. Site of aneurysms are as follows: ACoA: Anterior Communicating Artery; MCA: Middle Cerebral Artery; PCoA: Posterior communicating artery; PICA:
Each participant attended three study sessions, separated by 1-4 weeks, at Addenbrooke’s Hospital. A double-blind, within-subjects design was utilised, with the order of drug administration randomised across visits in order to counter balance for practice effects. Therefore, on each visit participants either received 200mg modafinil, 1.25mg physostigmine, or a lactose placebo, that was encapsulated to look identical. The testing session began two hours after modafinil administration, and thirty minutes after physostigmine administration, in order for the cognitive testing to occur at peak plasma concentration (Gibson, Moore et al. 1985; Wong, King et al. 1998). The difference in the time for modafinil and physostigmine to reach peak plasma concentration necessitated the placebo drug administration to be randomly assigned to either two hours, like modafinil, or thirty minutes, like physostigmine. The testing session lasted approximately 1.5 hours.

In order to identify if SAH patients were impaired on the cognitive tasks at baseline, their performance on the placebo visit was compared to controls that were matched for practice effects. This was done by testing the controls on three separate occasions, with the same time interval between the testing sessions as patients, and then each control was consecutively matched to a patient so that the data analysed was from the same visit. As the control participants and SAH group were not matched for age (see Table 4.2), this variable was used as a covariate in the analysis.

### Table 4.2 Demographics

<table>
<thead>
<tr>
<th></th>
<th>SAH</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>28</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.5 (1.64)</td>
<td>39.8 (3.44)</td>
<td>P=0.005</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>14</td>
<td>10</td>
<td>P=1.0</td>
</tr>
<tr>
<td>IQ (NART)</td>
<td>114 (1.7)</td>
<td>113 (1.2)</td>
<td>P=0.688</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.3 (0.3)</td>
<td>28.5 (0.3)</td>
<td>P=0.74</td>
</tr>
<tr>
<td>BDI</td>
<td>5.96 (1.0)</td>
<td>2.95 (0.7)</td>
<td>P=0.021</td>
</tr>
</tbody>
</table>

Values shown are mean (standard error of the mean). IQ is the predicted verbal IQ score from the National Adult Reading Test (NART). P values were derived from one-way ANOVAs, and χ² test for gender distribution. MMSE is the Folstein mini-mental state examination (1983). BDI is depression symptom score as indexed by the Beck Depression Index, with a score below 13 indicating minimal or no depression.
4.2.2. **Physiological measures**

Blood pressure and pulse measurements were taken at three time points: before drug administration, immediately prior to testing (either 2 hours or 30 mins post-drug), and on completion of the testing session.

4.2.3. **Subjective measures**

4.2.3.1. **Visual analogue scales**

Subjects were asked to complete visual analogue scales (Bond and Lader 1974) before administration of the drug, immediately prior to testing, and on completion of testing. At each time point subjects were asked to rate their feelings in terms of sixteen dimensions, as detailed in Section 2.3.1.3.

4.2.3.2. **Adverse events**

Suspected adverse events that occurred during the study visit were recorded. Participants were also contacted by telephone 24 hours after each study visit so that any further suspected adverse events/side effects could be documented.

4.2.4. **Neuropsychological measures**

Participants were tested using well-validated and sensitive tests from the CANTAB battery. The order of the tests was counterbalanced across participants. All computerised tests were run on an ADVANTECH computer, and responses were recorded either via the touch-sensitive screen or a response key, depending on the task. A brief description of the cognitive tasks is presented in Table 4.3, with a fuller description in Chapter 2.

4.2.5. **Statistical analysis**

The study used a within-subjects design. Full details of statistics used are provided in Chapter 2.
<table>
<thead>
<tr>
<th>Cognitive task</th>
<th>Description</th>
<th>Key References</th>
<th>Important Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAL</strong></td>
<td>A test of the ability to form visuospatial associations and of the number of reminder presentations required to learn all the associations.</td>
<td>Sahakian, Morris et al. (1988)</td>
<td>Total Errors Adjusted &lt;br&gt; First trial memory score (sum of patterns correctly located on first presentation)</td>
</tr>
<tr>
<td><strong>RTI</strong></td>
<td>Measures simple reaction time, where response to a single stimulus is measured and 5-choice reaction time, where the stimulus appears in one of five possible locations.</td>
<td>Sahakian and Coull (1993)</td>
<td>Reaction time &lt;br&gt; Movement time (On simple and choice trials)</td>
</tr>
<tr>
<td><strong>RVIP</strong></td>
<td>A test of sustained attention to detect infrequent 3-digit sequences among serially presented digits.</td>
<td>Park, Coull et al. (1994)</td>
<td>Mean latency &lt;br&gt; $A'$ (target sensitivity, a measure of ability to detect sequences) &lt;br&gt; $B''$ (response bias, a measure of the tendency to respond regardless of whether target is presented)</td>
</tr>
<tr>
<td><strong>SST</strong></td>
<td>A test of response inhibition, involving speeded left or right responses to a Go stimulus, but this response should be withheld on trials where a Stop signal (300Hz tone) is presented. Race model allows estimation of the time taken to internally suppress a Go response (SSRT).</td>
<td>(Aron, Fletcher et al. 2003)</td>
<td>SSRT (a measure of response inhibition) &lt;br&gt; Go reaction time (ms)</td>
</tr>
</tbody>
</table>

PAL: CANTAB paired associates learning test; RTI: CANTAB reaction time task; RVIP: CANTAB rapid visual information processing; SST: CANTAB stop signal task; SSRT: Stop signal reaction time.
4.3. **RESULTS**

4.3.1. **PATIENTS COMPARED TO CONTROLS**

Compared to the control group, the patients had a significantly slower median Go reaction time on the SST ($F_{2,39} = 9.713, p<0.001$). On the reaction time task (RTI) patients also had a slower reaction time on both the simple ($F_{2,47} = 4.859, p=0.012$) and five choice conditions ($F_{2,47} = 3.67, p=0.034$), and a slower movement time on the five choice condition ($F_{2,47} = 4.859, p=0.012$). Patients were significantly less likely to detect a target sequence on the rapid visual information processing (RVIP) task ($Z=-2.255, p=0.024$). On the PAL task, patients made more errors ($F_{2,47}=4.576, p=0.016$) and therefore completed fewer stages of the task ($Z=-2.386, p=0.017$). There were no other significant differences between patients and controls on the tasks (Table 4.4).

**Table 4.4  SAH versus controls**

<table>
<thead>
<tr>
<th>Variable</th>
<th>SAH</th>
<th>Healthy subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST Go reaction time</td>
<td>452.7 (14.66)</td>
<td>404.5 (11.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SSRT</td>
<td>224.04 (15.94)</td>
<td>210.08 (15.22)</td>
<td>0.815</td>
</tr>
<tr>
<td>RVIP A'</td>
<td>0.88 (0.01)</td>
<td>0.92 (0.01)</td>
<td>0.024a</td>
</tr>
<tr>
<td>RVIP B'</td>
<td>0.93 (0.01)</td>
<td>0.95 (0.01)</td>
<td>0.176a</td>
</tr>
<tr>
<td>RTI choice movement time</td>
<td>439.23 (21.79)</td>
<td>382.62 (14.83)</td>
<td>0.036</td>
</tr>
<tr>
<td>RTI choice reaction time</td>
<td>360.53 (9.81)</td>
<td>368.87 (10.72)</td>
<td>0.034</td>
</tr>
<tr>
<td>RTI simple movement time</td>
<td>450.79 (24.29)</td>
<td>393.77 (21.49)</td>
<td>0.112</td>
</tr>
<tr>
<td>RTI simple reaction time</td>
<td>360.74 (14.26)</td>
<td>347.43 (12.11)</td>
<td>0.012</td>
</tr>
<tr>
<td>PAL total errors adjusted</td>
<td>40.21 (8.6)</td>
<td>13.45 (3.03)</td>
<td>0.016</td>
</tr>
<tr>
<td>PAL Stages completed</td>
<td>4.54 (0.18)</td>
<td>5 (0.00)</td>
<td>0.017a</td>
</tr>
</tbody>
</table>

Values shown for each variable are the mean and standard errors of the mean for each group. For the current study the reported p-values were derived from one-way ANCOVAs, with age as a covariate, unless the overall distribution of the score within the cohort differed from normality, in which case the equivalent non-parametric Mann-Whitney U test was applied (denoted a). Abbreviations are as for Table 4.3.
4.3.2. Effects of Modafinil and Physostigmine in SAH Group

4.3.2.1. Physiological Effects

Physiological readings were taken at three time points during the experiment. Repeated measures ANOVAs showed a main effect of drug on heart rate ($F_{2,40} = 4.84, p=0.013$; Figure 4.1c) and systolic blood pressure ($F_{2,40} = 4.75, p=0.014$ Figure 4.1a). For heart rate, there was a significant time x drug interaction ($F_{4,84} = 3.11, p=0.019$), with the difference in heart rate between the conditions being seen at the baseline measurement (before drug administration) and not post-drug or post-test (Figure 4.1). In contrast, the effect of drug on systolic blood pressure did not change according to the time. On the modafinil visit, systolic blood pressure was consistently higher compared to the physostigmine visit systolic blood pressure, including at the pre-drug administration reading. Diastolic blood pressure increased over time ($F_{2,42} = 10.99, p<0.001$; Figure 4.1b), and there was not a main effect of drug or a time x drug interaction ($p>0.1$).

4.3.2.2. Subjective Measures

4.3.2.2.1. Visual Analogue Scales

Over time the participants reported feeling more drowsy ($F_{2,42} = 2.03, p=0.005$), more excited ($F_{2,42} = 5.71, p=0.006$), and there was a trend towards participants feeling more feeble over time ($F_{2,40} = 2.85, p=0.07$). There was no effect of drug, or time x drug interaction.

4.3.2.2.2. Adverse Events

Three participants reported having a headache within 24 hours of taking modafinil. This expected side effect was reported to the Medicines and Healthcare Products Regulatory Agency (MHRA).

4.3.2.2.3. Neuropsychological Effects

Performance on the cognitive tests did not differ significantly between the drug visits (Table 4.5).
**Figure 4.1** Physiological response to Modafinil and Physostigmine

a: Pulse; b: Diastolic blood pressure; c: Systolic blood pressure.
Table 4.5  Overall effect of drugs

<table>
<thead>
<tr>
<th>Task</th>
<th>Variable</th>
<th>Placebo</th>
<th>Modafinil</th>
<th>Physostigmine</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit span</td>
<td>Forwards span</td>
<td>8.61 (0.47)</td>
<td>8.61 (0.57)</td>
<td>8.18 (0.48)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Backwards span</td>
<td>7.00 (0.50)</td>
<td>6.77 (0.63)</td>
<td>7.04 (0.50)</td>
<td>N/S</td>
</tr>
<tr>
<td>SST</td>
<td>Median RT</td>
<td>452.7 (14.66)</td>
<td>455.33 (14.1)</td>
<td>471.44 (12.14)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Stop signal RT</td>
<td>224.04 (15.94)</td>
<td>223.31 (20.16)</td>
<td>200.54 (16.82)</td>
<td>N/S</td>
</tr>
<tr>
<td>RTI</td>
<td>Simple MT</td>
<td>450.79 (24.29)</td>
<td>458.38 (21.89)</td>
<td>458.43 (18.71)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Simple RT</td>
<td>360.74 (14.26)</td>
<td>357.98 (11.55)</td>
<td>371.05 (13.17)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Five-choice MT</td>
<td>439.23 (21.79)</td>
<td>431.88 (22.16)</td>
<td>448.99 (17.64)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Five-choice RT</td>
<td>360.53 (9.81)</td>
<td>359.88 (8.87)</td>
<td>365.95 (9.98)</td>
<td>N/S</td>
</tr>
<tr>
<td>RVIP</td>
<td>Mean latency</td>
<td>480.30 (18.49)</td>
<td>490.55 (20.04)</td>
<td>503.13 (17.02)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.93 (0.01)</td>
<td>0.93 (0.02)</td>
<td>0.93 (0.01)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.88 (0.01)</td>
<td>0.88 (0.01)</td>
<td>0.88 (0.01)</td>
<td>N/S</td>
</tr>
<tr>
<td>PAL</td>
<td>Mean errors to success</td>
<td>5.89 (0.86)</td>
<td>6.78 (0.80)</td>
<td>6.89 (1.00)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Mean trials to success</td>
<td>2.81 (0.211)</td>
<td>2.91 (0.18)</td>
<td>3.00 (0.24)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Stages completed</td>
<td>4.54 (0.18)</td>
<td>4.75 (0.10)</td>
<td>4.56 (0.14)</td>
<td>N/S</td>
</tr>
<tr>
<td></td>
<td>Total errors adjusted</td>
<td>40.21 (8.60)</td>
<td>35.64 (4.96)</td>
<td>38.74 (6.84)</td>
<td>N/S</td>
</tr>
</tbody>
</table>

RT is reaction time and MT is movement time. Values shown for each variable are the mean and standard errors of the mean for each group. For the current study the reported p-values were derived from one-way repeated measures ANOVAs comparing each of the drug conditions to the placebo condition. N/S indicated that the difference in performance was not statistically significant. Abbreviations are as for Table 4.3.

4.3.2.2.4. Regression analysis

As illustrated in Table 4.4, although the group as a whole was impaired on the PAL task, there was a great deal of variability in their baseline (placebo performance). Therefore, a regression analysis was carried out to determine if this variability was related to drug response. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity, and homoscedasticity.

4.3.2.2.4.1. Modafinil vs. placebo on PAL total errors adjusted

Linear multiple regression was used to assess the ability of baseline performance (placebo) on the PAL task, and the order in which placebo and drug were administered, to predict performance on modafinil. Therefore, the dependant measure was drug effect: the difference in total errors adjusted between the
placebo and modafinil condition. The independent or predictor variables were participants’ performance on placebo, and the order in which they performed the two conditions (placebo or modafinil). The model explained 42.6% of the variance in PAL performance on modafinil, and was found to be highly significant ($R^2=0.426, F_{2,26}=8.907, p=0.001$; see Figure 4.2). When the variance explained by all other variables in the model was controlled for, baseline performance made the strongest contribution to explaining performance on modafinil (Beta=0.489, $p=0.005$). The Beta value for order was slightly lower, indicating that it made less of a contribution (Beta=0.37, $p=0.026$). Aneurysm location and time since SAH did not significantly contribute to the variance, so were not included in the final model.

![Figure 4.2](image.png)

**Figure 4.2** Relationship between baseline performance and modafinil on PAL total errors adjusted

There was a significant relationship between baseline performance on the number of errors made on the PAL memory test and the change in performance on modafinil. Patients who performed badly at baseline tended to improve in the modafinil condition, whereas patients who performed well on the test at baseline got worse when taking modafinil.

### 4.3.2.4.2. Physostigmine vs. placebo on PAL total errors adjusted

Baseline performance was not found to be a predictor of performance on physostigmine, and the model was not significant ($p>0.1$).
4.4. **DISCUSSION**

4.4.1. **COGNITIVE IMPAIRMENT**

In our sample of SAH patients, patients showed impairments on measures of reaction time, attention, and memory, compared to the control group. Specifically, they had a slower reaction time to respond to the Go stimulus on the SST, were less sensitive to the target sequences on the RVIP, were slower to both respond to and touch the target on the RTI task, and made more errors and thus completed fewer stages on the PAL task.

There is evidence to suggest that slowing of Go reaction time can be modulated via the dopaminergic system. For example, rats with ibotenic acid lesions to the subthalamic nucleus show longer Go reaction times compared to controls and to rats with lesions in the orbital frontal cortex and infralimbic cortex, and this slowing is not related to impairment on the SSRT (Eagle, Baunez et al. 2008). Infusion or injection of the D1/D2 dopamine agonist α-flupenthixol into the rat dorsal prelimbic cortex selectively impairs Go response, whereas the α-2A-adrenergic guanfacine selectively prolongs SSRT (Eagle, Tufft et al. 2007; Bari, Mar et al. 2011). Therefore, this finding could reflect disruption to the dopaminergic system, either directly, or as a downstream effect of damage to other systems, for example cholinergic, GABAergic or glutamatergic (Lester, Rogers et al. 2010). In humans, responding to the Go stimulus on the SST relies on both motor and attention functions, so the slower reaction time in SAH patients could reflect either of these processes. The RTI task separates out motor and attentional functions, by measuring both movement and reaction time in response to a cue. SAH patients had both slower reaction time and movement time on the five choice phase of the task, suggesting patients have impaired attentional functions as well as motor slowing. This motor slowing agrees with previous studies that document motor impairments in SAH patients, as well as with animal studies that demonstrate motor slowing following experimental SAH.

Patients showed attentional impairment on both the RTI and RVIP tasks. Performance on both of these tasks is manipulated by the cholinergic system. The administration of the muscarinic receptor antagonist scopolamine reduces the number of sequences detected on the RVIP task in healthy adults, whereas nicotine (a nicotinic receptor agonist) prevents decline in sequence detection over time, as well as increasing participant reaction time (Wesnes and Warburton 1984). Furthermore, administration of nicotine improves performance on the RVIP task in patients with dementia of the Alzheimer’s type (Sahakian, Jones et al. 1989). Attentional performance on the RTI task is also intimately related to the cholinergic system. For example, damage to the basal forebrain cholinergic system leads to impairments on the five choice serial reaction time task (5-CSRT), the rat version of the RTI task (Muir, Dunnett et al. 1992), and cholinergic agents have been shown to remediate impairments on the RTI task, (Sahakian, Owen et al. 1993). These findings converge with the fact that SAH patients were impaired on the PAL task, a visuospatial episodic memory test that has been shown to be sensitive to pathology in the hippocampal formation (Sahakian, Morris et al. 1988; Blackwell, Sahakian et al. 2004; de Rover, Pironti et al. 2011).
Performance on the PAL task has been shown to be significantly improved following oral administration of a cholinesterase inhibitor (Greig, Sambamurti et al. 2005). Together, the pattern of findings is consistent with the hypothesis that the basal forebrain cholinergic system is vulnerable to damage in SAH patients (Mavaddat et al., 1999).

It was interesting that SSRT was no different between the SAH patients and healthy controls. This is in line with the mixed findings for executive impairment in SAH patients, who perform normally on attentional set shifting, planning, working memory, and the WCST (Shoqeirat, Mayes et al. 1990; Rousseaux, Godefray et al. 1996; Mavaddat, Sahakian et al. 1999). However, it has been demonstrated that SAH patients show increased risk taking, impaired risk adjustment, and impaired decision making on the CGT (Salmond, DeVito et al. 2006; Sheldon, Macdonald et al. 2012). Successful response inhibition has been shown to be dependent on the right inferior frontal gyrus, whereas decision making on the CGT is associated with the orbitofrontal cortex. It could be that executive impairments in SAH are related to disruption of the orbital or ventromedial aspects of the frontal lobe. Together, the pattern of impairments suggests that the behavioural changes seen in SAH patients could reflect increased risk taking rather than impulsivity per se. However, further research which looks in more detail at the executive function of SAH patients is required.

The conclusions that can be drawn regarding cognitive impairment in SAH are limited by the fact that the control group in this study was significantly younger than the SAH group. For example, performance on memory tasks, including the PAL task, declines across the lifespan (Robbins, James et al. (1994), unpublished data, Cambridge Cognition). Therefore, even though age was used as a covariate in the analysis, the age difference could still account for the impairments seen. Another limitation is the fact that the placebo visit was pseudo-randomly allocated across visits, so that ten patients received the placebo on their first visit, nine patients received the placebo on their second visit, and nine on their third visit. In order to account for this, the control group was also tested three times, and the corresponding visit was used for each control (for example, if the first SAH patient received placebo on the first visit, then the data from the first visit was used for the first control tested as well). Therefore, even though practice effects were controlled for, it is difficult to determine whether the SAH patients would have been impaired when the tasks were novel, or whether the findings represent impairment in learning. The sample size of the SAH group meant that analysis looking at the first visit only would have been underpowered. It also prevented comparison of the different aneurysm locations, which could have demonstrated differences in cognitive profile (Vieira, Azevedo-Filho et al. 2011). Another limitation of the comparison is that the SAH patients in this study were all high functioning, with MMSE scores nearly at ceiling. It is possible that high functioning individuals are more likely to take part in research, and therefore the group may not be representative of the SAH population as a whole.
Overall, the SAH patients were impaired on tasks of attention and memory, which is consistent with the hypothesis that the cholinergic system is vulnerable following aneurysm. However, these findings should be extended to incorporate a larger sample size, and age-matched controls.

4.4.2. Pharmacological Treatment

Physostigmine did not have an overall effect in the SAH group for the cognitive domains measured, which did not support the prediction that it would improve memory and attention. The lack of improvement on the tests of attention and memory using the cholinesterase inhibitor physostigmine could suggest a number of things. Firstly, this finding may suggest that the impairments in these cognitive domains are not the result of disruptions to the basal forebrain cholinergic system. However, the measures of attention used have been shown to be modulated by cholinergic agents, even in healthy adults who are not impaired (Wesnes and Warburton 1984), and therefore increasing Acetylcholine (ACh) levels should have improved performance on these tasks, even if the impairment was caused by another mechanism. Another explanation may be that damage to the cholinergic pathways is so extensive in the SAH patients that, even with the administration of the cholinesterase inhibitor physostigmine, levels of ACh are still too low to make an impact on cognition. In order to test this hypothesis, PET imaging could be utilised in this patient group, using 11C-nicotine and to assess nicotine binding sites, and 11C-bentropine to visualize muscarinic receptors, both with and without the presence of a cholinesterase inhibitor (Nordberg, Lundqvist et al. 1997). Because of the fact that physostigmine has a short plasma half-life of only 30 minutes, it is also very plausible that the drug was not present in sufficient levels when the patients completed the cognitive tests. The order of the cognitive tasks in the battery was randomised for each patient, meaning that if the drug was having no effect by the end of the battery, this was diluted across tasks. As no blood plasma samples were taken, it is impossible to determine the level of drug for each patient. A Cochrane review has suggested that the short half-life of physostigmine together with the high occurrence of adverse events in studies utilising it, make this drug impractical for clinical use (Coelho and Birks 2001). Therefore, an alternative cholinesterase inhibitor may prove to be effective in SAH, and thus this should be investigated.

Similarly, there was no overall effect of modafinil on cognition in the SAH patients, again, not supporting the hypothesis that modafinil would improve executive functions in this group. Past studies have also found mixed effects of modafinil on the cognitive tests utilised. On the RVIP task, a study of healthy volunteers showed an improvement in target detection on 200mg of modafinil, but not 100mg (Randall, Viswanath et al. 2005). However, an equivalent study did not find any effect (Turner, Robbins et al. 2003), and a further study that utilised a dose of 300mg also did not find an effect (Winder-Rhodes, Chamberlain et al. 2010). Modafinil does not improve performance on the RVIP task in trichotillomania patients (Chamberlain, Grant et al. 2010), but it does improve target sensitivity in ADHD patients when they do the task for the first time (Turner, Clark et al. 2004). Modafinil has been shown to modulate SSRT in healthy
adults in a dose-dependent manner (Turner, Robbins et al. 2003), although this has not always been replicated (Winder-Rhodes, Chamberlain et al. 2010).

Despite the lack of overall effect, it was found that patients who were impaired at baseline (placebo visit) on the PAL memory test improved in the modafinil condition, whereas patients who were unimpaired at baseline showed an increase in the number of errors made whilst on modafinil. This analysis was post-hoc, and was not initially predicted, so the results should be considered to be exploratory. Aneurysm location and years since bleed did not contribute to the relationship seen, although order of drug administration did, but to a lesser degree than baseline performance. This is the first time modafinil has been shown to modulate visuospatial episodic memory performance using the PAL test (Turner, Robbins et al. 2003). However, modafinil has been shown to increase neuronal activity in the hippocampus in both animals and humans (Engber, Koury et al. 1998; Kim, Yoon et al. 2007; Pierard, Liscia et al. 2007; Joo, Seo et al. 2008; Tsanov, Lyons et al. 2010; He, Peng et al. 2011; Gozzi, Colavito et al. 2012), as well as to decrease GABAergic (Ferraro, Antonelli et al. 1997; Huang, Zhang et al. 2008) and to increase glutamatergic (Ferraro, Antonelli et al. 1997) neurotransmission in this area. Modafinil’s effect on other neurotransmitter systems has not yet been investigated in the hippocampal formation (Scoriels, Jones et al. 2013). It is therefore plausible that modafinil should modulate performance on a task that is closely related to hippocampal function.

The baseline effect found is consistent with the Yerkes-Dodson principle, which generally takes the form of an inverted-U-shaped function linking level of arousal with behavioural performance. The inverted-U-shaped function proposes that cognitive performance at low or high values of arousal is relatively poor, and at intermediate values it is optimal. Baseline performance has been shown to predict drug-response in both humans and animals. For example, infusion of the partial D1 agonist SKF 38393 into the medial pre-frontal cortex (PFC) in rats improved the accuracy of detecting visual targets on the animal version of the RTI task, but only in rats whose performance was at a relatively low level (Granon, Passetti et al. 2000). It was proposed that the high performing rats had already recruited the D1 system to attain optimal performance and so were not susceptible to further boosting of accuracy. In humans, individuals with a low working memory capacity improve following the administration of the dopamine D2-agonist bromocriptine, although individuals who have a high working memory capacity show a decline in performance following drug administration (Mehta, Swainson et al. 2001). Bromocriptine has also produced similar results on another aspect of executive function, task-set switching, as well as distractibility, in participants who varied in baseline levels of impulsivity. The drug enhanced task-switching performance in individuals scoring high in impulsivity on the Barratt Scale, but, if anything, impaired performance in low impulsive participants (Cools, Sheridan et al. 2007). The inverted-U-shaped function has principally been applied to monoamine modulation of the prefrontal cortex, although it has been proposed that it is possible that this function could exist in comparable brain regions, and for other neurotransmitters (Iversen 2010). Therefore, it is difficult to determine by which mechanism modafinil affects PAL performance, although it is possible that it is through increased dopaminergic or...
glutamatergic activation in the hippocampus, or even acetylcholine modulation that could occur as a downstream effect (Scoriels, Jones et al. 2013). In this study, individuals may have varied in their degree of neurotransmitter tuning because of factors such as genetic polymorphisms, and degree or location of damage as a result of SAH. The relative dose of modafinil would have also made a difference to the level of stimulation in each individual, so this study could be extended by taking blood samples in order to determine the concentration of drug in the blood plasma.

In conclusion, the results of this study suggest that modafinil offers potential as a cognitive treatment, in respect of its effects on memory in SAH patients, but only in some patients. If confirmed in a larger sample, these finding may have clinical relevance because cognitive baseline assessments, using sensitive measures, could aid treatment decisions.
5. **ATOMOXETINE IN PARKINSON’S DISEASE**

5.1. **INTRODUCTION**

A significant minority of Parkinson’s disease (PD) patients develop Impulsive/Compulsive Behaviours (ICBs), which are reward or incentive based and repetitive in nature, and which have been linked to Dopamine Replacement Therapy (DRT) (Gallagher, O’Sullivan et al. 2007; Weintraub, Koester et al. 2010). Furthermore, even PD patients who do not develop ICBs show elevated impulsivity compared to healthy controls. Impulsivity is a concept that covers a wide range of “actions that are poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation and often result in undesirable outcomes” (Daruna and Barnes 1993). There is not one type of impulsivity; instead, there are several varieties of impulsivity which are influenced by different biological mechanisms (Evenden 1999). These include response inhibition, reflection impulsivity, delay discounting, and delay aversion. Apart from manipulating dopaminergic therapy, which can be detrimental to motor symptoms, there are currently no pharmacological treatments for impulsivity in PD. Therefore, the primary focus was to test the utility of the noradrenaline (NA) reuptake inhibitor atomoxetine as a treatment for impulsive behaviours in PD.

When considering impulsivity in PD, it is important to bear in mind that disease process and dopaminergic medications may have a differential effect. Traditionally, PD has been associated with low pre-morbid impulsivity that includes traits such as industriousness, punctuality, and lack of novelty seeking. It is thought that these traits reflect early damage to the dopaminergic system which predates the onset of the motor illness (Menza 2000). Indeed, the pathological hallmark of PD is a regionally specific loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Lees, Hardy et al. 2009). The most severe loss of dopaminergic neurons in PD occurs in the ventrolateral and caudal portions of the SNpc,
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diminishing dopaminergic projections to the dorsal striatum (Bernheimer, Birkmayer et al. 1973; Fearnley and Lees 1991). However, as the disease progresses, there is also a gradual degeneration of the Locus Coeruleus (LC), dorsal raphe, and cholinergic brainstem, which progressively compromises the noradrenergic, serotonergic, and cholinergic systems (Braak, Del Tredici et al. 2003). The LC represents the largest group of brain noradrenergic neurons, which send widespread projections to forebrain areas and is the only source of NA in the hippocampus and neocortex (Berridge and Waterhouse 2003). NA has been closely linked to impulsivity (see Section 1.5.1.), and therefore it is likely that the decrease in NA also influences impulsivity in PD groups. In support of this, impairments in task set switching, which involves suppressing one set of criteria guiding behaviour to respond to another set of criteria, seems to reflect disease severity, rather than dopaminergic status (Kehagia, Cools et al. 2009). Furthermore, PD patients discount future rewards more steeply than controls, both on and off medications (Milenkova, Mohammadi et al. 2011).

DRT, which is used to treat motor symptoms, is also linked to impulsivity in PD. It is thought that DRT has an overdosing effect on the relatively intact ventral striatum, leading to cognitive impairment in some domains (Cools 2006). A minority of patients who are administered DRT develop ICBs, which can have a profound effect on everyday function (Lawrence, Blackwell et al. 2007; Potenza, Voon et al. 2007). ICBs include motor stereotypes, such as punding (repetitive, stereotypical, and mindless behaviour, e.g. collecting, arranging, or dismantling), appetitive behaviours, such as hypersexuality, pathological gambling (PG), compulsive shopping, and binge eating, (Voon and Fox 2007), as well as the compulsive use of excessive DRT, termed ‘Dopamine Dysregulation Syndrome’ (DDS) (Lawrence, Evans et al. 2003). Patients with DDS show greatly enhanced drug-induced release of dopamine in the ventral striatum (Evans, Pavese et al. 2006), a finding also seen in PD patients with pathological gambling during decision making (Steeves, Miyasaki et al. 2009). It has been suggested that increased impulsivity is a central feature of ICBs, rather than the overvaluation of rewards (Housden, O’Sullivan et al. 2010). Patients with ICBs display increased impulsivity on delay discounting tasks, compared to those without ICBs (Housden, O’Sullivan et al. 2010; Voon, Reynolds et al. 2010).

Increased impulsivity is also seen in PD patients without ICBs. On an action selection task where participants are asked to make a free choice on which button to press, PD patients fail to inhibit repetitious moves, compared to healthy controls who avoid making the same action twice. This effect was found both ‘On’ and ‘Off’ medications, although patients ‘On’ dopaminergic therapy showed more perseverative behaviour than those ‘Off’ (Hughes, Barker et al. 2010). This mirrors the findings on the random number generation task, where PD patients are unable to suppress habitual counting behaviour, compared to controls who are able to produce random numbers (Dirnberger, Frith et al. 2005; Obeso, Wilkinson et al. 2011). Furthermore, PD patients on medications are significantly worse at inhibiting a proponent motor response on the stop signal task (SST) compared to controls. This effect was not related to general slowing, and was independent of global cognitive impairment and severity of PD (Gauggel, Rieger et al. 2004). The use of other tasks requiring inhibition of proponent responses also supports
impairment in inhibitory processes in PD, for example the Go No-Go tasks (Cooper, Sagar et al. 1994; Beste, Willemsen et al. 2010; Baglio, Blasi et al. 2011), anti-saccade (Rivaud-Pechoux, Vidailhet et al. 2007), flanker (Praamstra and Plat 2001; Wylie, Stout et al. 2005; Wylie, van den Wildenberg et al. 2009), Hayling (Bouquet, Bonnaud et al. 2003) and Stroop (Obeso, Wilkinson et al. 2011). There is evidence to suggest that response inhibition impairments may be related to the disease process, rather than a medication effect, as PD patients show significantly worse response initiation and inhibition both ‘on’ and ‘off’ levodopa medication, compared to healthy controls (Obeso, Wilkinson et al. 2011). This converges with findings in the animal version of the SST, where increasing DA availability by blocking its reuptake (Bari, Eagle et al. 2009) or by L-Dopa administration (Overtoom, Verbaten et al. 2003) does not affect Stop Signal Reaction Time (SSRT) performance in rats, although intrastriatal DA agonists do influence Go reaction time on the SST (Eagle, Wong et al. 2011). Evidence from animal studies instead suggests an important contribution of NA in modulating prefrontal areas during response inhibition (Robbins and Arnsten 2009; Bari, Mar et al. 2011).

Other facets of impulsivity include delay aversion and reflection impulsivity. The Cambridge Gamble Task (CGT) measures decision making under risk, and, importantly, also dissociates risk taking from impulsivity (Manes, Sahakian et al. 2002). The impulsivity measures include deliberation time and delay aversion. On the CGT, PD patients are more delay averse when ‘On’ their dopaminergic medications, as compared to ‘Off’ medications (Cools, Barker et al. 2003). Reflection impulsivity is the tendency to gather information before making a decision (Kagan 1966). Using the beads task, Djamshidian, O’Sullivan et al. (2012) have shown that patients with ICBs ‘jump to conclusions’ and sample less information than PD patients without ICBs in both fixed and costly conditions of the task. In the present study, reflection impulsivity was indexed using the Information Sampling Task (IST) (Clark, Robbins et al. 2006). Both former and current opiate and amphetamine users display significantly reduced information sampling on this task (Clark, Robbins et al. 2006). It is currently unclear whether tests of reflection impulsivity load primarily on the risk-taking dimension of impulsivity, or on response inhibition, as illustrated by impulsivity factor analysis (Meda, Stevens et al. 2009).

Given the degeneration in the LC, and the close link between NA and impulsivity, a drug with a noradrenergic action would be an ideal candidate for remediation of impulsivity in PD. Atomoxetine is a NA reuptake inhibitor that increases NA synaptic levels in the prefrontal cortex. Recently it has been shown that atomoxetine increases the phasic-to-tonic ratio of LC-evoked responses (Bari & Aston-Jones, 2012), which may effectively enhance LC signalling temporally linked to sensory events in downstream target areas. Atomoxetine also increases DA in the prefrontal cortex, via its effects on the NA transporter in the pre-frontal cortex (PFC) (Bymaster, Katner et al. 2002). Atomoxetine has been shown to improve response inhibition in healthy adults, and has been shown to be an effective treatment for impulsivity in patients with Attention Deficit Hyperactivity Disorder (ADHD) (Chamberlain, Del Campo et al. 2007; Chamberlain, Hampshire et al. 2009). In animal models, atomoxetine decreases impulsivity in high impulsive rats (Fernando, Economidou et al. 2012), and demonstrates better efficacy than placebo in
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preventing relapse to drug (Economomidou, Dalley et al. 2011). Atomoxetine reduces impulsivity in rats on the SST, on the 5-choice serial reaction time (5-CSRT) task and on a delay discounting task (Robinson, Eagle et al. 2008). In PD, atomoxetine has been shown to improve questionnaire rated executive function in twelve patients, in an 8 week open-label, flexible-dose trial (Marsh, Biglan et al. 2009). Atomoxetine has also been well tolerated by PD patients in two other studies (Jankovic 2009), one of which showed that the drug improved Mini Mental State Examination (MMSE) and sleepiness ratings (Weintraub, Mavandadi et al. 2010).

Therefore, a double-blind, placebo-controlled study was conducted to investigate the effects of a single dose of atomoxetine on impulsivity in PD patients without ICBs. A range of impulsivity measures were used: response inhibition, reflection impulsivity, and delay aversion. Patients stayed on their usual medications for the duration of the study, and a within-subject design was used to account for the heterogeneity of the population. It was hypothesized that atomoxetine would reduce impulsivity.

5.2. Materials and Methods

5.2.1. Patients and Procedures

Twenty-five participants with a diagnosis of PD were recruited through the Brain Repair Centre, School of Clinical Medicine, University of Cambridge. Exclusion criteria were: a history of neurological disorder other than PD; current psychiatric comorbidity; history of stroke or brain damage; anti-cholinergic or noradrenergic medications; uncontrolled hypertension; colour blindness; glaucoma; scoring in the dementing range (<23) on the MMSE at prior clinical assessment. The participants had a mean age of 64, an average IQ score of 115, as indexed by the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001), and a mean of 20 years of full time education (see Table 5.1). The study was approved by the Cambridge Local Research Ethics Committee (ref: 09/H0302/84) and written informed consent was given by all participants prior to testing.

All participants were tested on their best ‘On’; average levodopa equivalent units (LEU) are displayed in Table 5.1. As it was expected that atomoxetine would only be used clinically as an adjunctive treatment, all participants remained on their current medications for the duration of the study. Participants were screened with the South Oaks Gambling Screen (Lesieur and Blume 1987), the Mini-International Neuropsychiatric Interview (Sheehan, Lecrubier et al. 1998), and the Minnesota Impulse Disorders Interview (Christenson, Faber et al. 1994). Six participants reported past visual hallucinations which had disappeared after their medication was adjusted. None of the participants reported behaviour that was indicative of an Impulse Control Disorder.

A randomized double-blind, placebo-controlled, crossover design was used, with twelve participants randomized to receive a single oral dose of a lactose placebo on the first session followed by 40mg of atomoxetine on the second session (the P/A group) and thirteen participants randomized to receive drug first, followed by placebo (the A/P group). For each patient, testing sessions were separated by at least
five days (average = 10.16±4.6). Both groups were matched for age, IQ, education level, disease severity as indexed by the Unified Parkinson’s Disease Rating Scale (UPDRS) motor subscale, and LEU (F_{1,23}=2.73, P≥0.113) (Table 5.1).

A dose of 40mg was used, as there have only been three studies using atomoxetine in PD, all using a starting dose of 40mg or lower (Jankovic 2009; Marsh, Biglan et al. 2009; Weintraub, Mavandadi et al. 2010). In healthy volunteers, 40mg of atomoxetine has been shown to improve inhibitory control and increase activation in the right inferior frontal gyrus (Chamberlain, Hampshire et al. 2009). Furthermore, unpleasant subjective feelings of “sickness” and “badness” increase with larger acute doses (90mg) (Heil, Holmes et al. 2002). This dose could be considered to be conservative, compared to studies in healthy adults and adults with ADHD, which tend to use a dose of 60mg (Heil, Holmes et al. 2002; Chamberlain, Muller et al. 2006; Gilbert, Ridel et al. 2006; Chamberlain, Del Campo et al. 2007; Chamberlain, Muller et al. 2007). As peak plasma concentrations of atomoxetine have been shown to be approximately 1-2 hours following oral dosing (Sauer, Ring et al. 2005), participants spent 1.5 hours resting in a quiet room, before undertaking the neuropsychological tests which took approximately 2.5 hours.

5.2.2. PHYSIOLOGICAL MEASURES

Blood pressure and pulse measurements were taken at three time points: before drug administration, immediately prior to testing (1.5 hours post-drug), and on completion of the study (4 hours post-drug). Two blood samples were also taken: one immediately prior to testing (1.5 hours post-drug), and one on completion of the study (4 hours post-drug). These samples were used to estimate the drug plasma concentration for each participant during each session.

5.2.3. SUBJECTIVE MEASURES

Patients were asked to complete visual analogue scales before administration of the drug, and at intervals during the testing session: immediately prior to testing, halfway through the cognitive testing session, and on completion of testing. At each time point patients were asked to rate their feelings in terms of sixteen dimensions, as detailed in Section 2.3.1.3.
### Table 5.1 Patient demographics

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<td><strong>Years of Education</strong></td>
<td>14.31 (3.2)</td>
<td>14.58 (2.5)</td>
<td>0.06</td>
<td>0.813</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>28.62 (0.96)</td>
<td>28.75 (13)</td>
<td>0.09</td>
<td>0.769</td>
</tr>
<tr>
<td><strong>IQ</strong></td>
<td>105.3 (8.9)</td>
<td>106.7 (6.2)</td>
<td>0.19</td>
<td>0.667</td>
</tr>
<tr>
<td><strong>UPDRS (motor)</strong></td>
<td>26.38 (13.7)</td>
<td>17.18 (13.47)</td>
<td>2.73</td>
<td>0.113</td>
</tr>
<tr>
<td><strong>LEU (mg)</strong></td>
<td>1010.4 (524.45)</td>
<td>1311.5 (741.51)</td>
<td>1.39</td>
<td>0.250</td>
</tr>
<tr>
<td><strong>BDI</strong></td>
<td>7.75 (4.22)</td>
<td>7.17 (4.2)</td>
<td>0.12</td>
<td>0.738</td>
</tr>
<tr>
<td><strong>ESS</strong></td>
<td>9.75 (4.47)</td>
<td>11.08 (3.96)</td>
<td>0.60</td>
<td>0.448</td>
</tr>
<tr>
<td><strong>Verbal fluency</strong></td>
<td>51.07 (16.6)</td>
<td>54 (11.8)</td>
<td>0.26</td>
<td>0.619</td>
</tr>
<tr>
<td><strong>Semantic fluency</strong></td>
<td>19.76 (3.47)</td>
<td>21.33 (5.16)</td>
<td>0.80</td>
<td>0.379</td>
</tr>
<tr>
<td><strong>STAI State</strong></td>
<td>12.38 (6.63)</td>
<td>9.92 (8.13)</td>
<td>0.69</td>
<td>0.412</td>
</tr>
<tr>
<td><strong>STAI trait</strong></td>
<td>15.77 (6.10)</td>
<td>14.08 (11.30)</td>
<td>0.22</td>
<td>0.643</td>
</tr>
</tbody>
</table>

Values shown are the mean and standard deviations for each group. Education level is in years of formal education. MMSE is the score on the Folstein Mini Mental State Examination. IQ is the predicted verbal IQ score form the Wechsler Test of Adult Reading. UPDRS (motor) is the motor subscale of the Unified Parkinson’s Disease Rating Scale. LEU (mg) is the Levodopa equivalent daily dose of dopaminergic therapy in mg = levodopa (x 1.2 if COMT inhibitor (x1.2 if 10mg selegiline or x 1.1 if 5mg selegiline)) + [pramipexole x 400] + [ropinirole x 40] + [cabergoline x 160] + [ pergolide x 200] + [bromocriptine x 10] + [lisuride x 160]. BDI is the score on the Beck’s Depression Scale (Beck, Ward et al. 1961), ESS is the score on the Epworth Sleepiness Scale (Johns 1991). Verbal fluency is the number of words produced on the Controlled Oral Word Association Test (COWAT) FAS scale (Benton 1976). Semantic fluency is the number of words produced on the COWAT semantic scale, STAI State is the score on the State section of the State/Trait Anxiety Inventory (Spielberger, Gorsuch et al.), and STAU trait is the score on the trait section.

#### 5.2.4. Neuropsychological measures

Patients were tested on a neuropsychological test battery including tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB). Three tasks were used to measure different forms of impulsivity: the SSRT to measure response inhibition, the Cambridge Gamble Task (CGT) to measure delay aversion and deliberation time, and the IST to measure reflection impulsivity. The CGT and the IST both had parallel versions to allow the task conditions (ascend and descend; fixed win and decreasing win) to be counter-balanced. The order of the administration of these versions was counter-balanced.
across the atomoxetine/placebo and placebo/atomoxetine groups. All computerised tasks were run on a Paceblade touch screen computer and responses were registered either via the touch-sensitive screen or a button box. A brief description of the tasks is presented in Table 5.2, with fuller descriptions in Chapter 2.

5.2.5. Plasma atomoxetine analysis

Plasma levels of atomoxetine were analysed in all the pre- and post-scan active treatment samples obtained, with a high performance liquid chromatographic method with diode array detection on Agilent 1100 series chromatographic system (Agilent Technologies GmbH, Waldbronn, Germany). Blood samples were collected from all of the participants on the drug visit, meaning that a total of 25 x 2 samples were available for analysis. Separation of atomoxetine and mianserin (internal standard) was performed on an Agilent Zorbax Eclipse XDB C8 reversed phase column after sample preparation by liquid/liquid extraction. Detection wavelength was 218 nm, and the limit of quantitation was 2.0 g/L. The calibration function was linear within a range from 2 to 500 g/L (coefficient of correlation .995). Intra- and inter-day coefficients of variation were below 8.0%.

5.2.6. Cognitive data analysis

This study used a crossover within-subjects design. Full details of the statistics used are provided in Chapter 2.
<table>
<thead>
<tr>
<th>Cognitive task</th>
<th>Description</th>
<th>References</th>
<th>Important Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>A test of response inhibition. Participants make speeded left or right responses on Go trials but withhold their response on Stop trials (signalled by a 300Hz tone). Race model allows estimation of the time taken to internally suppress a Go response (SSRT).</td>
<td>Aron, Fletcher et al. (2003), Logan, Cowan et al. (1984)</td>
<td>SSRT (a measure of response inhibition), Go reaction time (ms)</td>
</tr>
<tr>
<td>CGT</td>
<td>A decision making task where participants decide whether a randomly hidden token is more likely to be in a red or blue box (the ratio of which varies within a display of ten boxes). They then place bets (in ascending and descending order) on their choice being correct.</td>
<td>Rahman et al. 2001</td>
<td>Deliberation time (time taken to make a probability decision), Delay aversion (difference in bets between the ascend and descend condition)</td>
</tr>
<tr>
<td>IST</td>
<td>A decision-making task where participants are presented with a 5 x 5 matrix of grey boxes which reveal themselves to be one of two colours when they are touched. The participant has to decide which colour is in the majority by opening as many boxes as they like in order to win points. In the fixed condition, 100 points are given for a correct response, in the descending condition, 250 points are available to begin with, but this decreases by 10 points for every box opened.</td>
<td>Clark et al 2006</td>
<td>Number of boxes opened per trial (for both fixed and decreasing condition), Probability of being correct at the time of decision (for both fixed and decreasing conditions)</td>
</tr>
</tbody>
</table>

SST is stop-signal task, SSRT is stop-signal reaction time, CGT is Cambridge gamble task, and IST is information sampling task.
5.3. **Results**

5.3.1. **Physiological effects**

Atomoxetine was generally well-tolerated at the 40mg dose, although some side effects were reported. Side effects associated with the drug visit were: feeling more emotional and tearful (N=2) and headache during the testing session (N=1). Side effects associated with the placebo visit were: raised blood pressure at the end of the testing session (N=1), and the development of an ear infection two days later (N=1).

Plasma levels of atomoxetine (average of pre- and post-testing values) were 308.9 ± 121.2 ng/mL, range 72.6 – 560.15, during active treatment and confirmed correct randomization (Table 5.3). There was a large variability in drug plasma concentrations, accompanied by a large difference in peak plasma time across participants (see Table 5.3). Notably, the drug plasma levels increased from sample one to sample two in seven participants, but decreased in the remaining eighteen.

5.3.2. **Neuropsychological effects**

The overall scores for the main behavioural measures, both in the placebo and atomoxetine condition, are displayed in Table 5.4. The between-subjects variability in plasma concentration was taken into account by regressing the change in performance (difference between the placebo and drug conditions) onto the drug plasma concentration for the atomoxetine visit. For each cognitive test, the blood sample that was taken nearest in time to that test was used. For the SST, the mean of the first and second blood sample was used as the test was halfway through the battery, and for IST and CGT the second sample was used. For the blood sample of interest, participants with a drug blood plasma level below 100 ng/mL were excluded from the analysis, as this concentration was considered to be too low to have an effect on cognition. For each analysis, the independent variable was drug plasma level, and the dependent variable was the cognitive difference score. Order of drug administration, UPDRS motor and LEU were initially added as independent variables, but as they did not contribute to the model they were not included in the final analysis.
### Table 5.3 Drug Blood Plasma Concentrations

<table>
<thead>
<tr>
<th>Participant</th>
<th>Sample 1 (1.5 hours)</th>
<th>Sample 2 (4 hours)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>575.2</td>
<td>324.3</td>
<td>449.75</td>
</tr>
<tr>
<td>2</td>
<td>n.d</td>
<td>291.2</td>
<td>145.6</td>
</tr>
<tr>
<td>3</td>
<td>77.5</td>
<td>317.1</td>
<td>197.3</td>
</tr>
<tr>
<td>4</td>
<td>45.3</td>
<td>146.8</td>
<td>96.05</td>
</tr>
<tr>
<td>5</td>
<td>604.7</td>
<td>188.3</td>
<td>396.5</td>
</tr>
<tr>
<td>6</td>
<td>n.d</td>
<td>72.6</td>
<td>36.3</td>
</tr>
<tr>
<td>7</td>
<td>190.4</td>
<td>368.2</td>
<td>279.3</td>
</tr>
<tr>
<td>8</td>
<td>489.7</td>
<td>267.1</td>
<td>378.4</td>
</tr>
<tr>
<td>9</td>
<td>424</td>
<td>133.1</td>
<td>278.55</td>
</tr>
<tr>
<td>10</td>
<td>189.4</td>
<td>277.1</td>
<td>233.25</td>
</tr>
<tr>
<td>11</td>
<td>409.7</td>
<td>239</td>
<td>324.35</td>
</tr>
<tr>
<td>12</td>
<td>650</td>
<td>344.8</td>
<td>497.4</td>
</tr>
<tr>
<td>13</td>
<td>436.4</td>
<td>131.3</td>
<td>283.85</td>
</tr>
<tr>
<td>14</td>
<td>106.1</td>
<td>590.3</td>
<td>348.2</td>
</tr>
<tr>
<td>15</td>
<td>523.9</td>
<td>264.5</td>
<td>394.2</td>
</tr>
<tr>
<td>16</td>
<td>502.6</td>
<td>229.2</td>
<td>365.9</td>
</tr>
<tr>
<td>17</td>
<td>412.9</td>
<td>135</td>
<td>273.95</td>
</tr>
<tr>
<td>18</td>
<td>346</td>
<td>330.4</td>
<td>338.2</td>
</tr>
<tr>
<td>19</td>
<td>463.7</td>
<td>131.6</td>
<td>297.65</td>
</tr>
<tr>
<td>20</td>
<td>253</td>
<td>156.1</td>
<td>204.55</td>
</tr>
<tr>
<td>21</td>
<td>454.1</td>
<td>320.9</td>
<td>387.5</td>
</tr>
<tr>
<td>22</td>
<td>551</td>
<td>130.6</td>
<td>340.8</td>
</tr>
<tr>
<td>23</td>
<td>312.7</td>
<td>91.8</td>
<td>202.25</td>
</tr>
<tr>
<td>24</td>
<td>550.7</td>
<td>276.1</td>
<td>413.4</td>
</tr>
<tr>
<td>25</td>
<td>723.8</td>
<td>396.5</td>
<td>560.15</td>
</tr>
</tbody>
</table>

Plasma levels of atomoxetine are shown in ng/ml. Atomoxetine was not detected (n.d.) in the first sample for two participants. Sample 1 is the first blood sample collected on the active drug visit, at the start of the cognitive testing, 1.5 hours after drug.
administration. Sample 2 is the second blood sample collected on the active drug visit, at the end of the testing session, which was 4 hours after drug administration.

<table>
<thead>
<tr>
<th>Cognitive Task</th>
<th>Measure</th>
<th>Atomoxetine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>SSRT (ms)</td>
<td>237.47 (17.3)</td>
<td>236.76 (15.07)</td>
</tr>
<tr>
<td></td>
<td>Go reaction time (ms)</td>
<td>458.31 (22.4)</td>
<td>436.38 (14.5)</td>
</tr>
<tr>
<td>CGT</td>
<td>Deliberation time (ms; ascending condition)</td>
<td>2875.99 (201.6)</td>
<td>2617.95 (151.9)</td>
</tr>
<tr>
<td></td>
<td>Deliberation time (ms; descending condition)</td>
<td>2743.11 (213.9)</td>
<td>2643.07 (163.2)</td>
</tr>
<tr>
<td></td>
<td>Delay aversion (sec)</td>
<td>0.228 (0.036)</td>
<td>0.25 (0.041)</td>
</tr>
<tr>
<td>IST</td>
<td>Number of boxes opened (fixed-win condition)</td>
<td>14.02 (1.48)</td>
<td>13.52 (1.56)</td>
</tr>
<tr>
<td></td>
<td>Number of boxes opened (decreasing-win condition)</td>
<td>9.40 (1.14)</td>
<td>9.06 (1.15)</td>
</tr>
<tr>
<td></td>
<td>Probability of being correct (fixed-win condition)</td>
<td>0.80 (0.03)</td>
<td>0.80 (0.03)</td>
</tr>
<tr>
<td></td>
<td>Probability of being correct (decreasing-win condition)</td>
<td>0.72 (0.03)</td>
<td>0.71 (0.02)</td>
</tr>
</tbody>
</table>

The abbreviations are as for Table 5.2. The values shown are given as mean (standard error of the mean).
Since one participant did not complete the SST, and two participants had a mean blood plasma concentration that was below 100 ng/mL, data from 22 participants was analysed. Blood plasma level significantly predicted the change in SSRT on drug compared to placebo $\beta = 0.509$, $t_{(21)} = 4.06$, $p=0.001$. Blood plasma level also explained a significant proportion of the variance for the SSRT difference score, $R^2 = 0.452$, adjusted $R^2 = 0.424$, $F_{(1,21)} = 16.481$, $p=0.001$ (Figure 5.1). No significant relationships were seen between drug plasma level and direction errors, Median Go reaction time, or Stop signal delay.

**Figure 5.1 Effect of atomoxetine on stop signal reaction time**

There was a significant relationship between drug plasma level and change in SSRT when on drug compared to placebo.
Two participants did not complete the CGT, and two participants had drug concentration of less than 100 ng/mL in their second blood plasma sample. Regression analyses were carried out using the remaining 21 participants. There was a trend towards blood plasma level predicting the change in deliberation time (in the ascend condition) on drug compared to placebo \( \beta = -3.33, t_{(20)} = -2.074, p = 0.052 \). There was also a trend towards blood plasma level explaining a proportion of the variance in deliberation time (ascend) difference score \( (R^2 = 0.185, \text{adjusted } R^2 = 0.142, F_{(1,21)} = 4.3, p = 0.052; \text{Figure 5.2}) \). No significant relationships were seen between drug plasma level and deliberation time in the descending condition, delay aversion, overall proportional bet, quality of decision making, risk adjustment, or risk taking.

**Figure 5.2  Effect of atomoxetine on CGT deliberation time (ascending condition)**

There was a trend towards a relationship between drug plasma level and change in deliberation time (in the ascend condition) when on drug compared to placebo.

The IST was the last cognitive task in the battery. As a result of this, five participants did not complete the task as they were fatigued. Two further participants were excluded as they had a drug concentration of less than 100 ng/mL in their second blood plasma sample. In the remaining 18 participants, there was a significant relationship between the difference score for the number of boxes opened per trial (fixed win condition), and drug plasma level \( (R^2 = 0.294, \text{adjusted } R^2 = 0.249, F_{(1,17)} = 6.65, p = 0.02; \text{Figure 5.3a}) \). Furthermore, there was a significant relationship between the difference score for the probability of
getting the problem correct at the time of making a decision (fixed win condition) and drug plasma level \( (R^2 = 0.324, \text{adjusted } R^2 = 0.281, F_{(1,17)} = 7.658, p = 0.014; \text{Figure 5.3b}) \). No significant relationships were seen between drug plasma level and discrimination errors, box opening latency, colour decision latency, sampling errors, and total correct for both the fixed win and decreasing win conditions, and number of boxes opened per trial and probability of getting the problem correct for just the decreasing win condition.

**Figure 5.3 Effect of Atomoxetine on the Information Sampling Task**

There was a significant relationship between drug plasma level and change in the number of boxes opened (a), and the probability of getting the problems correct (b), in the fixed win condition.
5.4. Discussion

In the present study, atomoxetine did not have an overall effect on impulsivity in PD patients, as predicted. However, secondary analysis showed that low levels of atomoxetine in the blood plasma samples were associated with improved response inhibition on the SSRT task, and reduced reflection impulsivity on the IST, and there was also a trend towards increased deliberation time before making a decision on the CGT. Conversely, higher drug levels in the blood plasma samples were associated with longer SSRTs, increased reflection impulsivity on the IST, and reduced deliberation time on the CGT. Thus, overall, low blood plasma concentrations of atomoxetine reduced impulsivity in the PD group, but high blood plasma increased impulsivity relative to baseline.

Consistent with the present results, several studies have shown that atomoxetine dose-dependently improves SSRT in both rats (Robinson, Eagle et al. 2008) and humans (Chamberlain, Muller et al. 2006). Furthermore, atomoxetine infusions into the orbitofrontal cortex and dorsal prelimbic cortex speeds SSRT, but does not improve reaction time on Go trials (Bari, Mar et al. 2011). These brain areas have bidirectional connections with the LC and may exert top-down control on the release of NA in forebrain areas, facilitating the influence of cognitive processes on behavioural output. In the PD patients who improved on drug, the beneficial effects of atomoxetine may have been due to LC neurons becoming more responsive to the Stop signal, which cues an interruption to on-going behaviour (Aston-Jones and Cohen 2005; Dayan and Yu 2006). Another mechanism by which atomoxetine may improve response inhibition on the SST is by enhancing the efficacy of frontal-basal ganglia networks for inhibitory control (Eagle and Baunez 2010), which are implicated in response inhibition in rats and humans (Eagle and Robbins 2003; Zandbelt and Vink 2010). Both of these processes would facilitate improvement in inhibiting the Go response in the presence of the Stop signal. Atomoxetine has been shown to increase dopamine in the prefrontal cortex (Bymaster, Katner et al. 2002). However, it is unlikely that dopamine effects were responsible for the changes in SSRT, as blocking dopamine receptors using the D1/D2 antagonist α-flupenthixol prolongs Go reaction time only, whereas infusions of the α2-adrenergic guanfacine selectively impairs stopping (Bari, Mar et al. 2011).

Atomoxetine also had an inverted-U-effect on reflection impulsivity, although this effect was not as strong as for SSRT. On the IST, PD patients with low plasma levels opened more boxes before making their decision, but only in the fixed win condition when there was no cost to sampling information, whereas PD patients with high plasma levels opened fewer boxes in the fixed win condition compared to placebo. The same relationship was seen for the probability that the participant would get the problem correct at the time of decision making, as this is related to the number of boxes they opened. Reflection impulsivity has received little research attention to date, so this is the first study to examine the effects of manipulating NA on this dimension of impulsivity in humans. Our findings indicate that NA may play a role in reflection impulsivity, but only when there are no local costs involved in sampling information. It could be that the
improvements in response inhibition underlie the increase in number of boxes opened on the fixed win trials, as factor analysis of reflection impulsivity shows that it loads on the response-inhibition dimension of impulsivity, as well as the risk-taking dimension (Meda, Stevens et al. 2009). In contrast to the finding in the present study, it has been shown that 5-HT manipulation in healthy humans affects the costly, but not the fixed win, conditions (Crockett, Clark et al. 2012). Tryptophan depletion led to participants sampling more information in the costly condition, suggesting that 5-HT promotes the avoidance of immediate aversive outcomes. There was also a trend towards an inverted-U-shaped relationship between blood plasma level and deliberation time on the CGT. It has been suggested that rapid decision making on the CGT is a component of reflection impulsivity (Deakin, Aitken et al. 2004), and therefore the findings on the IST and CGT may share the same underlying process. We did not find an effect of drug on delay aversion on the CGT, suggesting that this form of impulsivity is not modulated by NA.

Improvements were only seen in PD patients who had a low blood plasma level of drug. It is well known that NA has an inverted-U-effect on the prefrontal cortex, whereby either too little or too much impairs function (Arnsten and Jentsch 1997; Arnsten, Mathew et al. 1999). For example, in monkeys performing an oculomotor delayed response spatial working memory task, neurons with relatively low levels of memory-related firing under control conditions show enhanced firing after the iontophoresis of a low dose of atomoxetine, but a high dose of atomoxetine suppresses neuronal firing (Gamo, Wang et al. 2010). An inverted-U-effect is also found on extra-dimensional set-shifting in rats, whereby atomoxetine remediates the impairments induced by deafferentation of the medial prefrontal cortex, but produces detrimental effects in non-lesioned rats whose performance was nearly at optimal levels before administration of the drug (Newman, Darling et al. 2008). This effect may be driven by the fact that postsynaptic α1 and α2 receptors have an opposing role in the PFC. NA has a higher affinity for the α2 adrenergic receptors than the α1 or β subtypes (Arnsten 2000), so the type of receptor engaged is determined by the amount of NA available. Therefore, α2 mechanisms may prevail when NA availability is moderate, producing optimal prefrontal cortex function, whereas α1 mechanisms may predominate under conditions of high levels of NA release, contributing to PFC dysfunction. This pattern has been found for working memory performance (Arnsten and Goldman-Rakic 1985; Li and Mei 1994; Birnbaum, Gobeske et al. 1999), with blockade of α2 (Li and Mei 1994) or infusion of an α1 agonist impairing performance (Mao, Arnsten et al. 1999). It is less clear how the inverted-U-response works for response inhibition as it has recently been shown in rats that SSRT is mediated by NA binding to β-receptors, suggesting that a higher level of NA is optimal for this cognitive function (Eagle and Robbins, unpublished).

Interestingly, this inverted-U-effect has not previously been found for SSRT at either 40mg or 60mg of atomoxetine, either in healthy young adults, or in young adults with ADHD (Chamberlain, Muller et al. 2006; Chamberlain, Del Campo et al. 2007; Chamberlain, Hampshire et al. 2009). Impairment in the PD patients at a lower dose than younger, healthy adults could be due to the effect of the disease process, or could be part of normal ageing. It is also plausible that the patients in our study found the testing
experience more stressful than younger adults, thus increasing their tonic NA levels (Aston-Jones, Rajkowski et al. 1999). Determining the optimal dose of atomoxetine for individuals may be difficult, as it would depend on environmental demands, as NA neurons change their firing rate according to arousal state and relevance of events in the environment. For example, NA neurons in the LC have moderate tonic firing and pronounced phasic firing to relevant stimuli during non-stressed waking, but high tonic firing and dysregulated phasic firing during stress (Aston-Jones, Rajkowski et al. 1999). Other factors that may affect baseline NA levels, and therefore dosing of atomoxetine, include disease progress, age, genetics, and personality. It may also be the case that optimal levels of NA required for one aspect of cognition, such as response inhibition, may not be the same as for another, such as working memory. It should also be considered that late in the disease progress, when there is widespread degeneration in the LC, atomoxetine may not produce improvements as even inhibition of the reuptake of available NA may still leave levels of NA sub-optimally low.

In order to investigate whether atomoxetine is a suitable treatment for PD patients with ICBs, this study should be extended to include a group of ICB patients. It would also be interesting to include a group of healthy, age-matched participants, and to test the PD patients both at their best ‘On’ as well as ‘Off’ their dopaminergic medications. This would help to tease apart the age, disease, and medication influences on the drug effect. The conclusions that can be drawn from this study are also potentially limited by the fact that PD patients who are willing to take part in research are often well-medicated and high functioning, possibly making our group less representative of the PD population as a whole.

In summary, atomoxetine was well-tolerated by PD patients, and improved performance on response inhibition, but only in patients who had a low drug plasma concentration. This suggests that at the right dose, atomoxetine may be a potential treatment for impulsivity in the group. Atomoxetine also had marginal effects on reflection impulsivity, which need to be investigated further. However, the fact that atomoxetine can modulate a range of impulsive behaviours makes it an ideal candidate as a general inhibition-improving treatment. These preliminary findings should be extended and replicated before atomoxetine is considered as a suitable treatment for impulsivity in PD.
6. MODAFINIL IN SLEEP-DEPRIVED DOCTORS

6.1. INTRODUCTION

The detrimental effect of sleep deprivation on cognition is well-established (see Section 1.3.4. for a detailed discussion). In certain situations where there is a small margin for error, sub-optimal cognitive functioning can have dangerous consequences for both the individual and others: e.g. drivers, pilots, air-traffic controllers, military, and doctors. A meta-analytic study of sleep loss and performance literature from 1971 to 2003 concluded that sleep loss of less than 30 hours reduces doctors’ overall performance (Philibert 2005). Indeed, fatigued doctors have been shown to make poor judgments and to commit serious medical errors (Landrigan, Rothschild et al. 2004).

The most effective way of avoiding cognitive impairment related to sleep deprivation is restorative sleep which could be facilitated by reducing working hours, and by ensuring that the recommended eight hours of sleep are taken each day. Restrictions on working hours for doctors in training have been implemented: the extension of the European Working Time Directive in 2009 reduced their maximum weekly working hours to 48. Observational studies following the capping of working hours have produced mixed findings (Fletcher, Davis et al. 2004), with some studies reporting no change in the standard of patient care (McIntyre, Winfield et al. 2010). In contrast, a review of surgical service found a reduction in mortality, which was thought to be related to decreased doctor fatigue (Privette, Shackford et al. 2009). The findings from observational studies such as these may be clouded by the fact that violations of working hour rules are common (Landrigan, Rothschild et al. 2004). Nonetheless, capping working hours may not be adequate to address fully the sleep deficits and resulting impairments reported by doctors, as suggested by a survey of 3,604 house officers (Baldwin and Daugherty 2004). Rotating shift patterns can
also cause sleep disruption which is thought to be related to increases in medical errors (Chang, Wu et al. 2011).

Currently, caffeine is the stimulant of choice for the medical profession to counteract the effects of fatigue (Bonnet, Balkin et al. 2005). Caffeine is widely available, effective, and in common use. However, at the dose required for maximum effect (around 600 mg), common side effects include anxiety, nausea, and tremor, which are particularly undesirable in surgical situations where fine motor movements are vital (Nawrot, Jordan et al. 2003). Other professions, such as the military, have investigated the use of alternative pharmacological agents to keep individuals alert until a safe opportunity for sleep presents itself (Caldwell and Caldwell 2005; Eliyahu, Berlin et al. 2007; Gore, Webb et al. 2010). It has been suggested that the medical profession should also explore the possibility of the use of alternative pharmacological treatments for sleep deprivation (Nelson 2007; Sugden, Aggarwal et al. 2010). One such agent is modafinil (PROVIGIL), a proven wake-promoting drug originally licensed for use in narcolepsy patients for the treatment of excessive daytime sleepiness. Modafinil has also been approved by the Food and Drug Administration for the treatment of excessive sleepiness associated with shift work sleep disorder and obstructive sleep apnea.

Modafinil is an interesting drug because it has been shown to have effects on several different chemical systems in the brain, including catecholamines, serotonin, glutamate, GABA, orexin and histamine (see Section 1.4.1 for a more detailed discussion), with its exact mechanism not yet agreed. It is commonly described as an ‘atypical stimulant’, because of its low abuse potential (Jasinski 2000; Jasinski and Kovacevic-Ristanovic 2000). Traditional psychostimulants, such as amphetamine and cocaine, block the uptake of monoamines such as noradrenaline (NA), dopamine and serotonin, promoting prolonged wakefulness and increasing both cortical activation and behavioural arousal (Monti and Monti 2007). There is strong evidence to suggest that modafinil’s arousing properties are also due to monoamine modulation, but in a relatively weak way (see Section 1.4.1). During sleep deprivation, dopaminergic projections to the nucleus accumbens are hypoactive, which limits the amount of sensory input to the cortex. Enhancing dopaminergic transmission using a drug such as modafinil may allow enough sensory input to reach the cortex so that wakefulness can occur. It also seems plausible that modafinil’s awakening effects could be due to the fact that it increases levels of noradrenaline, which is closely linked to arousal, in the cerebral cortex, frontal cortex, forebrain, medial hypothalamus, and striatum, through binding to the adrenergic α1-receptor, and also possibly through binding to noradrenergic transporters. Modafinil also activates orexin and histamine neurons in the hypothalamus which provide excitatory input to the ‘wake-promoting’ areas of the brain, including the locus coeruleus (LC), pedunculopontine tegmental nucleus, lateral dorsal tegmental nucleus, ventral tegmental area, basal forebrain, and tuberomammillary nucleus. Indeed, mice lacking orexin neuropeptides display sleep dysregulation that is similar in presentation to human and canine narcolepsy (Chemelli, Willie et al. 1999). However, modulation of orexin and of histamine systems is not necessary for modafinil’s arousing properties, as it still promotes wakefulness in knockout mice without hypothalamic orexin neurons (Willie, Renthal et al. 2001).
Modafinil in Sleep Deprived Doctors

2005). By decreasing levels of GABA in the posterior hypothalamus (Ferraro, Tanganelli et al. 1996; Ferraro, Antonelli et al. 1997; Ferraro, Antonelli et al. 1999; Scammell, Estabrooke et al. 2000) modafinil reduces the inhibition of these areas, and thus enhances glutamate transmission from the thalamus to the cortex, increasing wakefulness (Touret, Sallanon-Moulin et al. 1994; Ferraro, Antonelli et al. 1997). These changes to GABA and Glutamate levels can occur as a downstream effect of dopaminergic and noradrenergic modulation.

Increases in alertness and arousal following the administration of modafinil are well documented in narcolepsy patients, in those who are sleep-deprived, and in those with shift work sleep disorder (Mitler, Harsh et al. 2000; Becker, Schwartz et al. 2004; Czeisler, Walsh et al. 2005; Rosenthal, Majeroni et al. 2008). Furthermore, a single dose of modafinil significantly improves feelings of alertness and energy in postoperative patients (Larijani, Goldberg et al. 2004). Modafinil has also been shown to modulate attention in both sleep-deprived and non-sleep-deprived healthy humans and rats. This could be due to its effects on the noradrenergic system. For example, several studies of military personnel who have been sleep-deprived for at least 35 hours have used attentional tasks, like simple choice reaction time, mental arithmetic or critical flicker fusion, all of which have shown that doses above 100mg improve attentional functions as well as sleepiness (Lagarde, Batejat et al. 1995; Pigeau, Naitoh et al. 1995; Baranski and Pigeau 1997; Wesensten, Belenky et al. 2002). In a non-human primate model of sleep deprivation, modafinil was able to postpone the decline in vigilance and in coordinated motor movements (van Vliet, Jongsma et al. 2008). However, rats deprived of REM sleep did not show less of an impairment on the 5-choice serial reaction time (5-CSRT) task than those who received a placebo (Liu, Tung et al. 2011). In non-sleep-deprived states modafinil improves attentional set-shifting in animal models of cognitive impairment (Dawson, Thompson et al. 2010), as well as in human schizophrenic patients (Turner, Clark et al. 2004). However, there is a lack of data on the effects of modafinil on set-shifting in sleep-deprived humans. On the stop signal task (SST), modafinil decreases the stop signal reaction time (SSRT) in rats with a slow baseline SSRT, without effecting the Go reaction time (Eagle, Tufft et al. 2007), which converges with evidence that modafinil reduces SSRT in non-sleep-deprived healthy humans (Turner, Robbins et al. 2003), but this has not always been replicated (Winder-Rhodes, Chamberlain et al. 2010). In healthy, non-sleep-deprived populations, modafinil also enhances cognition on tests of spatial planning, visual recognition, and short term memory (Turner, Robbins et al. 2003; Muller, Steffenhagen et al. 2004), as well as spatial planning in ADHD patients (Turner, Clark et al. 2004). Overall, administration of modafinil leads to an increase in alertness and also improvement in cognitive domains, such as attention, that are impaired in the sleep-deprived state.

This study aimed to investigate the effect of modafinil on the cognitive abilities of sleep-deprived doctors, compared to sleep-deprived doctors who had taken a placebo. Attentional set-shifting, memory, planning, response inhibition, and decision-making skills (which included components of impulsivity and risk taking) were assessed using tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Sahakian and Owen 1992). The tasks measured cognitive skills that were expected to be...
impaired by sleep deprivation. These tasks also assessed areas of cognition that have the potential to be modulated by modafinil, so it was hypothesised that doctors in the modafinil group would perform better on the cognitive tests following sleep deprivation, compared to doctors who received the placebo. Furthermore, these areas of cognition are all of importance for medical skill.

6.2. MATERIALS AND METHODS

6.2.1. SUBJECTS AND PROCEDURES

Forty male resident doctors (See Table 6.1 for demographics) were recruited via email through their affiliation with St Mary’s Hospital or Imperial College London. Exclusion criteria were: history of psychiatric illness; visual, auditory or motor impairment; cardiac or neurological illness; scoring greater than 10 on the Epworth Sleepiness Scale (ESS); history of drug or alcohol addiction; drinking more than 8 cups of coffee a day; moderate to severe hypertension, angina or cardiac arrhythmias; the concurrent use of any medication contra-indicated with modafinil. The study was approved by Cambridgeshire Research Ethics Committee (Ref: 09/H0304/24), and site-specific approval was given by Imperial College London. Written informed consent was given by all participants prior to testing.

<table>
<thead>
<tr>
<th>Table 6.1</th>
<th>Participant demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.16 (0.77)</td>
</tr>
<tr>
<td>IQ (NART)</td>
<td>117.84 (0.90)</td>
</tr>
<tr>
<td>PGY</td>
<td>3.74 (0.49)</td>
</tr>
<tr>
<td>ESS</td>
<td>5.42 (0.67)</td>
</tr>
</tbody>
</table>

Values shown are the mean (standard error of the mean) for each group. NART is the predicted verbal IQ from the National Adult Reading Test, PGY is the number of post-graduate years, and ESS is the score on the Epworth sleepiness scale.

Participants reported to the researchers at 08:00 am on the study day and committed to remain awake for the duration of the day. They attended the study centre (St Mary’s Hospital, Praed Street, London) for the overnight session at 08:00 pm. Between this time and the conclusion of the study at 08:00 am the following morning, participants were awake in a designated room and supervised at all times by a member of the research team. Non-strenuous activities, including watching television, reading books, and playing video games, were permitted. A maximum of two participants took part in the study each night.

A double-blind, between-subjects design was utilised, with participants randomised to receive either a single oral dose 200mg of modafinil, which was encapsulated in a black gelatin capsule, or a placebo, which consisted of an identical black capsule without a tablet inside. Groups were well matched for age,
NART verbal IQ (as indexed by the National Adult Reading Test, (Nelson and O'Connell 1978), training level and baseline sleepiness (as indexed by the ESS) (Table 6.1). At 03:00 am a single dose of either modafinil or the placebo was given. The testing session began at 06:00 am, as peak plasma concentrations have been obtained 2-3 hours after oral administration (Wong, King et al. 1998), and lasted for approximately 2 hours. Participants were instructed not to work for 24 hours following the study session.

6.2.2. PHYSIOLOGICAL MEASURES

Blood pressure and pulse measurements were taken at four time points: before drug administration, immediately prior to testing (3 hours post-drug), 1 hour into testing (4 hours post-drug) and on completion of the study (5 hours post-drug).

6.2.3. SUBJECTIVE MEASURES

6.2.3.1. VISUAL ANALOGUE SCALES (VAS)

Subjects were asked to complete visual analogue scales (VAS) (Bond and Lader 1974) before administration of the drug (03:00 am) and at intervals during testing: immediately prior to testing (06:00 am), 1 hour into testing (07:00 am), and on completion of testing (08:00 am). At each time point, subjects were asked to rate their feelings in terms of sixteen dimensions, as detailed in Section 2.3.1.3.

6.2.3.2. QUESTIONNAIRES

The Epworth Sleepiness questionnaire (Lee, Hicks et al. 1991) was given at the time of recruitment to measure baseline sleepiness in participants. This questionnaire comprised a list of situations: sitting and reading; watching T.V; sitting inactive in a public place; as a passenger in a car for an hour without a break; lying down to rest in the afternoon; sitting and talking to someone; sitting quietly after lunch without alcohol; in a car, while stopped in traffic for a few minutes. Participants had to rate how likely they were to doze on a 4 point scale. The placebo and modafinil group were matched for baseline sleepiness as indexed by this questionnaire (see Table 6.1).

The Stanford Sleepiness scale (MacLean, Fekken et al. 1992) was given before the administration of the drug (03:00 am) and also immediately prior to testing (06:00 am). Participants were asked to indicate where they were on an 8 point alertness scale, which ranged from ‘feeling active, vital, alert or wide awake’ to ‘asleep’.

6.2.4. NEUROPSYCHOLOGICAL MEASURES

Participants were tested using well-validated tests from the CANTAB battery. All participants received the same tests in the same order. All computerised tasks were run on a Paceblade touch-screen computer, and responses were registered either via the touch-sensitive screen or a response key, depending on the
Table 6.2  Summary of neuropsychological tests

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
<th>Reference</th>
<th>Important Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>IED</td>
<td>Rule acquisition and reversal learning, testing the ability to attend selectively to and set shift between shape and colour stimulus.</td>
<td>Rogers, Blackshaw et al. (1999)</td>
<td>Stages Completed&lt;br&gt;Total Errors</td>
</tr>
<tr>
<td>OTS</td>
<td>A spatial planning test, involving planning a sequence of moves to make an arrangement of coloured balls to achieve a goal arrangement without moving the balls.</td>
<td>Dagher, Owen et al. (1999)</td>
<td>Mean latency to correct&lt;br&gt;Mean attempts to correct</td>
</tr>
<tr>
<td>SST</td>
<td>A test of response inhibition, involving speeded left or right responses to a Go stimulus, but this response should be withheld on trials where a Stop signal (300Hz tone) is presented. Race model allows estimation of the time taken to internally suppress a Go response (SSRT).</td>
<td>Aron, Fletcher et al. (2003)&lt;br&gt;Logan, Cowan et al. (1984)</td>
<td>Stop Signal Reaction Time</td>
</tr>
<tr>
<td>CGT</td>
<td>A decision-making task, involving deciding under which coloured box a token will be hidden. Points can be gained if the right choice is made.</td>
<td>Rahman, Robbins et al. (2006)</td>
<td>Probability of choosing most likely outcome&lt;br&gt;% bet placed on decision&lt;br&gt;Delay aversion&lt;br&gt;Overall proportional bet&lt;br&gt;Deliberation time (ms)</td>
</tr>
<tr>
<td>SSP, Reverse</td>
<td>A test of spatial memory span to recall the reverse order in which a series of boxes were highlighted</td>
<td>Owen, Downes et al. (1990)</td>
<td>Errors&lt;br&gt;Span length</td>
</tr>
</tbody>
</table>

IED: Intra-Extra Dimensional Set Shift; OTS: One touch Stockings of Cambridge; CGT: Cambridge Gamble Task; SST: Stop signal task; SSP: Spatial span.
6.2.5. **Statistical Analysis**

The study used a between-subjects design. Full details of the statistics used are provided in Chapter 2.

6.3. **Results**

6.3.1. **Physiological Effects**

There was no overall main effect of group on blood pressure (systolic/diastolic) or heart rate.

6.3.2. **Subjective Effects**

6.3.2.1. **Visual analogue scales**

There was no overall main effect of group on the VAS. However, there was a main effect of time for eleven of the sixteen dimensions: Feeble ($F_{(3,93)}=4.83$, $p=0.004$), Clumsy ($F_{(3,93)}=3.11$, $p=0.03$), Discontented ($F_{(3,93)}=4.6$, $p=0.005$), Tranquil ($F_{(3,93)}=12.65$, $p<0.001$), Relaxed ($F_{(3,93)}=12.16$, $p<0.001$), Proficient ($F_{(3,93)}=6.6$, $p<0.001$), Sad ($F_{(3,93)}=4.47$, $p=0.006$), Amicable ($F_{(3,93)}=5.95$, $p=0.001$), Bored ($F_{(3,93)}=4.14$, $p=0.008$), Gregarious ($F_{(3,93)}=4.6$, $p=0.005$), Dreamy ($F_{(3,93)}=3.15$, $p=0.03$). This main effect of time differed according to the group for the dimensions drowsy, feeble, discontented, tranquil, quick-witted, proficient, sad, gregarious, and dreamy. No other interactions were significant.

Post-hoc analysis showed that at 06:00 am the modafinil group reported being less drowsy ($p=0.015$), less feeble ($p=0.005$), less bored ($p=0.014$), and less dreamy ($p=0.005$) than the placebo group, and more energetic ($p=0.008$) and more gregarious ($P=0.005$). However, group differences were not seen at 07:00 am or 08:00 am ($p>0.05$).

6.3.2.2. **Questionnaires**

Sleepiness as indexed by the Stanford Sleepiness Scale differed at the 03:00 am and 06:00 am time points (Main effect of time: $F_{(1,36)}=7.33$, $p=0.01$). There was a significant time x group interaction ($F_{(1,36)}=7.33$, $p=0.01$), with sleepiness in the modafinil group tending to decrease between 03:00 am and 06:00 am, and sleepiness in the placebo group remaining at the same level (see figure 6.1). However, post-hoc analysis did not reveal a significant group difference at either 03:00 am or 06:00 am ($p<0.2$).
6.3.3. Neuropsychological Effects

6.3.3.1. Intra-extra-dimensional Shifting

The participants in the placebo group were significantly less likely to pass the extra-dimensional shifting stage of the task, compared to the modafinil group, who all passed this stage ($F_{(1,38)} = 4.64, p=0.038$, see Figure 6.2a). There was no significant difference in total errors made (adjusted for the number of stages passed) between the placebo and modafinil group ($p=0.12$).

6.3.3.2. Cambridge Gamble Task

The drug had no effect on the probability of choosing the most likely outcome ($F_{(1,37)}=0.57, p=0.46$), the percentage bet placed on the decision ($F_{(1,37)}=0.39, p=0.54$), deliberation time ($F_{(1,37)}= 0.39, p=0.54$) and overall proportional bet ($F_{(1,37)}=0.01, p=0.92$). There was a significant difference between the placebo and modafinil group for delay aversion, with participants in the placebo group being less willing to wait when making bets, resulting in them making larger bets when bets were presented in the descending order compared to the ascending order ($F_{(1,36)} = 6.76, p=0.01$, see figure 6.2b). Delay aversion differed according to the ratio of red boxes to blue ($F_{(3,108)}=4.377, p=0.006$), but there was no group x ratio interaction ($p=0.311$).
6.3.3.3. REVERSE SPATIAL SPAN

The groups did not differ according to the span length that was reached (p=0.12). The placebo group made more errors, by touching boxes that were not in the highlighted sequence, more often than the modafinil group ($F_{(1,38)}=5.24, p=0.028$).

6.3.3.4. ONE TOUCH STOCKINGS OF CAMBRIDGE

Across both groups, the difficult problems, with a higher number of moves, took longer and required more attempts to solve. The groups did not differ for overall mean latency (p=0.20) and mean number of attempts (p=0.83). For the more difficult 5-move problems, the modafinil group took a shorter amount of time to solve the problems, compared to the placebo group. This difference was at trend level ($F_{(1,38)}=3.23, p=0.08$).

6.3.3.5. STOP SIGNAL TASK

No group differences were found for SSRT (see Table 6.3).
## Table 6.3 Cognitive Data

<table>
<thead>
<tr>
<th>Task</th>
<th>Variable</th>
<th>Placebo</th>
<th>Modafinil</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IED</td>
<td>Stages completed</td>
<td>8.6 (0.17)</td>
<td>9.0 (0.00)</td>
<td>p=0.04</td>
</tr>
<tr>
<td></td>
<td>Total errors (adjusted)</td>
<td>21.1 (4.53)</td>
<td>12.3 (0.91)</td>
<td>p=0.12</td>
</tr>
<tr>
<td>OTS</td>
<td>Mean latency (ms)</td>
<td>21198.1 (322.53)</td>
<td>17414.9 (1598.96)</td>
<td>p=0.20</td>
</tr>
<tr>
<td></td>
<td>Mean latency to correct, 5 move problems (ms)</td>
<td>36555.5</td>
<td>25589.8 (3236.40)</td>
<td>p=0.08</td>
</tr>
<tr>
<td></td>
<td>Mean attempts</td>
<td>1.2 (0.06)</td>
<td>1.2 (0.02)</td>
<td>p=0.83</td>
</tr>
<tr>
<td>CGT</td>
<td>Probability of choosing most likely outcome</td>
<td>0.98 (0.01)</td>
<td>0.97 (0.01)</td>
<td>p=0.46</td>
</tr>
<tr>
<td></td>
<td>Percentage bet placed on decision</td>
<td>0.61 (0.02)</td>
<td>0.63 (0.02)</td>
<td>p=0.54</td>
</tr>
<tr>
<td></td>
<td>Delay aversion</td>
<td>0.24 (0.04)</td>
<td>0.10 (0.03)</td>
<td>p=0.01</td>
</tr>
<tr>
<td></td>
<td>Overall proportional bet</td>
<td>0.57 (0.02)</td>
<td>0.58 (0.02)</td>
<td>p=0.92</td>
</tr>
<tr>
<td></td>
<td>Deliberation time (ms)</td>
<td>2234.5 (140.9)</td>
<td>2128.1 (232.2)</td>
<td>p=0.71</td>
</tr>
<tr>
<td>Reverse</td>
<td>SSP Errors</td>
<td>2.68 (0.32)</td>
<td>1.6 (0.34)</td>
<td>p=0.03</td>
</tr>
<tr>
<td></td>
<td>Span length</td>
<td>6.05 (0.27)</td>
<td>6.8 (0.37)</td>
<td>p=0.12</td>
</tr>
<tr>
<td>SST</td>
<td>Stop Signal Reaction Time</td>
<td>192.52 (12.47)</td>
<td>184.06 (12.30)</td>
<td>p=0.632</td>
</tr>
<tr>
<td></td>
<td>Median Go Reaction Time</td>
<td>382.16 (21.68)</td>
<td>378.6 (18.7)</td>
<td>p=0.96</td>
</tr>
<tr>
<td></td>
<td>Total Direction errors</td>
<td>7.95 (2.08)</td>
<td>4.25 (0.68)</td>
<td>p=0.395</td>
</tr>
</tbody>
</table>

IED: Intra-Extra Dimensional Set Shift; OTS: One touch Stockings of Cambridge; CGT: Cambridge Gamble Task; SSP: Spatial Span; SST: Stop Signal Task. Values shown are given as mean (standard error of the mean). The reported P values were derived from one-way ANOVAs, with group (placebo vs. modafinil) as a between subjects factor.
Figure 6.2 Cognitive Data

a. Intra-extra dimensional shift task (IED) stages completed. All participants on drug passed all stages of the test. Significantly fewer (p=0.04) participants on placebo passed the extra dimensional shift stage (stage 8) of the task.

b. Cambridge Gamble Task (CGT) delay aversion. Participants on placebo were more impulsive, and less willing to wait when making bets, resulting in them making larger bets when bets were presented in descending order compared to ascending order, compared to the modafinil group (p=0.013). In addition, there was a significant main effect of probability, with participants in both groups differing in the amount of time they were willing to wait according to the ratio of the coloured boxes (p=0.006).

Means are plotted, with error bars indicating standard error of the means.
6.4. **Discussion**

It was found that modafinil was effective at improving executive functions in sleep-deprived doctors. Doctors who received a 200mg dose of modafinil were significantly better at tasks assessing cognitive flexibility, working memory, and planning and at controlling impulsive responses, compared to a group who had received a placebo.

The finding that the placebo group was impaired at the cognitive tasks is consistent with previous findings of cognitive impairment in sleep-deprived adults. Of particular note, the placebo group was significantly less likely to pass the extra-dimensional shift stage of the IED task, which assesses an individual’s ability to flexibly shift responding from one stimulus dimension to another. The perseveration errors seen in this group are similar to the characteristic errors made by those with lesions to the prefrontal cortex (Owen, Roberts et al. 1991). Furthermore, the placebo group was more likely to make errors on the SSP, a working memory task, by touching boxes not in the highlighted sequence more often than the modafinil group. The SSP task requires individuals to hold and manipulate information in their working memory. This replicates findings that sleep deprivation can lead to inaccuracy on working memory tasks (Hagewoud, Havekes et al. 2010). Modafinil has been shown to improve performance on monotonous visuospatial memory tasks, especially on long delay conditions (Muller, Steffenhagen et al. 2004). These results suggest that modafinil has effects on maintenance and manipulation processes, which could be due to improvements in both attention and memory. On the OTS, a planning task, the placebo group took longer to solve difficult 5-move problems than the modafinil group (this difference was at trend level). However, the two groups did not differ either in terms of the number of attempts they had to make in order to solve the problems, or in the time taken to solve less complicated problems (i.e. those that took fewer than five moves to solve). Therefore, the placebo group took longer to plan difficult problems, but were just as likely to solve the problems correctly. Obviously in medical situations where decisions have to be made under pressure, speed in addition to accuracy may be critical. In non-sleep-deprived adults, modafinil has been shown to increase the time taken to solve the problems, but increase accuracy (Turner, Robbins et al. 2003). This increase in accuracy has been shown to be antagonized by prazosin, an alpha1-adrenoceptor antagonist, when administered at the same time as modafinil (Winder-Rhodes, Chamberlain et al. 2010), but no effect on time taken was seen. In our sample of sleep-deprived doctors, we only saw changes in length of time taken to solve the problems, supporting the evidence for a dissociation in neurochemical mechanisms underlying accuracy and time taken to solve a problem on the OTS.

All of the above improvements in cognition could be explained by a global improvement in attention and general alertness in the sleep-deprived doctors, which fits with the strong evidence for modafinil’s effects on the noradrenergic system (see Section 1.4.1.2). Indeed, it has been suggested that cognitive impairments found after sleep deprivation represent a global decline in attention and alertness (Killgore
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2010). It is interesting to note that although our results converge with data that suggest a noradrenergic mechanism of action, we nonetheless did not find changes in blood pressure or heart rate, which is consistent with the literature that describes ambiguous cardiovascular effects with modafinil (Turner, Robbins et al. 2003; Taneja, Diedrich et al. 2005). It has been suggested that modafinil may activate noradrenergic neurons in the LC, without affecting noradrenergic neurons involved in cardiovascular and salivary regulation (Hou, Freeman et al. 2005).

On the decision-making CGT, the placebo group were just as likely to choose the most likely outcome as the modafinil group, took just as long to make their decisions, and did not make significantly different percentage bets on their decisions. However, the placebo group was more delay-averse on the task than the modafinil group; they made higher bets in the descending condition and lower bets in the ascending condition. Thus, the placebo group waited for a shorter period of time before placing their bets. Delay aversion is considered to be a facet of impulsivity. This concurs with evidence that suggests that increased impulsivity may be associated with sleep deprivation (Hamidovic and de Wit 2009; Anderson and Platten 2011). However, in contrast to these findings there was no difference in SSRT between the two groups. Modafinil has previously been shown to reduce SSRT in healthy non-sleep-deprived adults and ADHD patients (Turner, Robbins et al. 2003; Turner, Clark et al. 2004), although this finding has not been replicated in trichotillomania patients (Chamberlain, Grant et al. 2010), which converges with the findings of this study. This incongruity could be due to differences in baseline performance, as in rats modafinil significantly decreases SSRT, but only in those with a slow baseline reaction time (Eagle, Tufft et al. 2007).

Modafinil is licensed for use in narcolepsy and shift work sleep disorder because of its ‘wake-promoting’ effects. Therefore, it is unsurprising that modafinil also helped to alleviate the doctors’ subjective feelings of tiredness, as indexed by the VAS and the Stanford Sleepiness scale. This agrees with previous findings that modafinil reduces sleepiness ratings on the Stanford scale in healthy non-sleep-deprived humans (Joo, Tae et al. 2008). The VAS ratings showed that the modafinil group felt less drowsy, feeble, bored, and dreamy, and more energetic and gregarious, compared to the placebo group at 06:00 am, immediately prior to testing. However, these reported differences on the VAS and Stanford sleepiness scale were no longer present halfway through testing (07:00 am), and after testing (08:00 am). Furthermore, there was no overall difference in VAS ratings between the groups when all the time points were taken together. This makes it unlikely that the cognitive findings of this study were due to a generalized arousal effect in the modafinil group. To confirm this, the VAS dimension of drowsy/alert was used as a covariate, and the differences on the cognitive tests remained. Therefore, although modafinil seemed to have arousing properties at peak plasma levels, this was not felt by participants throughout all of the testing.

This research could be extended by investigating the effects of modafinil on the performance of doctors in life-like situations, through using simulator tasks and role plays. It would be interesting to ascertain whether the cognitive improvements seen would translate to reduced medical errors and better
outcomes for patients. More research also needs to be conducted into the suitability of modafinil for use by the medical professional. It has been suggested that modafinil has a disruptive effect on self-monitoring under sleep-deprived conditions (64 hours), with participants on modafinil over-estimating their performance on a visual judgment and complex mental addition task (Baranski and Pigeau 1997). Overconfidence in one’s ability could be problematic in medical situations. The consideration of the use of modafinil by the medical profession also raises many ethical issues (see Chapter 7 for discussion). It is also important to keep in mind that agents such as modafinil cannot replace the function of restorative rebound sleep (Porkka-Heiskanen, Smith et al. 1995; Chapotot, Pigeau et al. 2003; Greene and Siegel 2004).

Overall, in comparison to the placebo group, doctors who received 200mg of modafinil 3 hours prior to testing were more efficient at solving planning problems, less likely to make working memory errors, less impulsive, and were better at flexibly shifting their attention. All of these cognitive functions are key components to reacting quickly and safely in high pressure environments, such as in accident and emergency departments or in surgical situations. The findings in the placebo group highlight some of the challenges faced by doctors whilst they are sleep-deprived. The implications of this are that if medical complications occur, doctors may be less able to think flexibly and quickly plan new solutions whilst they are sleep-deprived. Overall, these findings are of importance as they highlight the potential of pharmacological agents to aid cognition under conditions of sleep deprivation when cognitive skill and executive functions are vital.
7. **DISCUSSION**

The primary aim of this thesis was to quantify cognitive impairment across a range of populations including amateur boxers, subarachnoid haemorrhage (SAH) patients, Parkinson’s disease (PD) patients, and sleep deprived individuals, in order to examine possibilities for pharmacological treatment. Specifically, the potential efficacy of three pharmacological agents was investigated. A number of important findings in relation to the treatment of cognitive deficits were made. The wider implications of these findings and their relevance are discussed after a brief overview of the individual studies.

7.1. **MAIN CONTRIBUTIONS OF THIS RESEARCH**

7.1.1. **COGNITION AND WHITE MATTER DENSITY IN AMATEUR BOXERS**

The effect of amateur boxing on cognition and white matter density was investigated in this study. Associations were found between changes in response inhibition and changes in white matter density in the right inferior frontal gyrus (rIFG), which is known to subserve this cognitive function. Improvements were associated with an increase in white matter density, and decline in performance was associated with a loss of white matter density. Although the conclusions that can be drawn from this study are limited by the small sample size and lack of control group, it still makes an important contribution by illustrating the utility of combining diffusion tensor imagining with rigorous cognitive tests in order to relate subtle structural changes with cognition.

7.1.2. **MODAFINIL AND PHYSOSTIGMINE IN SUBARACHNOID HAEMORRHAGE**

In this study, SAH patients were impaired on measures of reaction time, attention, and episodic memory. However, they did not show elevated impulsivity on the stop signal task (SST), compared to controls. Administration of the cholinesterase inhibitor physostigmine did not modulate patients’ performance on the cognitive tasks utilised. Equally, the atypical stimulant modafinil did not have an effect on overall performance. Despite the overall lack of effect, modafinil did improve performance on a visuospatial episodic memory test in patients who were low performers at baseline (placebo condition). In contrast,
modafinil had a detrimental effect on SAH patients who were high performers at baseline. This result is consistent with an inverted-U-shaped function linking level of arousal or stimulation to behavioural performance, as will be discussed in greater detail below. The results from this study demonstrate that physostigmine may not be an effective treatment for cognitive impairment in SAH patients. Conversely, modafinil may have potential as a treatment in this group so long as cognitive baseline assessments are conducted in order to aid treatment decisions.

7.1.3. Atomoxetine in Parkinson’s disease

Atomoxetine was shown to have an effect on impulsivity in PD patients in this study. In a similar way to the SAH study, the effects seen suggested an inverted-U-shaped function. However, in this study blood samples were taken, enabling the concentration of the drug in blood plasma to be associated with change in impulsivity. Low levels of atomoxetine in the blood plasma samples was associated with improved response inhibition, whilst higher drug levels in the blood plasma samples were associated with a decline in the ability to inhibit proponent motor responses. The same pattern of results was also found, although to a lesser extent, on deliberation time before making a decision and reflection impulsivity. Atomoxetine was well tolerated by the group of PD patients in this study, indicating that at the optimal dose, it is a potential treatment for impulsivity in this patient population.

7.1.4. Modafinil and sleep deprivation

In this study, we endeavoured to induce cognitive impairment by depriving healthy young adults of sleep. Following 24 hours without sleep, modafinil was shown to improve executive functions compared to placebo. Administration of modafinil enabled participants to successfully complete an attentional set-shifting task, whereas the placebo group were more likely to fail the task at the extradimensional set-shift stage. Modafinil was also associated with fewer errors on a task of working memory. On a decision-making task, the placebo group were more averse to delays, which is a component of impulsive behaviour. The group of healthy adults used were all medical doctors, who often work in high-pressure situations under sleep-deprived conditions. The impairments seen in the placebo group highlight some of the challenges faced by doctors when they are sleep-deprived. Modafinil showed potential as an agent with which cognitive impairments related to sleep deprivation can be remediated. The findings from this study also have clinical relevance as they illustrate that modafinil can successfully remediate executive impairments in states where they are impaired, such as neuropsychiatric and neurological disorders.

7.1.5. Summary of the main contributions of this research

The main contribution of this research was to demonstrate that both modafinil and atomoxetine have the potential to be repurposed and used as novel treatments in certain patient populations. Using the existing understanding of cognitive impairments in the cohorts investigated, atomoxetine and modafinil were identified as potential treatments, based on their known mechanisms of action.
A number of preliminary studies had suggested that the relatively selective noradrenaline reuptake inhibitor atomoxetine would be effective at modulating impulsivity in PD, as this agent has been shown to improve response inhibition in humans (Chamberlain, Del Campo et al. 2007; Chamberlain, Hampshire et al. 2009), and in a dose-dependent manner in rats (Robinson, Eagle et al. 2008), and represents the first non-stimulant-based medication that has proven efficacy in attention deficit hyperactivity disorder (ADHD) (Del Campo, Chamberlain et al. 2011). Importantly, atomoxetine does not affect dopamine in the striatum or nucleus accumbens (Bymaster, Katner et al. 2002), so it is unlikely to interfere with dopamine replacement therapy (DRT) in PD. Using a rigorous double-blind, placebo-controlled study whereby PD patients were randomised to receive either placebo or atomoxetine on their first visit, and returned for a second visit, it was shown for the first time that atomoxetine has an effect on response inhibition in this group. Decreased impulsivity was seen across a number of tasks, but only in participants with a low drug plasma concentration. This finding is particularly important in view of the development of impulsive-compulsive behaviours (ICBs) in a minority of PD patients (Voon and Fox 2007).

In contrast, the atypical stimulant modafinil, which also has noradrenergic actions, did not have an effect on impulsivity as indexed by the SST in SAH patients and sleep-deprived healthy adults. However, it was demonstrated that modafinil is a potential treatment for other executive impairments, such as those in delay aversion, working memory, planning, and attentional set-switching, which are caused by sleep deprivation. In contrast, modafinil was shown to have limited cognitive effect in SAH patients, but it was shown for the first time that modafinil modulated performance on a test of paired associates learning (PAL).

Importantly, in both the SAH and PD patients, an inverted-U-shaped function was seen in response to the administration of modafinil and atomoxetine respectively. This highlights the difficulty of determining optimal doses in clinical populations, as some patients may benefit from a treatment, whereas others might not. This heterogeneity can be caused by disease factors, medications, genetics, and also arousal states. These findings suggest that instead of repositioning drugs for disease populations, it may be more successful to target specific facets of cognition that can be applied across a range of disorders. Furthermore, within each patient population, patients should be treated more on an individual level, taking into account their personal cognitive profile.

### 7.2. Integration of Findings

#### 7.2.1. Cognitive Impairment

This thesis aimed to quantify cognitive impairments in the populations investigated. Using well-validated cognitive tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB), cognition was tested and then either monitored over time, as in the amateur boxers, or compared to a control group, as in Chapter 4 for the SAH patients. In Chapter 5 a within-subjects design was used, meaning that PD patients were tested both ‘On’ and ‘Off’ atomoxetine. The addition of a healthy, matched control group
would enable the cognitive impairments in this group to be better understood. In PD it is also difficult to separate out the effects of disease and medication on cognition, unless patients are tested both ‘On’ and ‘Off’ their medications. In Chapter 6 doctors were deprived of sleep and then a between-subjects design was used, with half of the participants receiving modafinil, and the other half a placebo. This design enabled the administration of tests of executive function, such as the Intra-extra Dimensional Set Shift (IED) task, that rely on novelty. However, this design could be considered to be problematic as a non-sleep-deprived measure of cognition, which would have allowed the effect of sleep deprivation on cognition to be seen, was not obtained.

One way in which cognitive impairment can be conceptualised is in terms of neurocognitive endophenotypes, such as impulsivity, that are derived from brain measures as well as behaviour, and using them transdiagnostically across disorders (Robbins, Gillan et al. 2012). This thesis utilised the SST as a measure of behavioural dyscontrol in all of the populations investigated. Impulsivity is a personality trait seen in healthy individuals, but extreme forms of excessive impulsivity are a component of many neuropsychiatric and neurological disorders, for example juvenile and adult forms of ADHD, mania, substance misuse disorders, behavioural addictions, such as gambling, and related borderline personality disorders. Furthermore, as illustrated by the presence of ICBs in PD, impulsivity can occur in disorders where they are not normally a major symptom. It has been shown that impulsivity, as indexed by the SST, fulfils the criteria for an endophenotype. For example both Obsessive-compulsive disorder (OCD) patients and their unaffected first-degree relatives exhibit impaired motor inhibitory control, indicated by a prolonged stop signal reaction time (SSRT), and this is associated with increased grey-matter volume in areas of the striatum, cingulate, and parietal cortex (Menzies, Achard et al. 2007). This endophenotype is not restricted to OCD, with individuals with stimulant drug addiction, and their biological siblings with no history of chronic drug use, both showing increased impulsivity on the SST that are also related to changes in the brain structure, including the inferior frontal cortex and putamen, both of which are key nodes in the neural network that mediates response inhibition (Ersche, Jones et al. 2012). Furthermore, in animal studies, rats with a consistent tendency to respond prematurely on the 5 choice serial reaction time (5CSRT) task are identified as ‘high impulsive’. These rats tend to escalate intra-venous cocaine self-administration, and also tolerate aversive events such as foot shock, in order to ‘seek’ the drug, and also show enhanced relapse of cocaine self-administration behaviour following abstinence (Dalley, Everitt et al. 2011). Therefore high impulsivity may be a risk factor for, rather than a product of, these disorders.

It is expected, therefore, that across disorders which are characterised by excessive impulsivity there will be similarities in brain structural and functional abnormalities. Identifying disorders which share an ‘impulsivity’ endophenotype would help to target behavioural and pharmacological treatments more effectively. In this way, transdiagnostic treatments can be used across what appear to be very different diagnostic entities.
In this thesis, all the participants were tested on the SST and therefore impulsivity can be compared across the groups in a post-hoc manner. All the testing sessions were run by the same investigator, under similar test conditions, although, because of the different populations investigated, the groups are not matched for age (Mean in years for boxers: 22; SAH: 53; PD: 65; Sleep deprived: 28), or estimated verbal IQ (Mean for boxers: 119; SAH: 114; PD: 106; Sleep deprived: 117). Therefore, the comparisons made were qualitative, in order to explore the degree to which the functioning of these groups differed in terms of response inhibition and reaction time. Table 7.1 shows details of comparisons between the healthy controls used in Chapter 4 (N=20; Mean age=40; Mean estimated IQ=113) and the populations investigated in each of the chapters. The SST shows limited practice effects; therefore the issue of the difference in the level of prior test exposure should be minimal compared to other tests of executive function. As expected, the young adults show faster Go reaction times compared to controls, but their SSRT is no different. After one year of boxing, the same group shows a shorter SSRT and a faster Go reaction time compared to the older controls. Young, healthy sleep-deprived doctors do not differ in SST performance compared to the older healthy controls. The SAH patients show a slowing of Go reaction times, and there is a trend towards the same effect in the PD patients. Neither the SAH patients nor the PD patients differ in terms of SSRT compared to the healthy controls that were younger than them. Examination of these results, however, does not give any indication of the extent to which the groups differ from each other.

<table>
<thead>
<tr>
<th>Table 7.1 Performance on the stop signal task compared to controls</th>
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<tr>
<td><strong>Group</strong></td>
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<td>Young adults</td>
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<td>Boxers 1 year</td>
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<td>Sleep-deprived</td>
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<td>SAH</td>
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<td>PD</td>
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SSRT is the stop signal reaction time measure from the stop signal task. Median GO reaction time is the median reaction time in response to Go stimuli on the stop signal task. P-value was derived from independent samples t-tests. Mean age is given in mean whole years. Controls are the control group from Chapter 4. Young adults are the amateur boxers investigated in Chapter 3, at their baseline assessment. Boxers 1 year are the amateur boxers at their one year follow-up assessment. The sleep-deprived group are the sleep-deprived doctors who received a placebo in Chapter 6. SAH are the subarachnoid haemorrhage patients on the placebo visit, who were investigated in Chapter 4. PD is the placebo visit for the Parkinson’s disease group in Chapter 5.
Figure 7.1  Illustration of differences in performance on the stop signal task for each of the patient groups (on placebo) compared to the healthy volunteers from Chapter 4, expressed in terms of effect size (Cohen’s d)

The groups are as described under Table 7.1. Dark blue bars mark values where responses were faster compared to the control group, so this indicates a shorter stop signal reaction time or a faster Go reaction time. Light blue bars indicate longer stop signal reaction times or slower GO reaction time. An effect size of 0.2 is considered to be small, 0.5 medium, and 0.8 large (Cohen 1988), these cut-offs are indicated by the three thick gridlines. The line at the 0.5 effect size is approximately the point at which significant differences are seen.
The effect size gives an indication of the relative magnitude of the differences between groups. Cohen’s $d$, one of the most commonly used effect size statistics, presents the differences between groups in terms of standard deviation units (Cohen 1988). Effect sizes are loosely classified as small if $d=0.2$, medium if $d=0.5$, and large if $d=0.8$. Figure 7.1 illustrates the degree to which the individual groups differ from the healthy volunteer group in terms of effect size. Dark blue bars indicate better response inhibition and faster response times compared to controls, whereas light blue bars indicate worse response inhibition and slower response time compared to controls. The differing levels of performance across groups are most probably due to the underlying differences in development and pathology. The differences seen are in line with what would be expected. The SAH and PD groups are both slower to respond compared to controls and the younger groups. Interestingly, this motor deficit is more pronounced in the SAH group, despite the characteristic motor symptoms of PD. Conversely, all of the younger groups show speeded Go reaction time compared to controls, with the young healthy adults showing an increase in reaction time after one year of boxing. Although the sleep deprived group show faster Go reaction time than the controls, this is not to the same extent as the young adult group, which may suggest a slowing related to sleep-deprivation. On the SSRT measure of response inhibition, the PD, SAH and young adults showed elevated impulsivity. The effect size is largest for the PD group, then the SAH group and then the young adults. This fits with the hypothesis that increased impulsivity is a feature of PD, as discussed in Chapter 5. Interestingly, the sleep-deprived doctors, and the boxers after one year of training, both showed better response inhibition than the controls. The change in the boxers could be due to development, although the effect size is large, suggesting that boxing training may have an overall beneficial effect. This complements the findings of Chapter 3, which illustrated that, although the majority of boxers improved over time, a number of them show increased impulsivity that was related to a loss of white matter density in the right inferior frontal gyrus (rIFG). Overall, it can be seen that it is possible to quantify a specific dimension of a cognitive symptom, such as impulsivity, across a range of populations.

Looking at a specific cognitive domain across groups can highlight new therapeutic avenues that are targeted at specific symptoms. For example, in this thesis, atomoxetine, which has been shown to reduce impulsivity in ADHD, was shown to modulate impulsivity in the PD group. In turn, this suggests that atomoxetine may be a suitable treatment for other disorders where impulsivity is a characteristic feature, such as substance abuse. The limitation to this approach, however, is that there is always more than one cognitive impairment present in a disorder, and so an effective specific treatment for impulsivity might be overall less effective than a pharmacological cocktail, such as modafinil, with many potential therapeutic actions. In order to adequately define the nature of neuropsychiatric and neurological disorders, it is important to characterise all of the dimensions that contribute to a deficit, as well as their relative contributions in the individual patient.
7.2.2. Pharmacological Treatment of Cognitive Impairment

In this thesis, the treatment potential of three pharmacological agents was explored: atomoxetine, a noradrenaline re-uptake inhibitor, modafinil, an atypical stimulant, and physostigmine, a cholinesterase inhibitor. It was hypothesised that these agents would have distinguishable effects on cognition due to their different pharmacological profiles. However, it was expected that the actions of modafinil and atomoxetine would overlap, as they have both been shown to modulate the noradrenergic and dopaminergic systems. For example, atomoxetine increases extracellular levels of noradrenaline (NA) and dopamine (DA) in the prefrontal cortex (PFC), but does not alter NA or DA in the nucleus accumbens or the striatum (Bymaster, Katner et al. 2002). It is thought that atomoxetine’s primary mode of action is noradrenergic. The mechanism of action of modafinil is less well-known. However, there is converging evidence for the role of NA in its actions. For example, decreased activity is seen in the locus coeruleus (LC) after administration of modafinil (Minzenberg, Watrous et al. 2008), and administration of Prazosin antagonises the beneficial effects of modafinil on some aspects of cognition in healthy humans (Winder-Rhodes, Chamberlain et al. 2010). The following sections briefly describe the effect of each of these drugs on cognition in this thesis, particularly comparing the effects of modafinil in the two studies in which it was utilised, and discussing the differences in profile between atomoxetine and modafinil.

7.2.2.1. Specific Effects of Physostigmine

In Chapter 4, physostigmine was administered to SAH patients, as it was hypothesised that cognitive impairments in this patient group would be related to damaged cholinergic neurons. Although the cognitive impairments seen were consistent with this hypothesis, physostigmine did not improve performance. This may have been due to limitations in the study design, but these findings do not indicate that physostigmine is an effective treatment in this group.

7.2.2.2. Specific Effects of Modafinil

Modafinil was also administered to the SAH patients in Chapter 4, and was shown to improve performance on the CANTAB PAL episodic memory test, but only in patients who were impaired on the task at baseline. In agreement with this, modafinil also improved memory in the sleep-deprived doctors who feature in Chapter 6. The two memory tests used in these studies are different, with PAL assessing visuospatial episodic memory and learning, and the CANTAB reverse spatial span (SSP) task used in the sleep deprivation group testing short term memory capacity as well as working memory. However, successful performance on both of the tasks requires medial temporal lobe memory function, as well as frontal executive function (Fletcher, Frith et al. 1995). The mechanism by which modafinil improves performance on these tasks could be due to its effects on the dopaminergic and/or noradrenergic systems. For example, administration of the α-2a agonists guanfacine and clonidine facilitates learning on the PAL test (Jakala, Sirvio et al. 1999), and the DA-NA re-uptake inhibitor methylphenidate improves
performance on the SSP task in healthy young adults (Elliott, Sahakian et al. 1997). Modulation of either NA or DA would particularly affect the executive component of these tasks. Indeed, DA modulation has been shown to be important for the forward version of the SSP task, as the D2 receptor agonist bromocriptine (Mehta, Swainson et al. 2001) has been shown to improve performance, and PD patients are impaired when ‘Off’ their dopaminergic medication (Lange, Robbins et al. 1992). In healthy young adults, modafinil does not improve span length or errors on the forwards version of the SSP task (Turner, Robbins et al. 2003). However, modafinil does improve SSP performance in adults with ADHD (Turner, Clark et al. 2004). Furthermore, the dopamine-noradrenaline reuptake inhibitor methylphenidate improves performance of healthy young adults on SSP, but only on the first instance that they encounter the task (Elliott, Sahakian et al. 1997).

Given the previous findings for the role of NA in modafinil’s actions, it was interesting that modafinil did not effect response inhibition as measured by the SST in either the sleep-deprived doctors or SAH patients. Previous studies have found improved response inhibition after administration of modafinil. Specifically, in a group of healthy adults, modafinil has been shown to improve response-inhibition in a dose-dependent manner, with relatively high doses of 200mg improving response inhibition more than a lower dose of 100mg (Turner, Robbins et al. 2003). Modafinil also improves performance response inhibition in adult ADHD patients (Turner, Clark et al. 2004). Therefore, it was expected that modafinil would improve successful inhibition on the SST. In line with our findings, previous studies have not always found an effect of modafinil on SSRT. For example, some studies have failed to replicate the findings in healthy adults (Winder-Rhodes, Chamberlain et al. 2010), and there is also no effect of modafinil on SSRT in schizophrenic patients (Turner, Clark et al. 2004). One explanation for these mixed findings is demonstrated by a study looking at the effects of modafinil in alcohol-dependent patients, which found that response-inhibition on the SST task was only improved in patients who had higher impulsivity at baseline, whereas low impulsive patients showed diminished performance after drug administration (Schmaal, Joos et al. 2012). Furthermore, a similar relationship is seen in rats, with modafinil decreasing SSRT, but only in rats with slow baseline SSRT, whereas performance in rats with a fast SSRT is not affected (Eagle, Tuft et al. 2007). This is something that could potentially be explored further in the sleep-deprived doctors and SAH patients using post-hoc analysis.

**7.2.2.3. Specific effects of atomoxetine**

As hypothesised, atomoxetine modulated measures of impulsivity in the PD patients. However, the effect seen followed an inverted-U-shaped function as discussed in Chapter 5 and in section 7.2.2.5 below. The findings suggest that for some PD patients, atomoxetine may be a potential treatment for symptoms of impulsivity, subject to further research.
7.2.2.4. COMPARISON BETWEEN ATOMOXETINE AND MODAFINIL

In this thesis, atomoxetine was administered whilst PD patients completed the Cambridge gamble task (CGT) and SST, and modafinil was administered to SAH patients who were tested on the SST, and also to sleep-deprived doctors who were tested on the SST and CGT. The effect of these agents would have been influenced by the characteristics of the group they were administered to, as these differed in age and underlying pathology. However, it was hypothesised that given the effects of both atomoxetine and modafinil on the noradrenergic system, some similarities between the effect of the drugs would be seen. Table 7.2 summarises the effect of atomoxetine and modafinil on these tasks.

As expected, this thesis confirmed the role of NA in response inhibition by modulating SSRT in the PD patients. Contrary to predictions, modafinil did not have an effect on SSRT in either the SAH or sleep-deprived group, as discussed above. In contrast to this, modafinil was shown to decrease delay aversion on the CGT in the sleep-deprived doctors examined in Chapter 6. Delay aversion is considered to be a facet of impulsivity, with ADHD children displaying increased delay aversion (DeVito, Blackwell et al. 2008). Interestingly, this measure of impulsivity is manipulated by dopaminergic status in PD patients, with PD patients ‘On’ their medication showing an increase in delay aversion in comparison to ‘Off’ medication (Cools, Barker et al. 2003). In contrast to this, in Chapter 5, atomoxetine modulated deliberation time on the CGT in PD patients, but not delay aversion. Taken together, this could indicate that deliberation time is more dependent on NA stimulation, as compared to delay aversion which is dependent on DA stimulation. Taken together, these findings suggest that while atomoxetine’s primary mode of action is through NA, modafinil may be primarily affecting the dopaminergic system, either directly or as a downstream effect.

However, this explanation is oversimplified. Modafinil’s effect on PAL in the SAH patients in Chapter 4 illustrates that modafinil has more of a ‘cocktail’ effect whereby multiple neurotransmitter systems are modulated, as there is limited evidence for a role of DA in performance on the PAL test. For example, levodopa withdrawal in PD patients is not associated with changes on this task (Lange, Robbins et al. 1992), and administration of the DA D2 receptor antagonist sulpiride, or dietary induced tyrosine depletion, in healthy young volunteers does not induce impairment on this task (Mehta, Sahakian et al. 1999; Harmer, McTavish et al. 2001). There is more evidence to suggest that modafinil’s effect on NA, Ach or GABA may account for these changes. For example, PAL performance can be altered by NA manipulation by the administration of a high dose of the α2-adrenoceptor clonidine (Coull, Middleton et al. 1995), and the α2-adrenoceptor idazoxan improves PAL performance in a dose dependant manner in patients with dementia of the frontal type (Coull, Sahakian et al. 1996). Furthermore, PAL is improved by the α2-agonists clonidine and guanfacine (Jakala, Sirvio et al. 1999). There is evidence to suggesting that ACh modulates PAL performance (Greig, Sambamurti et al. 2005; Bartko, Vendrell et al. 2011).
Overall, the findings of this thesis converge with the current thinking that atomoxetine has relatively specific effects on the noradrenergic system (although some increase in DA is seen in the PFC after administration of the drug), whereas modafinil seems to modulate a number of neurotransmitter systems. It has recently been proposed that a parsimonious explanation for modafinil’s action may be that it increases glutamatergic activation (Scoriels, Jones et al. 2012), so this may be an area for future research. The difference between the profiles of atomoxetine and modafinil suggests that they may have a distinct utility in clinical practice. Atomoxetine may be an effective drug when only specific symptoms of impulsivity characterise a disorder, whereas modafinil may be a more effective treatment in disorders where multiple cognitive domains are affected.
7.2.2.5. **Inverted-U-shaped function**

Inverted-U-shaped effects were seen in response to both atomoxetine and modafinil administration. The inverted-U-shaped function, first described by Yerkes and Dodson, has proven to be a useful concept in cognitive neuroscience, particularly because of the parsimonious way in which it can be applied to many dose relationships found. One criticism the model has received is that some of the effects it explains may actually be caused by regression towards the mean, whereby participants performing badly in the first testing session are likely to improve on the second testing session, and patients who initially perform well are likely to subsequently perform less well. More simply put, ‘When you’re at the top, the only way is down, but when you’re at the bottom the only way is up’ (Mehta 2002). However, the accumulating evidence for the inverted-U-shaped function indicates that this is a real and important effect. The following section will discuss the concept of the inverted-U-function in relation to the findings of this thesis.

![The generalised Yerkes-Dodson relationship](figure7.2)

**Figure 7.2 The generalised Yerkes-Dodson relationship**

The inverted-U-shaped function shows how performance efficacy may vary as a function of activation. Optimal levels of activity may be different for different tasks, for example those that differ in terms of difficulty. This is illustrated by the blue and tan curves.

The general principle of the inverted-U-shaped function is that efficiency of behavioural performance, particularly those behaviours linked to the PFC, is related to the level of activity in the ascending monoaminergic (mainly dopaminergic and noradrenergic) systems (Figure 7.2). Importantly, the optimal level of activation may differ depending on the task, or the difficulty of a task. This is indicated in Figure 7.2 by the blue and tan curves, with the blue curve representing a task or a level of difficulty that requires a lower level of activation for optimal performance, whereas the tan line represents a task or a level of difficulty that requires a higher level of activation for optimal performance. For example, the selective DA D2 receptor agonist bromocriptine increases activation by enhancing DA in the PFC. Administration of
1.25 mg of bromocriptine to healthy volunteers improves their performance on the CANTAB SSP test, but impairs performance on a reversal learning task (Mehta, Swainson et al. 2001). Although this relationship has been almost exclusively demonstrated in relation to PFC function related to monoamines, it is not impossible that inverted-U-shaped functions also exist for the subcortical systems, or for other neuromodulators such as ACh (Robbins 2010).

7.2.2.5.1. **Baseline effects**

In Chapter 4, the SAH patients responded to modafinil in a baseline-dependent manner. Specifically, participants who performed poorly at baseline improved on drug. Conversely, the SAH patients who performed better at baseline showed a detrimental response to modafinil. This is illustrated in Figure 7.3 in terms of the inverted-U-shaped function. It is presumed that individuals who do not perform as well at baseline have a lower level of activation, indicated by the green spot. By increasing activation in this group they are moved to a more optimal point on the inverted-U. In contrast to this, it is presumed that those who perform better at baseline are already performing at an optimal level, and therefore increasing activation will shift them to the downward slope of the inverted-U-curve. In this way, baseline performance has been shown to predict response to pharmacological agents in both animals and humans (Kimberg, D'Esposito et al. 1997; Granon, Passetti et al. 2000; Mehta, Swainson et al. 2001).
Baseline levels of activation differ between individuals as a result of a number of factors including genetics, pathology, medication, and arousal (a). This determines the effect of increasing activation, caused by drug administration, arousal or increasing task load, on task performance (b).

There are many factors that determine an individual’s level of activation at baseline, including genetics, arousal, and level of pathology and medications in patients. Variation in PFC processing, including cognitive function, have been associated with functional polymorphisms of the catechol-O-methyl transferase (COMT) gene (Val^{108/158} Met), which is important in DA metabolism. Humans with the val/val genotype have hypothetically more rapid inactivation of release of PFC DA than those with the met/met genotype, with those with the val/met heterozygote intermediate between these two. These changes in PFC DA function have been shown to be related to performance on tasks that are sensitive to frontal lobe function; COMT polymorphism, for example, predicts 4% of Wisconsin Card Sort Test (WCST) performance (Barnett, Jones et al. 2007), and also produces a predictable pattern of effects on working memory performance (Mattay, Goldberg et al. 2003). Interestingly, the COMT phenotype modulates the L-dopa response in PD, with met/met individuals exhibiting the greatest degree of deficit on tests of
planning and recognition memory, especially in response to dopaminergic medications (Foltynie, Goldberg et al. 2004; Williams-Gray, Hampshire et al. 2007), although this effect is only seen early on in the disease (Williams-Gray, Evans et al. 2009). These findings fit with the proposed overdosing hypothesis whereby early on in the disease process regions with extensive DA depletion, such as the putamen, would have their functions optimally titrated by DA medications, but regions which are relatively spared in the early stages, such as the caudate and ventral striatum, would be disrupted by DA medication, following the Yerkes-Dodson principle. This hypothesis has been supported by the fact that early on in the disease process medicated PD patients are impaired at tasks associated with ventral striatal and orbitofrontal function, but are improved on tests of spatial working memory (Wang, Vijayraghavan et al. 2004). Furthermore, withdrawal of dopaminergic medication in PD patients improves probability reversal performance, which is a test associated with ventral striatal-orbitofrontal circuitry (Cools, Barker et al. 2001). Therefore met/met individuals who have higher baseline levels of DA activation may be more vulnerable to this overdosing effect.

These findings show that there is a complex interplay between pathology, medication, and genetics, which together with environmental factors affect the level of activation, and thus response to pharmacological treatment. The findings in the SAH patients in this thesis need further investigation in order to be fully understood. However, accurate baseline measurements, such as PAL used in Chapter 4, can be used to determine whether or not a patient may benefit from treatment.

7.2.2.5.2. DOSE EFFECTS

Following the same logic as discussed above, the dose of a pharmacological agent can determine the level of activation, and thus cognitive performance. In Chapter 5, atomoxetine was shown to modulate impulsivity in PD patients, but in a dose-dependent manner with low doses improving performance and high doses impairing performance. This is illustrated in Figure 7.4. As discussed in Chapter 5, atomoxetine has been shown to dose-dependently reduce SSRT in rats, without affecting the Go reaction time (Robinson, Eagle et al. 2008). An inverted-U-shaped function has been found for other neuromodulators. In animals, for example, the full D1 receptor antagonist SKF-81297 has been shown to improve working memory function at low doses, but high doses impaired performance, especially at short delays (Chudasama and Robbins 2004).

In clinical practice, determining the correct dose may be difficult due to individual variation in baseline, and the fact that different cognitive functions may have differing levels of optimal function. For example, doses of atomoxetine that improve SST performance have also been shown to impair attentional set shifting (Newman, Darling et al. 2008).
The amount of activation produced as a result of administering a pharmacological agent is dependent on the dose of the drug. Low doses will increase activation less than high doses.

**Figure 7.4  Effect of Drug Dose**

**7.2.2.6. Ethical Considerations of Cognitive Enhancement**

The investigation of pharmacological enhancement, particularly in healthy populations such as the sleep-deprived doctors in Chapter 6, raises some ethical considerations. This is especially important considering the fact that pharmacological agents have the potential to not only compensate for existing deficits, but to also enhance normal functioning. The use of cognitive-enhancing drugs has gone up over recent years, although many individuals who take them do not suffer from neuropsychiatric disorders.

Modafinil is one of the agents which appear to be increasingly used in healthy populations, along with other stimulant drugs such as methylphenidate. Evidence for increased use can be seen in trends suggesting that between 1993 and 2001 there was a clear increase in the 12-month prevalence rates of nonmedical use of prescription drugs in college students (National Institute on Drug Abuse, 2005). In the United States, studies indicate that up to 16% of students on some college campuses use stimulants, while 8% of university undergraduates report having illegally used prescription stimulants (Babcock and Byrne 2000). There is also a trend for increasingly younger students to use such drugs with one report indicating 2.5% of eighth graders (13–14 years) abused methylphenidate as did 3.4% of tenth graders (National Institute in Drug Abuse, 2005). Stimulant drugs for off-label use are obtained from the internet, as well peers and family (McCabe and Boyd 2005). The most commonly reported motives for drug use are to aid concentration, increase alertness, to counteract the effects of jet lag, and to deal with demanding and important mental challenges (Sahakian and Morein-Zamir 2007).

This thesis suggests that modafinil may remediate temporary cognitive impairment in healthy adults. Previous research has also shown that modafinil improves performance on a range of cognitive domains.
Modafinil in Sleep Deprived Doctors

in non-sleep deprived healthy adults (Turner, Robbins et al. 2003), and administration of an acute dose of atomoxetine improves response inhibition in healthy adults (Chamberlain, Hampshire et al. 2009). However, although improvements have been seen in acute studies in the laboratory, it has yet to be determined how these drug-induced enhancements in cognition translate to the real world, and what the long-term efficacy of these drugs is for healthy people. One concern is that some stimulants, such as methylphenidate, have a well recognised side-effect profile that include the risk of addiction (Lynskey and Hall 2001). Modafinil is considered to have a low side-effect profile, although it has been shown to bind to dopamine transporters in the nucleus accumbans, albeit to a lesser degree than other stimulant drugs (Volkow, Fowler et al. 2009). As well as the potential side effects that healthy individuals could be exposing themselves to, this thesis has illustrated that the degree of enhancement varies between individuals, with some individuals showing a decline in performance as a result of taking pharmacological agents (see section 7.2.2.5). Therefore, taking a ‘cognitive enhancing’ drug does not guarantee enhancement, and may actual cause impairment compared to baseline (Elliott, Sahakian et al. 1997).

There are also more global ‘neuroethical’ issues that are raised by the use of pharmacological agents by healthy individuals. These include issues relating to coercion, whereby individuals may feel pressured to enhance their cognitive abilities pharmacologically, either by peers or employers. It also raises issues of fairness, cheating and defining the self (Bostrom and Sandberg 2009). These factors all need consideration and public debate as the use of these agents increases.

7.3. LIMITATIONS OF THE CURRENT WORK

As well as the limitations discussed in each individual chapter, there are also several overarching factors that might limit the conclusions that can be drawn from this thesis. These are discussed in the following section.

7.3.1. PRACTICE EFFECTS

Practice effects can make the interpretation of results difficult. For example in Chapter 3, a longitudinal study was conducted in order to determine the effects of amateur boxing. One of the strengths of the study was that de novo boxers were recruited, giving a pre-boxing baseline. However, this design means that any changes seen are confounded by practice effects. In order to remedy this, a control group should be tested at the same time intervals, so that the difference in the change over time can be established between groups. Because of the known issue of practice effects, the SST was used in this study, which has limited learning effects compared to other tests of executive function. Despite the confounding factor of practice effects biasing the results towards improvement over time, it was interesting that some of the boxers showed a decline in performance over time, despite the fact that they had prior experience of the task.

In Chapters 4 and 5 a cross-over design was utilised due to the heterogeneity of the patient populations investigated. However, because of this it is possible that there was an interaction between the drug effect
and order of administration. For example, for the SAH patients investigated in Chapter 4, regression analysis revealed that their baseline performance on the PAL task was associated with the change in performance after the administration of modafinil. However, the order of drug administration also contributed to this model, although it did make a smaller contribution compared to baseline performance as indicated by a lower Beta value, and the model remained significant when order was excluded. Despite this, the order effect does make the results more difficult to interpret. A cross-over design was also used for the PD study in Chapter 5, although order of drug administration did not contribute to the regression models. For both of these studies, where possible, parallel versions of the tasks were used and the order of the different task conditions was counterbalanced.

### 7.3.2. Difficulty Effects

An important consideration is the extent to which floor or ceiling effects might impact upon results. As described in Chapter 2, the cognitive tests used are well-validated and have been shown to be sensitive to pharmacological manipulation in healthy volunteers, as well as patient populations. However, the nature of the populations tested in Chapter 3 and Chapter 6 meant that they were all high functioning, and together their average estimated verbal IQ was 118, and their average years of education was 18.5, with most of the boxer cohort remaining in education over the course of the study. This is slightly higher than previous studies utilising modafinil in healthy volunteers (Turner, Robbins et al. 2003; Winder-Rhodes, Chamberlain et al. 2010).

A concern with healthy volunteer studies, especially when they are already high functioning, is the possibility of ceiling effects, meaning that cognitive tasks would not be sensitive to subtle impairment (for example in the boxers, or the sleep deprived placebo group), or to any potential improvements as a result of drug administration, such as modafinil administration in the sleep deprivation study. Indeed, the impairments seen in healthy adults following a year of amateur boxing, or one night’s sleep deprivation, was relatively mild, and in the boxers a number of participants continued to improve on the tests. The sleep deprived doctors on placebo did show cognitive impairment relative to the modafinil group, although they were still relatively high functioning. It is likely that the impairments seen would have been more pronounced if harder problems had been used for the One Touch Stockings (OTS) and reverse SSP tasks. Previous studies utilising OTS in healthy adults have shown that modafinil reduces errors on this task, as well as increasing the time taken to solve a problem (Turner, Robbins et al. 2003; Winder-Rhodes, Chamberlain et al. 2010). In contrast to these previous findings, no effect of modafinil was seen on errors on the OTS in the sleep-deprived doctors. The more difficult version of SSP was used, where participants have to touch the boxes in the reverse order to that in which they were highlighted by the computer (Section 2.3.3.1), in order to try and remove ceiling effects. Although the placebo group made more errors on this task compared to the modafinil group, they still passed all of the stages of the task. A more adaptive version of the test, where the span length keeps increasing until the participant fails, would have helped to illuminate the degree of remediation associated with modafinil.
At the other end of the spectrum, severely debilitated patients might have been unable to understand the task or be too fatigued to perform correctly. In Chapter 5 a number of patients did not complete the tests either because of time constraints or fatigue. In order to avoid floor effects and to ensure all the data was of a good quality, all participants were told they could take breaks whenever needed, and if participants reported feeling fatigued towards the end of the testing session it was terminated. Most participants did report that they enjoyed the testing sessions, and the visual analogue scales (VAS) did not suggest that the patients suffered from a lack of motivation. It is therefore unlikely that fatigue accounted for some of the difference in responses. However, one consequence of not continuing the session once participants reported feeling fatigued was that for some participants data was not collected from the complete range of tests.

7.3.3. Concurrent Medications

In the SAH and PD studies, the issue of concurrent medications was a potential confound to the results. This is particularly the case in the PD study, where all patients were receiving DRT. Patients were tested on their usual medications since it was expected that atomoxetine would only be used clinically in conjunction with dopaminergic medications.

Measures were put in place in order to try and control for concurrent medications. Specifically, a within-subjects design was used; patients were tested at the same time for both sessions, and were told to take their medication regime as usual. The testing sessions were timed so that patients were at their best ‘On’ during the session. This was both to ensure that participants were conformable, and also to standardise as far as possible the effect of medications across visits. When analysing the results in the PD study, Levodopa equivalent units (LEU) were entered into the regression model as an independent variable for each analysis, and were shown not to contribute to the model. Nevertheless, the PD patients in Chapter 5 were all well medicated which limits the generality of the results in PD, and therefore an area for future work would be to assess the effects of atomoxetine on a wide range of patients with varying degrees of impairments and concurrent medications.

7.3.4. Heterogeneity of Groups

The heterogeneity of a group could also influence the results, and this was an issue for all of the groups studied. The SAH patients were mixed in terms of the site of their aneurysm; the majority of patients (N=19) had an aneurysm located in the anterior communicating artery, whilst the remaining 9 patients had a mixture of aneurysm locations, covering the middle cerebral artery, posterior communicating artery, posterior inferior cerebellar artery, vertebral artery and basilar artery. It is likely that the different sub-groups of SAH patients may have respond differently to pharmacological intervention, as their cognitive profile has been shown to differ (Mavaddat, Kirkpatrick et al. 2000; Salmond, DeVito et al. 2006; Vieira, Azevedo-Filho et al. 2012). The small numbers of subtypes in each sample did not permit a comparison of the responses made by each of the different subtypes. Therefore, this represents an area for future research.
PD patients are known to be heterogeneous due to the nature of the neurodegenerative disease, and the variations in their medication status. It has been proposed that there are distinct subgroups of patients that are identifiable in the early stages of the disease. Using a data driven approach, Lewis, Foltynie et al. (2005) identified distinct cohorts of patients: younger onset, tremor dominant, non-tremor dominant, and rapid disease progression. It is likely that the different clinical subgroups have different pathological processes and foci, which may have different aetiological bases (Brooks 1999; Jellinger 1999). The PD participants in Chapter 5 also varied in the duration and severity of disease as indexed by the Unified Parkinson’s Disease Rating Scale (UPDRS) motor subscale. PD is a progressive neurodegenerative disease, so therefore the pathology varies greatly between patients (Braak and Del Tredici 2008). Variation in the dose of dopaminergic medication would also have contributed to the heterogeneity of the PD patients, as it has been demonstrated that medication has a profound effect on cognition in PD (Swainson, Rogers et al. 2000; Cools, Barker et al. 2001; Cools, Barker et al. 2003). In order to account for both disease severity and medication status, both of these were entered into the regression model in Chapter 5, and were shown to not explain the variance seen in change in impulsivity.

Young healthy adult populations tend to be less heterogeneous than patient populations. However, the amateur boxers investigated in Chapter 3 would have had varying boxing experience, as it was difficult to control for the number of sparring sessions they had during training, and hence it would have been difficult to quantify the amount of injury. In order to navigate these difficulties a university amateur boxing club was used to recruit the cohort, where training only took place in term time and in order to be eligible to fight the boxers had to have attended all of the training sessions. Furthermore, boxers were each used as their own baseline, and then their change in performance was examined in relation to their white matter density.

7.4. Directions for future work

There are several directions that emerge as promising areas for future research following the studies in this thesis. Discussed below are several of these avenues, which are all additional to the more immediate extensions discussed in the individual chapters, such as increasing the sample size, addition of appropriate controls, and testing additional patient subgroups.
7.4.1. **GENETICS OF COGNITIVE RESPONSE**

As discussed in section 7.2.2.5.1 genetic subtypes have an important effect on cognitive response to pharmacological intervention, and thus this represents an important avenue for future research. An understanding of which genes are specific to a disorder and aspects of cognitive function is important to determine whether a functional polymorphism has an effect on response to treatment, and to establish why only some patients respond to treatment. The effects of modafinil could be examined in patients genotyped for DA and NA polymorphisms. This would help to interpret effects such as those seen in the SAH patients in this thesis, and would help to develop a personalised approach to treatment in the future.

7.4.2. **TRANSLATION TO CLINICAL OUTCOMES**

There is a diverging pathway from neurochemistry to cognition, and then from cognition to behaviour. Because of this, it is essential to determine whether changes seen in laboratory studies will affect clinical outcome and quality of life for patients, particularly in the long-term.

Connected with this is the need to predict which patients are most likely to respond to treatment. This can be utilised by a focus on endophenotypes which are seen across disorders, rather than disorders which can be heterogeneous in nature. Therefore, future studies should focus on fractionating cognitive domains based on finer-grained neuropsychological analysis. The genetic, neural and neurochemical substrates of these domains need to be investigated, and the potential of these domains to supplement traditional diagnostic criteria should be examined.

7.4.3. **MAPPING PSYCHOTROPIC DRUG ACTION**

Understanding the precise pharmacokinetic and pharmacodynamic properties of pharmacological agents can help to contribute to both the understanding of neuropsychiatric and neurological disorders and their treatment. A potential area of exploration with agents such as atomoxetine and modafinil is the visualisation of their effects in vivo.

Using radioactively labelled tracers that bind to or are metabolised by specific molecules positron emission tomography (PET) and single photon computed tomography allow the direct assessment of neurotransmission in vivo, at baseline or in response to pharmacological challenges. For many years there has been a lack of suitable NA tracers, however there has been a new development recently with the successful deployment of NA transporter radioligands in humans (Takano, Varrone et al. 2008; Ding, Singhal et al. 2010; Hannestad, Gallezot et al. 2010). Using (S,S)-[\(^{11}\)C]methylreboxetine PET in healthy volunteers, methylphenidate that shown to significantly reduce NA transporter availability in a dose dependant manner in NA rich regions, such as the locus coeruleus (Hannestad, Gallezot et al. 2010). This paves the way for future investigations. Given the findings that modafinil modulates the NA system, but the fact that it does not always show an effect on SSRT, it would be interesting to see if its benefits can be
convincingly dissociated with respect to cortical DA and NA. It would also be interesting to determine whether modafinil and atomoxetine induce similar changes to NA transporter availability. Furthermore, as atomoxetine is used as a treatment in ADHD, and has the potential to be used to treat other disorders characterised by impulsivity, is would be interesting to see whether it induces similar changes in NA transporter availability compared to more traditional ADHD medications, such as methylphenidate. Functional imaging can also be utilised to assess the impact of treatment regimes. The effects of atomoxetine on functional activation has already been measured by fMRI, with atomoxetine modulating rIFG activation during inhibitory control (Chamberlain, Hampshire et al. 2009). However, this finding could be extended to investigate the effect of atomoxetine in ADHD and PD patients both on and off medication. It would also be interesting to visualise the effect of chronic administration of atomoxetine. Functional imaging can also be used for ‘genetic imaging’ whereby the effect of a functional polymorphism, such as COMT, can be shown to modulate activity, for example it has been shown that individuals homozygous for the met allele show stronger functional coupling between the PFC and hippocampus (Schott, Seidenbecher et al. 2006). This technique could be used to further investigate the effect of COMT on medication response in PD.

7.4.4. Future studies

This thesis has highlighted the potential for both atomoxetine and modafinil to be repurposed and used in novel ways. Atomoxetine has been shown to have the potential to modulate impulsivity in PD patients, and modafinil has been shown to remediate executive impairments under sleep-deprived conditions. These agents could be used transdiagnostically to treat these symptoms in other patient groups.

For example, individuals with substance abuse disorders may benefit from atomoxetine. Animal studies have shown that increased impulsivity is a risk factor for developing compulsive drug addiction. Specifically, rats who show impulsive behaviour on the SCSR task tend to escalate intra-venous cocaine self-administration, and will tolerate foot shock in order to obtain the drug (Dalley, Everitt et al. 2011). Studies in humans have identified impulsivity as an endophenotype that may be a risk factor for developing a substance-abuse disorder. Substance-dependent individuals and their biological siblings both report greater imperative tendencies as assessed by the Barratt impulsivity scale, in comparison to healthy controls (Ersche, Turton et al. 2010). Furthermore, prolonged SSRTs are seen in both stimulant-dependant individuals and their non-dependent siblings, compared to controls (Ersche, Jones et al. 2012), and children of alcohol dependent individuals also display cognitive control impairments on a Stroop task, with corresponding abnormalities in the rIFG (Silvery, Rogowska et al. 2011). This thesis suggests, in accordance with previous findings, that atomoxetine modulates impulsivity, particularly response inhibition as indexed by the SST. It would therefore be of interest to investigate the effect of atomoxetine on response inhibition in individuals with substance abuse disorder and their first degree relatives. Atomoxetine could also be used to treat impulsivity in other disorders. In this thesis, a minority of the amateur boxers showed a lengthening of SSRT over time, which was associated with white matter
changes in the rIFG. The frontal lobes are particularly vulnerable to damage following TBI, and this is reflected by the fact that disinhibition and personality changes are one of the characteristic impairments seen in this population. The increases in impulsivity that are related to TBI could be remediated using atomoxetine. This is another potential avenue for future research.

There are also novel ways in which the atypical stimulant, modafinil, could be used. In Chapter 6, modafinil was shown to improve executive functions under conditions of sleep deprivation. Sleep disturbances are a common feature of all neuropsychiatric and neurodegenerative disorders, so this finding suggests that modafinil may be of benefit to many other patient populations, including PD patients. A recent study has shown that modafinil increases ratings of task enjoyment in healthy adults during testing session (Muller.64.490). This, together with its arousal properties, suggests that modafinil could be a potential treatment for apathy in, for example, normal pressure hydrocephalus.

7.5. **Achievements of the Aims of this Thesis**

Broadly, this thesis has achieved the aims set out in Chapter 1, and also has addressed the hypotheses detailed in the individual chapters. The first aim of this thesis was to quantify cognitive impairment in a range of disorders, as this is the crucial first step in identifying novel treatments. The second aim of this thesis was to determine the potential of three pharmacological agents (modafinil, physostigmine, and atomoxetine) to be repurposed for new clinical use, based on their known pharmacological effects.

Cognition was probed using well-validated tests from the CANTAB battery. There was a particular emphasis on impulsivity, as this cognitive domain has been shown to be an endophenotype that is present across a number of disorders such as head injury, substance abuse, and ADHD. The effect of amateur boxing on a facet of impulsivity, response inhibition, was investigated in a longitudinal study. Although this study should be considered to be preliminary, an important association was demonstrated between SSRT and white matter density in the rIFG, an area that has been shown to be crucial for successful response inhibition on this task.

This thesis also demonstrated that both atomoxetine and modafinil have potential as pharmacological treatments in disorders for which they are not currently approved. The most promising finding was that atomoxetine was shown to be a potential treatment for impulsivity in PD. Given the fact that a significant minority of PD patients develop ICBs, possibly due to their dopaminergic therapy, this finding has real clinical relevance. This paves the way for further studies that should include PD patients with ICBs and also explore the possibility of chronic use in this patient group. Modafinil was also shown to improve executive functions in sleep-deprived individuals, and most strikingly improved performance on the extra-dimensional stage of a set-shifting task, which is the first time that this effect has been demonstrated. This finding suggests that modafinil may have real clinical utility in disorders where sleep disturbance is common.
Comparing the results obtained with modafinil and atomoxetine has indicated that there are differences in their mode of action. It was hypothesised that both modafinil and atomoxetine would affect cognitive domains that are neuromodulated by the noradrenergic system, although this hypothesis was not completely supported, as modafinil did not modulate SSRT in either the sleep-deprived healthy adults or the SAH patients. Overall, the findings of this thesis converge with the current thinking that atomoxetine has relatively specific effects on the noradrenergic system, whereas modafinil seems to modulate a number of neurotransmitter systems. The differences between the profiles of atomoxetine and modafinil suggest that they may have a distinct utility in clinical practice. Atomoxetine may be an effective drug when only specific symptoms of impulsivity characterise a disorder, whereas modafinil may be a more effective treatment in disorders where multiple cognitive domains are affected.

Another significant finding of this thesis was the individual variation seen across participants in terms of drug response. These findings have been considered in relation to the Yerkes-Dodson inverted-U-Shaped function. This has important clinical implications, suggesting that a drive towards personalised treatments is necessary. Overall, the main contribution of this research is to demonstrate that both modafinil and atomoxetine have the potential to be repurposed and used as novel treatments in certain patient populations.
References


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