Neuroanatomical Correlates of Cognitive Dysfunction in Obstructive Sleep Apnoea

Thesis submitted to Imperial College, London for the degree of Doctor of Philosophy in the Faculty of Science

Martin Glasser, MBBS

CID: 00234721

Clinical and Academic Unit of Sleep and Breathing

National Heart and Lung Institute, Imperial College London

Royal Brompton Hospital, Fulham Road, London

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Copyright Declaration

Declaration of Originality

I, Martin Glasser, hereby declare that this thesis contains the results of my own work except where otherwise acknowledged. The studies presented in this thesis were conceived and designed with the assistance of my supervisors, Professor Morrell and Professor Simonds and Dr Ivana Rosenzweig. In addition, I acknowledge the invaluable assistance of Dr Alison McMillian who randomised the patients in Study 2 (Chapter 4) and collected the sleep data in Study 3 (Chapter 5). Peter Drivas set up the scanning sequences for Study 2 (Chapter 4) on the Royal Brompton scanners and captured the images. The physicists, radiographers and radiologists at Charing Cross Hospital were similarly involved in Studies 1 and 3 (Chapter 3 and 5).

The MRI scans for Study 1 (Chapter 3) had already been collected prior to my re-analsyis in the study. Statistical support was received from Dr Milan Milosovic for studies 1 and 2 (Chapter 3 and 4). Chi Cheng Tsai assisted in the statistical analysis of study 3 (Chapter 5).

Information derived from the work of others and discussed in this thesis is referenced in the text and listed in the bibliography.

The results of studies contained within this thesis have previously been presented. A list of publications arising from this work follows.

PUBLICATIONS ARISING FROM THIS THESIS

Published Original Research Papers, Reviews and Editorials

M. Glasser, N. Bailey, A. McMillan, E. Goff, M.J. Morrell, Sleep apnoea in older people. Breathe 2011; 7: 248-256 (Review)

Morrell MJ, **Glasser M**. The brain in sleep-disordered breathing: a vote for the chicken? Am J Respir Crit Care Med. 2011 May 15;183(10):1292-4. (Editorial)

Morrell MJ, **Glasser M**, McMillan A, Rosenzweig I. CPAP for the treatment of cognitive dysfunction in obstructive sleep apnoea. Neuropsychiatry News. 2012 Winter.

Rosenzweig I, Kempton MJ, Crum WR, **Glasser M**, Milosevic M, Beniczky S, Corfield DR, Williams SC, Morrell MJ. Hippocampal hypertrophy and sleep apnea: a role for the ischemic preconditioning? PLoS One. 2013 Dec 13;8(12):e83173

Rosenzweig I, **Glasser M**, Polsek D, Leschziner GD, Williams SC, Morrell MJ. Sleep apnoea and the brain: a complex relationship. Lancet Respir Med. 2015 May;3(5):404-14. (Review)

Rosenzweig I, **Glasser M**, Crum WR, Kempton MJ, Milosevic M, McMillan A, Leschziner GD, Kumari V, Goadsby P, Simonds AK, Williams SC, Morrell MJ. Changes in Neurocognitive Architecture in Patients with Obstructive Sleep Apnea Treated with Continuous Positive Airway Pressure. EBioMedicine. 2016 May;7:221-9. doi: 10.1016/j.ebiom.2016.03.020. Epub 2016 Mar 25

Abstracts

Glasser M, Rosenzweig I, McMillan A, Drivas P, Satkunam K, Man WDC, Simonds AK, Morrell MJ. Neuroanatomical Correlates Of Cognitive Dysfunction In Obstructive Sleep Apnoea: An Ongoing Study. A109. Sleep disordered breathing: Cardiovascular, metabolic and Neurocognitive outcomes. May 1, 2013, A2319-A2319. First published online May 09, 2013 as doi:10.1164/ajrccm-conference.2013.187.1_MeetingAbstracts.A2319

Other publications completed during the Thesis

Carlisle T, Carthy ER, Glasser M, Drivas P, McMillan A, Cowie MR, Simonds AK, Morrell MJ Upper airway factors that protect against obstructive sleep apnoea in healthy older males. . Eur Respir J. 2014 Sep;44(3):685-93

Prizes

NIHR BRU/BRC Experimental Training Camp 2011, MPHRP Project Winner

American Thoracic Society Abstract Scholarship Award, 2013

Abstract

Obstructive sleep apnoea (OSA) has been reported to be associated with brain hypotrophy and cognitive dysfunction; however, whether these normalise after treatment is unclear. The overall aim of this thesis is to investigate the relationship between OSA and brain structure using FreeSurfer (a new automated technique that reliably measures brain structures). I have investigated changes in brain morphology and the newly described phenomenon in OSA of ischaemic preconditioning. Chapters 4 and 5 will also assess brain structural response to CPAP, and investigate the association between brain structure and cognitive function in OSA.

Chapter 3 reports an observational study investigating brain structure. FreeSurfer analysis of magnetic resonance imaging (MRI) found OSA patients had hypertrophy in the right hippocampus (p=0.03) and right choroid plexus (p=0.02) but hypotrophy of the corpus callosum (p=0.04) compared to healthy controls.

Chapter 4 reports a randomised controlled trial of CPAP in OSA. At baseline hypotrophy was seen in the corpus callosum (p=0.03) and pallidum (p=0.03) of OSA patients compared to healthy controls. Hypertrophic changes in the right thalamus were seen in the CPAP group after 1 month (p=0.06), associated with improvement in verbal memory (p=0.04).

Chapter 5 reports a randomised controlled trial of CPAP in older patients with OSA. A significant decrease in left fimbria volume was seen in the CPAP group (p=0.01). A significant increase in the left presubiculum volume was seen in the best supportive care group (p=0.03). No hippocampal hypertrophy was seen in the CPAP group.

In summary, young and middle-aged OSA patients had evidence of brain hypotrophy, but also areas of hypertrophy that may signify dendritic sprouting and increased connectivity as a result of ischaemic preconditioning. This allows recovery of brain hypotrophy after CPAP treatment. This was not seen in older OSA patients suggesting an age-related difference which may have implications for OSA treatment in older people.

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I would like to thank my PhD supervisors, Professor Mary Morrell and Professor Anita Simonds as well as my honorary third supervisor Ivana Rosenzweig for their invaluable support and assistance. I know I haven't always made it easy for you, but you have made it as easy as it could be for me.

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My parents have always encouraged my search for knowledge and professional qualifications and this is perhaps the pinnacle of that quest. Their support, financial, emotional and with childcare has been limitless and I hope I can be as good a role model to my children as you both continue to be to me.

Azriella, Tsofia and Ashira no matter how difficult a day I am having you always make me smile. I love you more than you will ever know and you make me so proud.

Finally, I would like to dedicate this thesis to my wife and best friend, Lucinda. You are everything I wish I could be; abundantly kind, excessively giving, and meticulously organised. You have been my bedrock throughout this whole process and I could not have done it without you.

Table of contents

Abbreviations

ACER – Addenbrooke's Cognitive Examination – revised

AD – Alzheimer's disease

AHI – Apnoea Hypopnoea Index

ANCOVA – Analysis of Covariance

ASV - Bilevel Ventilation and Adaptive Servoventilation

Auto-CPAP – Autotitrating CPAP

BDNF - Brain Derived Neurotrophic Factor

BRU - NIHR Respiratory Biomedical Research Unit

BSC – Best Supportive Care

CPAP – Continuous Positive Airway Pressure

DBM - Deformation based morphometry

DG - Dentate Gyrus

ECG – Electrocardiogram

EEG – Electroencephalogram

EMG - Surface Electromyogram

EOG – Electro-oculogram

ESS – Epworth Sleepiness Scale

FS – FreeSurfer

ICV - Intracranial Volume

IH – Intermittent hypoxia

LM – Logical Memory

MMSE – Mini Mental Score Examination

MRI – Magnetic Resonance Imaging

NREM – Non-rapid Eye Movement

OSA – Obstructive Sleep Anoea

OSAHS – Osbstuctive Sleep Apnoea-hypopnoea syndrome

PLM – Periodic Limb Movements

RF – Radiofrequency

SDB – Sleep Disordered Breathing

SWA – Slow Wave Activity

TLR4 - Toll-like Receptor 4

TMT – Trail Making Test

VAS – Visual Analogue Scale

VBM - Voxel Based Morphometry

Chapter 1

Introduction

1.1 OVERVIEW

Obstructive sleep apnoea (OSA) is the name given to repetitive episodes of sleep-related upper airway collapse, which occlude the airway and lead to hypoxaemia plus hypercapnaemia. These periods of sleep apnoea are frequently terminated by arousal from sleep, which can lead to symptoms of sleepiness, as well as cardiovascular disease and neurocognitive impairment. The cognitive impairment associated with OSA is the particular focus of the studies presented in this thesis.

Chapter 1 outlines the aetiology, pathophysiology and epidemiology of OSA, as well as a brief discussion of some of its sequelae. The neurological consequences of OSA will be reviewed focusing on cognitive impairment. The chapter will also describe the cognitive domains that are thought to be affected by OSA, as well as the functional outcomes e.g. driving impairment. Possible mechanisms of cognitive impairment will be considered. These will focus on a) attention deficits as a result of sleepiness, b) changes in brain structure as a result of either intermittent hypoxia or sleep fragmentation. The different methods of assessing cognitive function, brain structure and sleepiness will be included in Chapter 2. The final section of Chapter 1 will list the specific aims of the thesis.

1.2 OBSTRUCTIVE SLEEP APNOEA

OSA is at the severe end of a disease spectrum referred to as '*sleep disordered breathing'* (**Fig 1.1**). Snoring and nocturnal airflow limitation (sometimes called '*upper airway resistance syndrome'*) is at the mild end, and complete obstruction resulting in cessation of airflow (apnoea) is at the other. Partial obstruction of the upper airway is in the middle of the disease severity spectrum, and results in reduction of airflow termed 'hypopnoea'. The definition of what constitutes a hypopnea varies but it is often considered that a 50% reduction in airflow, in combination with a 4% decrease in arterial oxygen saturations or an arousal from sleep is classed as a hypopnoea (see Chapter 2 for more details).

OSA severity is described by the apnoea-hypopnoea index (AHI). This is the mean number of apnoeas and hypopnoeas per hour of sleep. OSA is defined as 5 events or more per hour. Mild OSA is usually an AHI less than 15 events per hour. Moderate OSA is defined as an AHI between 15 and 30 events per hour, and an AHI greater than 30 events per hour is considered severe (**Fig 1.1**). Obstructive sleep apnoea-hypopnoea syndrome (OSAHS) is defined as the presence of OSA in combination with excessive daytime sleepiness.

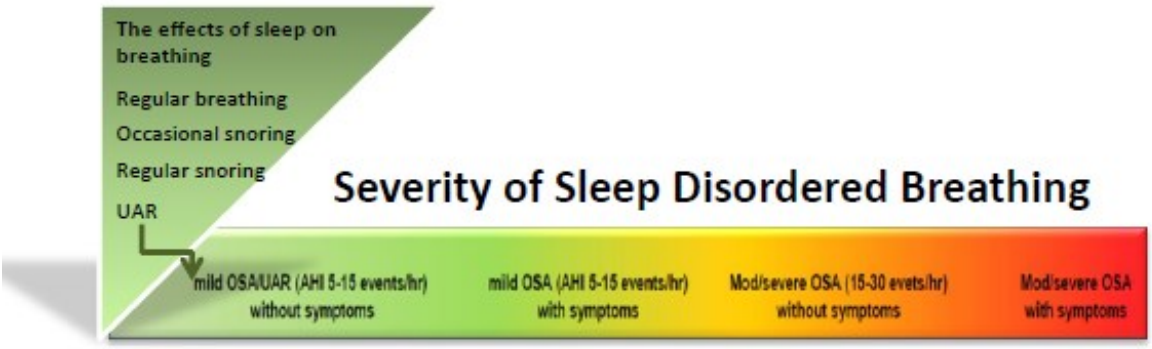

Figure 1.1: The spectrum of disease referred to as '*sleep disordered breathing'.* UAR: Upper airway resistance, OSA: Obstructive sleep apnoea, AHI: Apnoea/Hypopnoea Index

1.2.1 **The aetiology of OSA**

The aetiology of OSA is complex and multi-factorial. In simple terms the cycle of OSA is produced by upper airway occlusion leading to apnoea, which in turn produces changes in blood gases, and a subsequent arousal from sleep that triggers the airway re-opening and the resumption of airflow (**Fig 1.2**). This section will give an overview of the factors that contribute to the development of OSA.

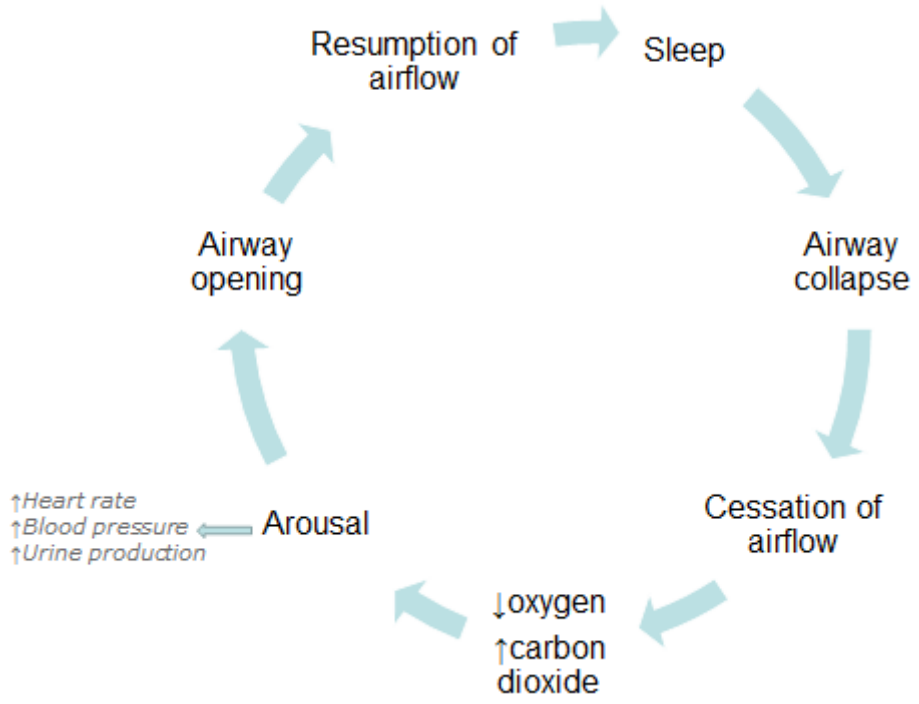

Figure 1.2 The cycle of obstructive sleep apnoea

Upper airway narrowing in OSA: The most common site of upper airway collapse in patients with OSA is the retroglossal airway; (**fig 1.3**). However, narrowing can occur at different points along the airway and any condition that leads to upper airway narrowing will predispose to OSA (Dempsey *et al.*, 2010), including craniofacial abnormalities (Mixter *et al.*, 1990; Pijpers *et al.*, 2004), enlargement of upper airway soft tissue structures (Hwang *et al.*, 2013) and external compression due to obesity (Pahkala *et al.*, 2013) or oedema (Redolfi *et al.*, 2009). However, it is important to note that people with anatomical narrowing of the upper airways are able to maintain airway patency during wakefulness, and it is only during sleep that airway obstruction occurs.

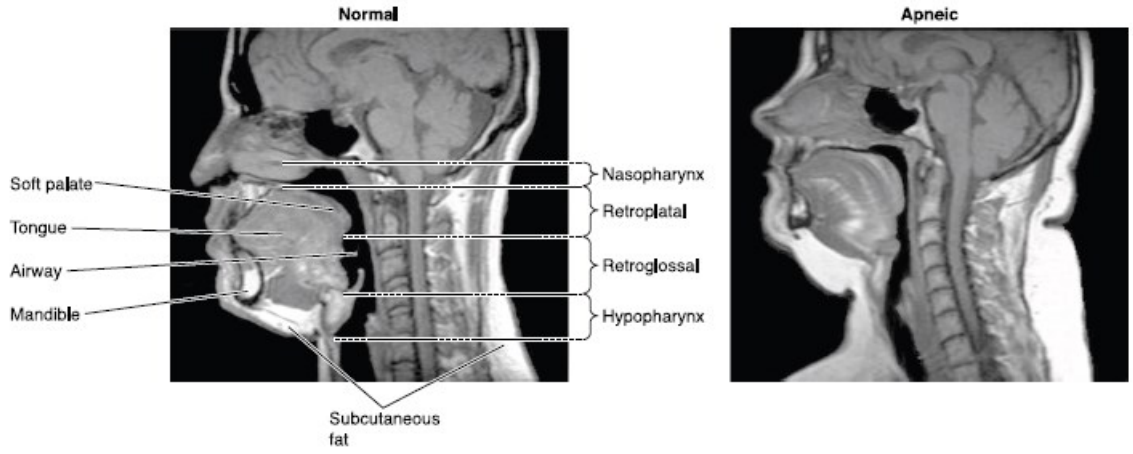

Figure 1.3 Midsagital MRI in a normal subject (left) and a patient with Severe OSA (Apnoeic – Right). Highlighted are the 4 regions of the pharynx, the upper airway soft tissues and craniofacial structures that can contribute to OSA. Fat tissue is shown in white. Note that the OSA patient has more fat tissue around the neck, and in the tongue. He/She also has a smaller retroplatal and retroglossal airway (Dempsey *et al.*, 2010).

Hypoventilation during sleep: The respiratory minute volume in healthy individuals has been shown to be up to 15% lower during sleep, compared to wakefulness (Douglas *et al.*, 1982). A number of factors contribute to this reduction. During wakefulness there is a ventilatory drive from the cortex called the '*Wakefulness Drive to Breathe'*. This cortical drive is absent during sleep (Fink, 1961; Morrell *et al.*, 1993; Wellman *et al.*, 2004). Furthermore, a simultaneous sleep-related reduction in chemosensitivity also occurs (Dempsey & Skatrud, 1986). These two factors reduce the ventilatory drive to the respiratory pump muscles, which produces a relative hypercapnia during sleep (Dempsey & Skatrud, 1986; Leevers *et al.*, 1994).

Reduced muscle tone during sleep: During sleep muscle tone is reduced throughout the body. Reduced diaphragm and accessory respiratory muscle tone contributes to the reduction in minute volume that has been recorded during sleep (Orem *et al.*, 2002). The reduced tone in the upper airway

dilator muscles results in an increased resistance to airflow in the pharynx. This hypotonia can produce upper airway collapse in predisposed individuals as a result of negative inspiratory pressure generation in the thorax, and positive extra-luminal pressure, produced by gravitational forces, and adipose tissue. The critical collapsing pressure of the upper airway (termed Pcrit) is ultimately determined by the transmural pressure (Dempsey, 2010).

Apnoeas caused by upper airway obstruction are terminated by an arousal from sleep that leads to increased tone in the airway dilator muscles and a return to waking-state ventilatory control; hence regular breathing. The physiological conditions that induced the airway obstruction return on resumption of sleep, and OSA patients can experience multiple cycles of sleep-apnoea throughout the night (**Fig 1.2**).

1.2.2 **The prevalence of OSA**

The prevalence of OSA appears to be rising. This is likely to be due to the aging population, and an obesity epidemic in developed countries. Both obesity and aging are high risk factors for OSA. This section describes the way in which the prevalence of OSA has increased over the past 20 years.

The most widely quoted figures for the prevalence of OSA are taken from the Wisconsin Sleep Cohort (Young *et al.*, 1993). During the period between 1988 and 1994 the prevalence of moderate-severe OSA (AHI ≥ 15 events/hour) in the Cohort was estimated to be 8.8% in males aged 30-70. By comparison, during the period between 2007 and 2010 the estimated prevalence had risen to 13%. OSA is less common in women, but a similar increase has occurred from 4% in the period between 1988 and 1994, to 6% in the period between 2007 and 2010 (Young *et al.*, 1993; Peppard *et al.*, 2013). As mentioned above the most likely reason for the increasing prevalence of OSA is the increasing prevalence of obesity. In 1993, 15% of the UK adult population was obese (BMI >30 Kgm^2). In 2011 this had risen to 25% (Health and Social Care Information Centre; http://www.hscic.gov.uk).

OSA is also more common with increasing age. The Wisconsin Sleep Cohort data showed that 10% of men aged 30-49.9 years had moderate-severe OSA, compared to 17% of men aged 50-70 years. This age distribution is even more pronounced in women where the prevalence increases from 3% in 30-49.9 year olds, to 9% in women from 50-70 years. The increased prevalence of OSA in older people is supported by other epidemiological studies, which estimate the prevalence of OSA in people over 65 years to be approximately 20%, with some estimates as high as 70% (Ancoli-Israel *et al.*, 1989; Young *et al.*, 2002b; Hader *et al.*, 2005b).

The factors that contribute to the age-related increase in the prevalence of OSA include a reduction in pharyngeal muscle function (Worsnop *et al.*, 2000b) and a more collapsible upper airway (Kirkness *et al.*, 2008b). Age-related differences in upper airway structure include a decrease in the size of the upper airway lumen (Carlisle *et al.*, 2014), associated with an age-related changes to the position of the hyoid bone (Pae *et al.*, 2008). An increase in arousal frequency, has an associated change in the stability of breathing (Browne *et al.*, 2003a). Perhaps the most important change is an increase in the prevalence of co-morbidities. For example, patients with chronic heart failure are more likely to have both obstructive and centrally mediated sleep apnoea (Vazir *et al.*, 2007).

1.2.3 **The Sequelae of OSA**

OSA is known to impact multiple organ systems, and the most commonly investigated are cardiovascular and metabolic changes. However, other conditions as diverse as skin cancer, Alzheimer's disease and retinal dysfunction have been linked with OSA. This section will briefly outline the cardiovascular and metabolic effects of OSA. The association between OSA and brain function, which is the focus of this thesis will be covered in Section 1.3.

Cardiovascular: The association between OSA and hypertension has been known for many years (Fletcher *et al.*, 1985; Strohl *et al.*, 1994; Bixler *et al.*, 2000). Longitudinal data from the Wisconsin Sleep Cohort suggests that hypertension is a consequence of OSA, with a three fold increased risk of hypertension in OSA patients with severe disease (Peppard *et al.*, 2000). OSA patients are also at increased risk of coronary artery disease and heart failure (Mooe *et al.*, 1996; Mooe *et al.*, 2001; Shahar *et al.*, 2001).

The mechanisms that lead to increased cardiovascular disease in OSA have been studied in both animal and human models. The difficulty in demonstrating causality is that both OSA and cardiovascular disease are linked with obesity. The most likely factors by which OSA (independent of obesity) causes cardiovascular disease are increased sympathetic nervous system activity, hypoxic and oxidative stress, systemic inflammation, and mechanical factors secondary to intrathoracic pressure oscillations, e.g. reduced left ventricular stroke volume, systemic arterial pressure, cardiac output and heart rate (Somers *et al.*, 2008), (Drager *et al.*, 2011).

Metabolic: OSA is strongly associated with obesity and therefore as with cardiovascular disease, the extent to which there is an independent association between OSA and metabolic disease is debated. Current data appear to show a particular link with insulin resistance and type-2 diabetes mellitus (Ancoli-Israel *et al.*, 1989; Strohl *et al.*, 1994; Punjabi *et al.*, 2002). Specifically the link with insulin resistance may be a combination of both central and visceral obesity, although sympathetic drive from

frequent arousals, intermittent hypoxia and sleep fragmentation may also play a role (Punjabi *et al.*, 2004; Spiegel *et al.*, 2009).

Additional metabolic factors have also been studied, including blood lipid levels (triglycerides, low density lipoproteins, non-high density lipoproteins and total cholesterol). The impact of OSA on triglycerides as a marker of cardiovascular disease has also been evaluated, with 2 months of treatment leading to a reduction in triglyceride and cholesterol levels (Phillips *et al.*, 2011). Recent data from the UK show that diabetic patients with OSA are more likely to have diabetic retinopathy and neuropathy, and an ongoing treatment trial is investigating the role that reducing OSA could have on diabetic retinopathy (Harsch *et al.*, 2004; Kosseifi *et al.*, 2010; Mason *et al.*, 2012; Rudrappa *et al.*, 2012).

1.2.4 **The Treatment of OSA**

There are many treatments for OSA and their application depends in part on the severity of the disease (**Fig 1.4**). For patients with mild disease modifying lifestyle (weight loss, stopping smoking, and cardiovascular exercise), increasing total sleeping time, and improving the sleeping environment (e.g. remove digital screens etc. from the bedroom) can help. Additionally, improving diet and reducing stimulants, such as caffeine, before bedtime is advisable. For patients who only have OSA when sleeping in the supine position, positional aids can help. Optimising medical management of co-morbidities is another key part of treatment e.g. pain control, and optimising cardiorespiratory function. In patients with mild to moderate disease, oral mandibular advance devices are often helpful. The most commonly used treatment for OSA is positive airway pressure, which acts as a pneumatic splint blowing air into the upper airway and preventing collapse (**Fig 1.5**). There are different forms of pressure delivery, including continuous positive airway pressure (CPAP), autotitrating CPAP (auto-CPAP), bilevel ventilation and adaptive servoventilation (ASV). OSA is most effectively treated with CPAP, and this section will summarise the reasons why CPAP is considered the mainstay of treatment for patients with moderate to severe OSA.

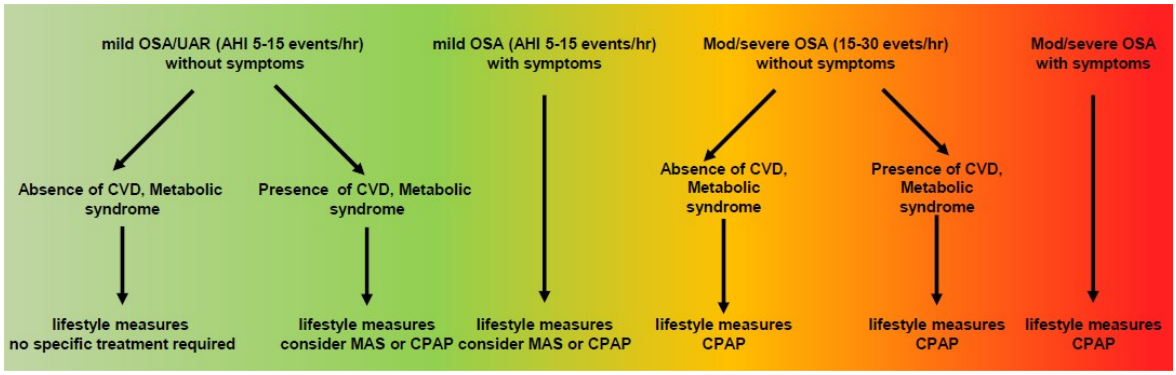

Figure 1.4 Algorithm for the treatment of *mild, moderate, and severe obstructive sleep apnoea (OSA). Upper airway resistance (UAR), cardiovascular disease (CVD).*

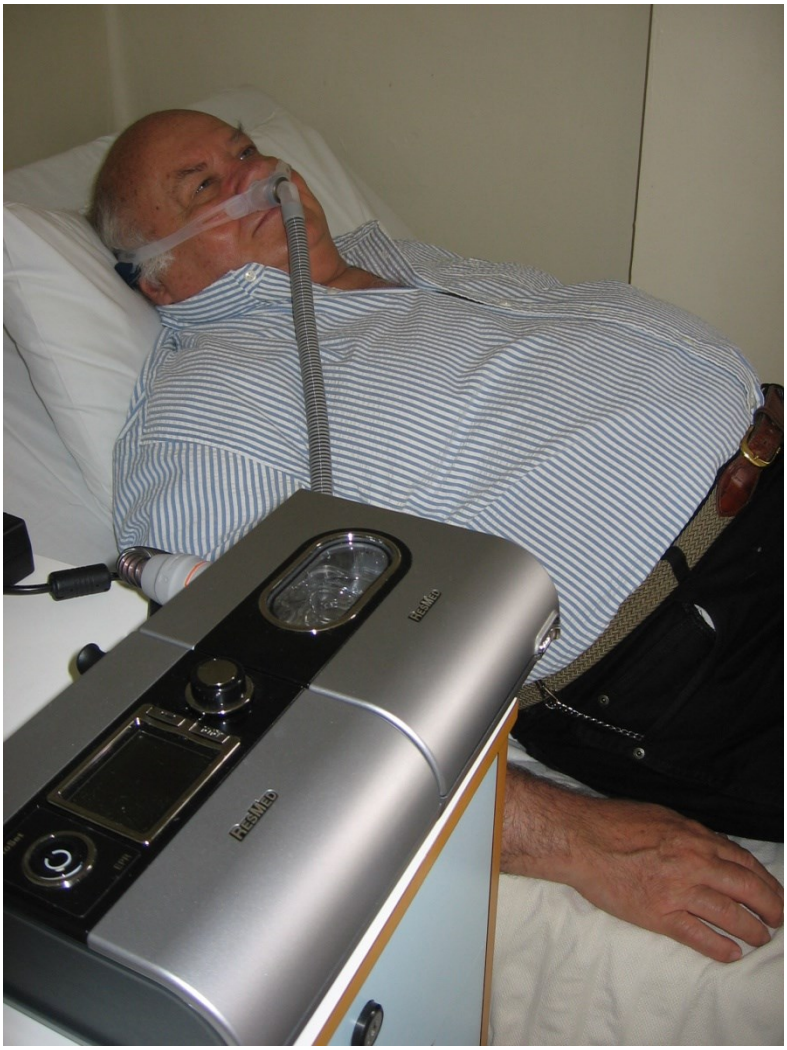

Figure 1.5 A patient using CPAP

The recent National Institute for Health and Care Excellence (NICE) Health Technology Appraisal of the use of CPAP concluded that it was an efficacious and cost-effective treatment for OSA syndrome

(McDaid *et al.*, 2009). The benefit of CPAP is measured in most trials as an improvement in sleepiness as measured by the Epworth Sleepiness Scale (ESS; See Chapter 2, Section 2.3.1). The ESS is a subjective measure of sleepiness and treatment with CPAP (versus conservative treatment) reduces sleepiness by approximately 2.7 points (95% confidence interval -3.45 to -1.96). The reduction in sleepiness is proportional to the severity of the disease and therefore patients with severe OSA often feel more relief of symptoms than those with mild disease.

The cost of CPAP treatment is relatively cheap (compared to no treatment) per quality adjusted life year (QALY) gained at <£4,000 (McDaid *et al.*, 2009). However, the majority of the cost is associated with a reduction in the incidence of cardiovascular disease, stroke, and road traffic accidents associated with sleepiness. Thus in patients who are not sleepy, and have a low cardiovascular and/or cerebrovascular risk CPAP may not be cost-effective.

The effectiveness of CPAP therapy is also related to the adherence to treatment. Adherence to CPAP is estimated to range from 46% to 83%, with an average nightly usage of 2.39 hours per night in minimally symptomatic middle-aged OSA patients (Craig *et al.*, 2012) and 2 hours 22 mins in older OSA patients at 12 months (McMillan *et al.*, 2014). This is somewhat lower than the 4 hours per night on 70% of nights which is commonly used as the benchmark for CPAP adherence, and less than the optimal outcomes achieved with a usage of at least 5 hours per night. Factors that impact CPAP compliance are shown in Table 1.1 (adapted from the Oxford Handbook of Medicine chapter submitted for publication). The percentage of patients who continue using their CPAP falls over time, e.g. from 84% at the end of the first year to 68% after 4 years, remaining at this level for a further 3 years. The weighted average for studies that report discontinuation rates over 3 years was estimated to be 3.8% per annum

Table 1.1: Predictors of poor CPAP adherence include
Patient characteristics – increased nasal resistance, depression
Disease characteristics – either severe or mild minimally symptomatic disease
Psychological or social – less self-efficacy, poor social support, limited disease or treatment knowledge
Technical – lack of heated humidification and flexible pressure

1.3 COGNITIVE FUNCTION AND OSTRUCTIVE SLEEP APNOEA

There have been many studies of OSA and cognitive function. These have shown an association between OSA and a broad range of neurocognitive impairments (Bucks *et al.*, 2013). A recent study by our group demonstrated deficits in verbal memory (Twigg *et al.*, 2010), while other studies have found deficits in spatial memory (Varga *et al.*, 2014) (Daurat *et al.*, 2008), reaction time (Bedard *et al.*, 1991; Bedard *et al.*, 1993) and numerical memory (Verstraeten *et al.*, 2004). Even in cognitive tests where no impairment has been detected, functional imaging has demonstrated that sleepy OSA patients utilise spare capacity in brain regions not utilised by healthy controls to achieve normal cognitive function (Ayalon *et al.*, 2006). However, despite these data, the impact of OSA on cognitive function remains controversial. Indeed, it has recently been argued that there is only a weak correlation between (subjective) cognitive complaints, and objective cognitive function in patients with OSA (Vaessen *et al.*, 2014). The wide variation in the reported cognitive dysfunction of patients with moderate to severe OSA was the starting point for the studies reported in Chapters 3-5 of this thesis.

1.3.1 Impaired Cognitive function in OSA

A recent meta-analysis determined that deficits in attention/vigilance, delayed long-term visual and verbal memory, visuospatial/constructional abilities and executive dysfunction have been consistently reported in patients with OSA (Twigg *et al.*, 2010; Bucks *et al.*, 2013). The cognitive impairment appears to be associated with the severity of hypoxaemia, while the attention and vigilance dysfunction is more likely to be associated with the degree of sleep fragmentation (Bucks *et al.*, 2013). Working memory, short-term memory, and global cognitive functioning and language ability appear to be largely unaffected by OSA (Bucks *et al.*, 2013).

There are several possible explanations for the wide range of results seen in these studies: The subjective tests used may not be specific enough to detect accurately the cognitive deficits (see

Chapter 2, Section 2.4 for a detailed explanation of the cognitive tests used in this thesis), indeed the objective tests used are frequently designed to detect deficits in patients with brain-injuries, and therefore may not be able to capture the milder impairments that occur in OSA (Vaessen *et al.*, 2014). Similarly, one day (or night) of testing provides only a 'snapshot' of the patient's ability. This kind of testing is unable to take account of time-dependent factors, such as fluctuation over time and various circadian influences. Moreover, cognitive domains frequently involve more than one construct. Therefore only carefully deconstructed analyses of the different cognitive test results can accurately assess patient deficits (Olaithe *et al.*, 2014). Finally, the cognitive dysfunction may be a sign of psychological distress, rather than impairment (Pullens *et al.*, 2010; Vaessen *et al.*, 2014). Of note, cognitive reserve and genetic vulnerability (e.g. apolipoprotein e4 genotype) are likely to be factors in the susceptibility to cognitive dysfunction (Rosenzweig *et al.*, 2013c; Olaithe *et al.*, 2014; Rosenzweig *et al.*, 2014). Some other important factors considered during the design of the studies presented in this thesis were the duration of exposure to the disease, the role of the blood-brain barrier and co-morbidities such as hypertension, metabolic dysfunction and systemic inflammation.

1.3.2 Treatment of Cognitive Function in OSA

Both pharmacological and non-pharmacological treatments for OSA have been shown to improve cognitive function in patient with OSA. Several meta-analyses suggest that CPAP treatment reduces sleepiness and improves mood, and that these changes are associated with improvements in objective cognitive functioning (Giles *et al.*, 2006; Marshall *et al.*, 2006; Kylstra *et al.*, 2013; Vaessen *et al.*, 2014). Although less successful, drugs such as donepezil, phylostigmine, and fluticasone have also been used to try to improve cognitive outcomes in treated OSA patients (Kohler *et al.*, 2009; Mason *et al.*, 2013).

Of interest for the studies presented in this thesis, CPAP treatment also appears to reverse some, but not all, neuroanatomical changes. Specifically the study by Canessa *et al*, 2011 showed that 3 months of CPAP treatment improved cognitive function in several domains and that these improvements were correlated to grey matter volume increases in frontal and hippocampal regions (Canessa *et al.*, 2011). Another recent study showed significant improvements involving memory, attention, and executive functioning that correlated with white matter changes after 12 months of treatment with CPAP (Castronovo *et al.*, 2014). However, in these studies not all areas showed complete reversal of tissue damage, or deficits in cognition, suggesting that initiation of prolonged treatment may be needed as early as possible in the disease process (Kushida *et al.*, 2012; Prilipko *et al.*, 2012; Ferini-Strambi *et al.*, 2013; Yaffe *et al.*, 2014).

To investigate the effect of disease duration on cognitive function, studies have been carried out looking into the cognitive performance and effects of treatment in children with OSA (Gozal, 1998; Biggs *et al.*, 2014). In a recent study of children with sleep disordered breathing (SDB), followed-up for four years, treatment led to improvements in several aspects of neurocognition, collectively categorised as performance IQ (Biggs *et al.*, 2014).

Performance IQ represents fluid intelligence and is reflective of incidental learning. It describes the subject's ability to adapt to new situations (Cattell, 1967). In the study by Biggs et al, improvements were recorded in tasks associated with spatial visualisation, visuo-motor coordination, abstract thought and non-verbal fluid reasoning (Biggs *et al.*, 2014). However, overall improvements in the academic ability or behaviour were less clear. Furthermore, worsening of verbal IQ, which, unlike performance IQ, is more likely to be affected by formal education and learning experiences, was noted in a treated group (Biggs *et al.*, 2014). No conclusive explanation for this finding was provided, and no statistically significant association between the reduction in verbal IQ performance and treatment was demonstrated (Biggs *et al.*, 2014).

Conversely, in the seminal study by Gozal in 1998, younger children with SDB were followed for 12 months after treatment and significant improvements in academic performance were recorded (Gozal, 1998). The different neurodevelopmental ages of children, and different test parameters used may account for the differences in findings between the two groups. Nonetheless, similarities may be emerging from these studies of children and earlier work. Specifically, associations between performance IQ and slow wave activity (SWA) during the non-rapid eye movement (NREM) sleep (Biggs *et al.*, 2012; Biggs *et al.*, 2014).

In healthy adults, sleep progresses through NREM stages N1 to N3 followed by a period of REM sleep occurring approximately 60- 90 minutes into sleep (Vyazovskiy & Delogu, 2014). It has been argued that cognitive improvements in treated OSA patients may reflect increased stability of brain activity during sleep, allowing crucial synaptic repair and maintenance to occur during SWA, and counteracting the toxic effects of OSA mediated by arousal and hypoxia (Neubauer & Fink, 2009; Biggs *et al.*, 2014). This argument is supported by findings showing that sleep presents a crucial period during which the brain can restore cellular homeostasis (Fig 1.6), increase signal to noise ratio, and reinforce neuronal circuitry for subsequent cognitive processing demands (Poe *et al.*, 2010; Abel *et al.*, 2013; Tononi & Cirelli, 2014).

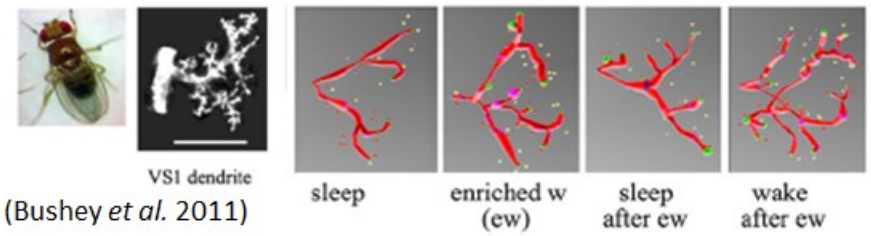

Figure 1.6: "Sleep is the price we pay for [neural] plasticity" Tononi & Cirelli 2014. Figure shows neural connections in a fruit fly (left) following sleep, enriched wakefulness, sleep after enriched wakefulness and wake after enriched wakefulness with no sleep. Note the smallest number of synapses occur following sleep, and the most following sleep deprivation. New learning occurs during wakefulness via synaptic potentiation. Neurones strengthen synapses during enriched wakefulness, while interacting with the environment e.g. fear conditioning, cue-reward learning etc. Neurones renormalize synapses in sleep when the brain is off line; synapses have a high energy cost and neural 'space' may be reduce if sleep does not occur.

1.3.3 Sleep Deprivation and Cognitive Function in OSA

Sleep alters the molecular signalling pathways that regulate synaptic strength (**Fig 1.6**), plasticity-related gene expression and protein translation (Abel *et al.*, 2013). Moreover, sleep deprivation can impair neuronal excitability, decrease myelination and lead to cellular oxidative stress and misfolding of cellular proteins (Abel *et al.*, 2013; Picchioni *et al.*, 2014).

The frequent arousals and ensuing fragmented sleep that occur in OSA, have been shown to impact on cognitive function the following day, in a manner similar to that of total sleep deprivation (Daulatzai, 2013). Studies of the effects of sleep deprivation on cognition in the general population suggest comparable cognitive impairments to those seen in OSA (Killgore, 2010). Furthermore, an association has been shown between excessive daytime somnolence and cognitive impairment and an increased risk of cognitive decline and dementia (Yaffe *et al.*, 2014). In a prospective cohort study (The Honolulu-Asia Aging Study), lower nocturnal oxygenation and a reduction in SWA NREM sleep were associated with the development of micro-infarcts and brain atrophy. Conversely, men with longer SWA sleep showed slower cognitive decline (Gelber *et al.*, 2014).

The impact of OSA on selected sleep stages may be particularly important, as each sleep stage is associated with its own functional learning and memory processes (Poe *et al.*, 2010). In OSA patients, stage N2 NREM sleep has been shown to increase, while stages N1, N3 and REM sleep decrease **Fig 1.7** (Andreou *et al.*, 2014).

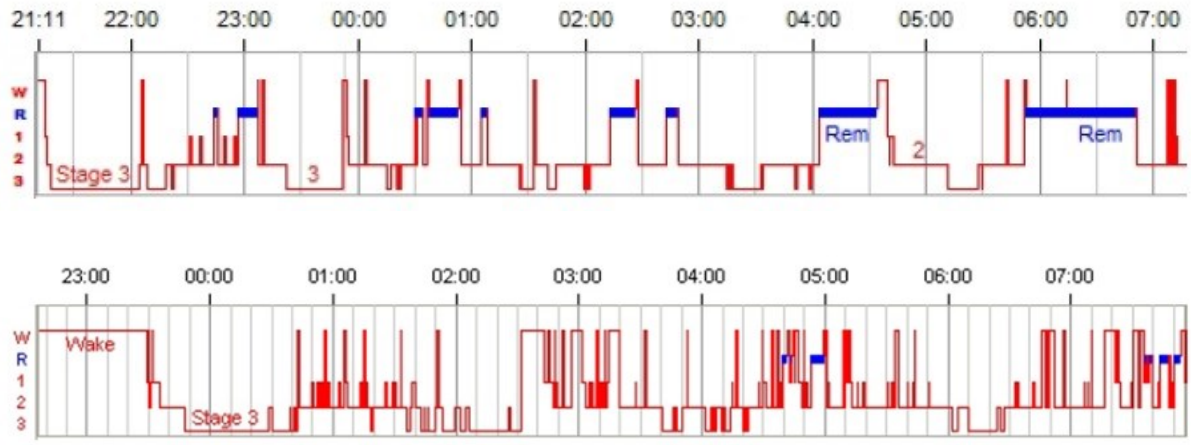

Figure 1.7: Overnight sleep patterns (hypnogram), obtained from electroencephalography, illustrating sleep cycles in a young healthy person (Top panel). Note that NREM is the first sleep, and the first REM sleep occurs after approximately 90 minutes; throughout the night there are occasional brief arousals from sleep. The Bottom Panel illustrates sleep cycles in an OSA patient. Note that throughout the night there are frequent, brief arousal from sleep with a reduction in both NREM stage 3 and REM sleep. W: wake; R: REM sleep (marked in blue); 1, 2, 3: NREM stage 1, 2 and 3 sleep.

The consequences of sleep fragmentation in OSA are difficult to assess. In one study of mild OSA, where sleep fragmentation did not significantly reduce the scoring of N3 Sleep, the exponential decay function of SWA was demonstrated as significantly slower in patients than in controls (Ondze *et al.*, 2003). This was due to the more even distribution of SWA throughout the night. These results show that mild sleep fragmentation can alter the dynamics of SWA, without decreasing significantly the amounts of SWA and REM sleep. This means that to fully understand the impact of sleep deprivation in OSA it may be necessary to perform SWA decay analysis (Ondze *et al.*, 2003). In the same study, a decrease of spindle activity was observed in N2 and N3 which was not attributable to an increase of SWA (Ondze *et al.*, 2003; Schonwald *et al.*, 2012). Such a reduction in total spindle density has also been reported in sleep maintenance insomnia, and is likely to be related to sleep fragmentation (Ondze *et al.*, 2003; Schonwald *et al.*, 2012).

REM Sleep: Traditionally, obstructive events during NREM sleep were viewed as associated with greater cognitive deficits or impaired quality of life, whilst REM sleep events were shown to be associated with greater sympathetic activity, hypertension and cardiovascular instability in patients with OSA (Mokhlesi *et al.*, 2014) (Mokhlesi & Punjabi, 2012). Recently, a role for fragmented REM sleep in spatial navigational memory problems in OSA patients was suggested (Varga *et al.*, 2014). During this study, patients spent two different nights in the laboratory, during which they performed timed trials, before and after sleep, on one of two unique 3D spatial mazes (Varga *et al.*, 2014). The normal consolidation of sleep was achieved with use of therapeutic CPAP throughout the first night, whereas during the second night CPAP was reduced only during the REM stages. Here, patients showed improvements in maze performance after a night of normal sleep, and those improvements were significantly reduced following a night of REM disruption without changes in psychomotor vigilance. Cognitive improvements were significantly positively correlated with the mean REM run duration across both sleep conditions (Varga *et al.*, 2014).

In some OSA patients, reduction of REM sleep can lead to dissociation of REM traits to other sleep stages, further impacting on critical sleep windows for memory formation and consolidation (Poe *et al.*, 2010). Of particular note, several studies have now demonstrated that, if high homeostatic demands are not fully met during sleep, microsleeps can occur in highly active regions of the brain during the subsequent wake period (Vyazovskiy & Delogu, 2014). This can lead to the concomitant disability of that region (Tononi & Cirelli, 2014; Vyazovskiy & Delogu, 2014). To what extent this takes place in OSA patients, and whether this also contributes to attention/vigilance dysfunction and the higher rate of road traffic accidents seen in OSA patients has yet to be fully understood. Previously reports of retarded SWA decay through the night in even mild OSA patients further supports the notion of non-restorative sleep in OSA (Ondze *et al.*, 2003) .

Sleep Spindles: Intriguingly, in a study of spindle frequency changes in OSA, it has been shown that, unlike healthy controls, OSA patients continued displaying a significant proportion of slow spindles in frontal, central and parietal regions during the night. This may suggest that deregulated spindle formation contributes to cognitive deficits in OSA patients (Schonwald *et al.*, 2012). In another study, sleep architecture of patients with mild OSA showed a high degree of sleep fragmentation resulting in a different time course of SWA and a decreased sleep spindle index when compared to controls (Ondze *et al.*, 2003).

Taken together, these studies further highlight the possible role for OSA brain injury in the acceleration, or even initiation, of cognitive decline especially in older people (Sforza & Roche, 2012; Daulatzai, 2013; Pan & Kastin, 2014; Yaffe *et al.*, 2014).

1.4 ISCHAEMIC CONDITIONING AND NEURO-INFLAMMATION IN OSA

In OSA, repetitive occlusions of the upper airways lead to intermittent hypoxia and recurrent hypoxaemia, typically characterized by short cycles of hypoxia and reoxygenation (Almendros *et al.*, 2014). These cycles can lead to either adaptive or maladaptive processes (Almendros *et al.*, 2014) and the outcome will vary depending on the dynamic interplay between the specific type and amount of reactive oxygen/nitrogen species produced, their duration and frequency, the intracellular localization, and the micro-environmental antioxidant activity (Lavie, 2014). Additional interplay depends on factors such as the genetic makeup, nutrition and other lifestyle related variables, which all affect the redox status (see also (Almendros *et al.*, 2014; Lavie, 2014)). A variety of studies to date suggest that severity of hypoxia, its duration, and cycle frequency, are fundamental determinants of outcomes (Ayalon *et al.*, 2010). For example, it has generally been acknowledged that short, mild, and lower cycle frequency may generate beneficial and adaptive responses in brain, such as ischaemic preconditioning (Almendros *et al.*, 2014). Conversely, chronic moderate-to-severe, and high frequency intermittent hypoxia can induce maladaptive disruption of homeostatic mechanisms, leading to dysfunction and sterile neuroinflammation (Almendros *et al.*, 2014; Lavie, 2014).

1.4.1 Ischaemic preconditioning

Ischaemic preconditioning represents a generalized adaptation to ischaemia by a variety of cells (Lavie & Lavie, 2006; Dirnagl *et al.*, 2009). In OSA, induction of ischaemic preconditioning is thought to be due to the activation of several gene programs, including the hypoxia inducible factor-1, vascular endothelial growth factor, erythropoietin, atrial natriuretic peptide and brain derived neurotrophic factor (Brzecka, 2005; Nanduri *et al.*, 2008).Various end-mechanisms and pathways have been shown to play a role, including that of long-term facilitation of diaphragmatic motor output, chemo-reflex activation, vascular remodelling, neo-angiogenesis, productive autophagy, reactive gliosis, various synaptic alterations. Modulation of adult hippocampal neurogenesis has also been suggested (Haddad & Yu, 2009; Aviles-Reyes *et al.*, 2010; Tsai *et al.*, 2011; Papadakis *et al.*, 2013; Tsai *et al.*, 2013; Lavie, 2014).

CPAP treatment of OSA has been shown partially to reverse the damage in the hippocampus, and to ameliorate some of the associated cognitive deficits, possibly also by modulating adult neurogenesis (Canessa *et al.*, 2011). It is proposed that at any given time ongoing maladaptive neuro-inflammatory processes probably exist alongside adaptive mechanisms of increased brain plasticity and ischaemic preconditioning (Ferriero, 2005; Lledo *et al.*, 2006; Dirnagl *et al.*, 2009; Seki, 2011; Rosenzweig *et al.*, 2013a). In a recent study that compared the cognitive performance of patients with high and low

hypoxemia after controlling for demographic factors and other aspects of OSA severity, an unexpected advantage of higher hypoxemia on memory was demonstrated in a carefully matched clinical cohort (Hoth *et al.*, 2013).

Another powerful central neuroprotective adaptive mechanism for ischaemic events was demonstrated following the activation of the intrinsic neurons of the cerebellar fastigial nucleus (Reis *et al.*, 1997). Neurostimulation of these nuclei appears to provide protective reduction in excitability of cortical neurons during the subsequent ischaemic episodes, and to lead to reduced immunoreactivity of cerebral microvessels by down-regulating their production of intracellular cell adhesion molecule (ICAM-1) and consequently preventing the release of pro-inflammatory cytokines (Rosenzweig *et al.*, 2014). The compensatory entraining of the cerebellum by hypertrophic hippocampi may also occur in younger patients with mild OSA (See Chapter 3).

Although there are no direct monosynaptic anatomical connections between hippocampi and cerebellum, their connectivity is thought to be important for the control of movement under states of heightened emotion and novel conditions, and for associative learning. Of note, failed adaptation of cerebellar networks to injury was shown to lead to cognitive deficits and hyperactivity, distractibility, ruminative behaviour, dysphoria and depression in some patients (Rosenzweig *et al.*, 2014). Several studies also suggest that, under certain conditions, intermittent hypoxia can increase immune defences without exacerbating inflammation (Almendros *et al.*, 2014; Lavie, 2014). Moreover, in animals, short-lasting hypoxic exposures mimicking OSA were associated with recruitment of bone-marrow derived pluripotent stem cells, which exhibited up-regulation of stem cell differentiation pathways, particularly involving central nervous development and angiogenesis (Almendros *et al.*, 2014).

1.4.2 Neuro-inflammation

Intermittent hypoxia can lead to maladaptive effects, including neuro-inflammation. Although the exact neurocellular sources for associated processes are still incompletely understood, it is likely that activation of astroglia is important (Dale *et al.*, 2014; Lavie, 2014). In addition, the oligodendrocytes, myelin-producing cells of the CNS, were shown to be selectively sensitive to hypoxia and sleep fragmentation (Rosenzweig *et al.*, 2012; Bellesi *et al.*, 2013). The subsequent loss of buffering functions can ultimately contribute to pathological processes, such as increased glial proliferation and microglial activation (Dale *et al.*, 2014). Astroglial and microglial cells play critical roles in regional blood flow regulation and inflammatory processes in the brain, as well as critical coordination of bioenergetics through lactate transport (Dale *et al.*, 2014). Under normal conditions, microglia in the

healthy CNS exhibit a surveillance phenotype that synthesizes and releases neuroprotective growth/trophic factors (Dale *et al.*, 2014). However, severe and prolonged hypoxia can activate microglia toward a toxic, pro-inflammatory phenotype that triggers pathology, including change in hippocampal structure, impaired synaptic plasticity, and cognitive impairment (Dale *et al.*, 2014) (See Chapter 5). Neuro-inflammation has been shown independently to raise the brain's sensitivity to stress, resulting in stress-related neuropsychiatric disorders, such as anxiety and depression (Skaper *et al.*, 2014).

Dynamic changes in transcription of inflammatory genes were demonstrated following exposure to intermittent hypoxia, with most inflammatory markers increasing over time (Dale *et al.*, 2014). Increased concentrations of prostaglandin E2 in neural tissue have also been demonstrated in hippocampal and cortical regions accompanied by lipid peroxidation of polyunsaturated fatty acids (Dale *et al.*, 2014). Similarly, it was shown that increased carbonylation- and nitrosylation-induced oxidative injury emerges in susceptible brain regions and promotes increased daytime somnolence (Dale *et al.*, 2014; Lavie, 2014). Recently, toll-like receptor 4 (TLR4) expression and activity was demonstrated to be increased in monocytes of patients with OSA (Unnikrishnan *et al.*, 2014). Expression of TLR4 was also demonstrated in the microglia of the cortex and brainstem after chronic intermittent hypoxia where it was postulated to play region specific and differential adaptive or maladaptive role (Unnikrishnan *et al.*, 2014). This finding is of particular interest since TLR4 has also been strongly implicated in several inflammatory and neurodegenerative disorders, including vascular dementia and Alzheimer's disease (Unnikrishnan *et al.*, 2014). In line with this, in cognitively healthy adults, intermittent hypoxia was correlated with increases in phosphorylated and total tau and amyloid$^{\beta}_{1\text{-}42}$ concentrations in cerebral spinal fluid, which are key components of Alzheimer's pathology (Yaffe *et al.*, 2014). Similarly, cerebral amyloidogenesis and tau phosphorylation along with neuronal degeneration and axonal dysfunction were shown in the cortex and brainstems of animals exposed to intermittent hypoxia (see Chapter 5) (Daulatzai, 2013).

Taken together, these findings support the role for neuro-inflammatory processes in cognitive and emotional deficits of OSA patients. They further suggest a close association between hypoxaemia-induced maladaptive processes and dementia.

1.5. DESCRIPTION AND STRUCTURE OF THESIS

The primary aim of this thesis is to investigate the effects of OSA on brain structure. The thesis will also investigate whether treatment of OSA with CPAP leads to resolution of any changes in brain structure, possibly mediated by ischaemic preconditioning. Finally the thesis will investigate whether these changes in brain structure are associated with corresponding changes in cognitive function.

The general methods used for all the studies in this thesis will be described in **Chapter 2**. Additional background to the individual studies will be presented in each chapter, with further details of study protocols, data analysis and statistical analyses where necessary.

In **Chapter 3** I will describe the reanalysis of brain imaging data gathered previously by the group using more modern techniques. The aim of this study was to investigate the effect of intermittent hypoxia on brain structure in OSA patients. I was interested in brain hypertrophy, suggestive of ischaemic preconditioning, as well as the hypotrophy demonstrated in other studies.

In **Chapter 4** I will describe a randomised controlled trial of CPAP in OSA. Brain structure and cognitive function were measured in younger people newly diagnosed with OSA before and after randomisation to CPAP or best supportive care. This aim of this study was to investigate the association of brain structural changes with cognitive dysfunction in OSA and to investigate whether these changes were reversible.

Chapter 5 will describe an analysis of brain structure in older people with OSA before and after randomisation to CPAP. The aim of this study was to investigate whether neuroplasticity still existed in older patients with OSA, or whether any brain structural changes caused by OSA were irreversible.

In **Chapter 6** the results of the studies presented in this thesis will be discussed, with some suggestions as to how the results, and areas of uncertainty identified during this research, could be developed in the future

CHAPTER 2

General Methods

2.1 PARTICIPANT AND LABORATORY INFORMATION

OSA patients were recruited from The Royal Brompton (Chapters. 3, 4 and 5), Harefield (Chapter 4) and Charing Cross (Chapter 3) Hospitals and Queen Elizabeth Hospital, Woolwich (Chapter 4). Controls were recruited from the Healthy Volunteers Database in the Academic Department of Sleep and Ventilation and from hospital staff at The Royal Brompton Hospital. Sleep studies were performed at The Royal Brompton as part of routine clinical practice. In addition, research nocturnal polysomnography was carried out (Chapter 4) in the NIHR Respiratory Biomedical Research Unit (BRU) at The Royal Brompton Hospital. MRI scans were performed at both The Royal Brompton Hospital (Chapter 4), and Charring Cross Hospital (Chapter 3 & 5). In the study presented in Chapter 4, I was the first investigator to use the Respiratory BRU Clinical Research Facility for overnight studies and helped to develop the pathway for future overnight studies. The study in Chapter 4 was also the first collaboration between the Respiratory BRU and the Cardiology BRU at The Royal Brompton and the first study to use the Cardiology BRU MRI scanners to perform brain imaging. I set up this collaboration, and arranged the sharing of knowledge and expertise between our previous imaging collaborators and the physicists and radiographers at The Royal Brompton to ensure the correct MRI sequences were used and the images obtained were of appropriate quality. I also set up two new collaborations, to recruit patients from Harefield Hospital and Queen Elizabeth Hospital, Woolwich to Royal Brompton sleep research projects for the first time.

All studies were carried out with Research Ethics Committee approval, and all participants gave written informed consent. Note, the IRAS ethical approval numbers are given in each experimental chapter.

2.2 NOCTURNAL POLYSOMNOGRAPHY

Polysomnography was performed in all OSA patients using the SOMNOscreen system (SOMNOmedics GmBH, Germany). This system allows data to be was transmitted wirelessly in real time to a computer, ensuring that the patient has as comfortable a night's sleep as possible (**Fig 2.1**).

Figure 2.1: Studies presented in this Thesis used nocturnal polysomnogram to diagnose the severity of the OSA. Note that the patient is able to move freely as the system used is wireless.

2.2.1 Assessment of sleep : EEG, EOG and EMG

Electroencephalogram (EEG), electro-oculogram (EOG) and surface electromyogram (EMG) are used to monitor sleep and wake, using standard sleep scoring criteria (Reschtschaffen, 1968). EEG was measured using 10mm gold cup electrodes (Grass technologies, Rhode Island, USA). The electrodes were sited according to the International ten twenty EEG electrode system. This system uses the nasion and inion as reference points, with EEG electrodes placed at increments of 10 or 20% along transverse and sagittal lines linking these landmarks. Electrodes were placed in the C3 and C4 positions, with a common reference electrode in the Cz position (**Fig 2.2**).

The electrode positions were marked with a chinagraph pencil and the site was cleaned with an abrasive paste (Nuprep, Weaver and Company, Colorado, USA) and an alcohol soaked swab. This process reduced the electrical impedance between the electrode and the skin. It also helped the attachment of the electrode. The gold cup electrodes were then filled with conducting paste (Ten20 conductive paste, Weaver and Company, Colorado, USA) and fixed in place with adhesive (Collodion, SLE Ltd., South Croydon, UK). Reference electrodes were placed over left and right mastoid processes (A1 and A2 respectively) and a ground electrode was placed on the centre of the forehead (**Fig 2.2**).

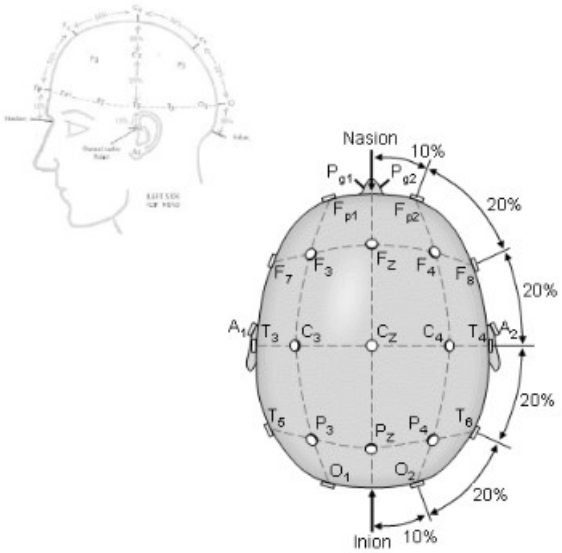

Figure 2.2. A view of the head showing the electrode positions of the international ten twenty system for recording of sleep using electroencephalography (EEG). (Figures adapted from Malmivo & Plonsey. Bioelectromagnetism: Principles and Applications of Bioelectric and Biomagnetic Fields. New York: Oxford University Press; 1995)

Self-adhesive electrodes were used to measure EOG activity. These were placed 1 cm lateral and superior to the right outer canthus, and 1 cm lateral and inferior to the left outer canthus and referenced to the A1 mastoid electrode. By placing the EOG electrodes asymmetrically conjugate vertical eye movements, which produce opposing deflections in the right and left EOG signals, can be distinguished from electrode artefact or high voltage which produce in-phase deflections in both EOG signals. Self-adhesive electrodes were also used to record EMG activity. The skin was prepared as for the EEG and electrodes were positioned 2 cm posterior to the inferior edge of the mandible, and 2 cm to either side of the midline.

After application of all electrodes, an impedance test was performed to ensure all electrodes had a low resistance for optimal detection of brain electrical activity. An impedance of <10,000 Ohms was considered acceptable, and where this was exceeded, further conducting paste was added to gold cup electrodes, or the electrode was replaced until impedance was satisfactory.

The EEG and EOG signals were digitally sampled at a rate of 128 Hz, whilst the EMG signals were sampled at a rate of 256 Hz (Silber *et al.*, 2007). The signals were processed by differential amplifiers to generate a conventional EEG montage (C3/A2; C4/A1, Right EOG/A1 and Left EOG/A1) for sleep scoring (Reschtschaffen, 1968). Conventional filter settings (High pass filter: 0.2 Hz; low pass filter: 35 Hz) were applied to the differential amplifier output to remove signals outside of the frequencies of interest.

2.2.2 Measurement of airflow using nasal pressure transducer

Airflow from the nose and mouth was monitored using a 'nasal pressure' signal, generated by changes in pressure at the nares. The pressure changes were measured by a nasal cannula (Embla, Colorado, USA) connected to a differential pressure transducer that was referenced to atmospheric pressure. The voltage output of the transducer was proportional to the pressure change across a membrane located within the transducer.

The measurement of nasal pressure provides a semi-quantitative measure of the velocity of air passing through the nostrils (**Fig 2.3**). However, because the airflow is most commonly non-linear, nasal pressure overestimates apnoeas compared to other sensors (Redline *et al.*, 2007). For example, if the amplitude of the nasal pressure signal falls below 10% (the commonly used criteria to score apnoea) when nasal airflow decreases to 30%. The Bernouilli equation states that the velocity of a gas is proportional to the square root of the gas pressure. Therefore, the nasal pressure signal is equivalent to the square of the airflow; for example, when nasal airflow decreases to 70% of baseline, the nasal pressure will decrease to 49%. It has been suggested that a square root transformation of the nasal pressure signal could be used in this case. However, in practice this is not often used.

The within-breath shape of the nasal pressure airflow signal can also provide information about the occurrence of airflow limitation that does not fulfil the requirements to be scored as apnoeas or hypopnoeas (**Fig 2.3**).

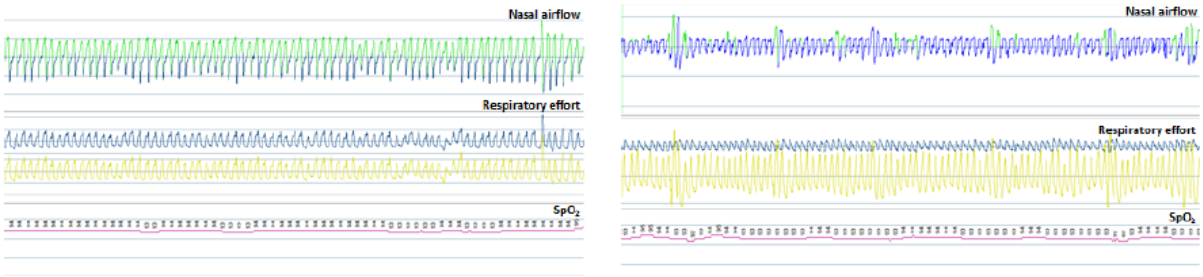

Figure 2.3: Nasal airflow, respiratory effort and oxygen saturation (SpO_2) measured during sleep in a health person (left) and a patient with airflow limitation (right). Notice the flattened inspiratory airflow profile (green) in the patient with flow imitation, compared to the healthy subject.

2.2.3 Measurement of respiratory effort using respiratory inductance plethysmography

Respiratory effort was measured by respiratory inductance plethysmography. This requires placement of a belt around the upper chest and a further belt around the abdomen, midway between the inferior ribs and the iliac crest (**Fig 2.1**). The belts were sized according to patient girth and were fastened with

Velcro. To ensure the belts did not become displaced during the sleep study they were further secured with tape.

The respiratory inductance plethysmography bands measure changes in current, caused by an alteration in cross sectional area enclosed by a wire. A magnetic field is created by passing alternating current through a thin zigzagging wire which has been sewn into the RIP belt (Sleepsense, SLP Inc., Illinois, USA). This magnetic field is affected by changes in the cross-sectional area enclosed by the wire and this, in turn, alters the frequency of the current. The change in current can be measured, which provides a metric of respiratory and abdominal expansion during inspiration as shown in **Fig 2.3**.

2.2.4 Measurement of arterial oxygen saturation using pulse oximetry

Finger pulse oximetry (Nonin 8000 Softsensor, Nonin, Minnesota, USA) was used to measure haemoglobin oxygen saturation. This measurement represents the average oxygen saturation of haemoglobin. It relies on the principle that the light absorption characteristics of haemoglobin are altered by the binding of oxygen. One arm of the clip contains LEDs that emit light at red (660nm) and infrared (910nm) wavelengths. Light transmitted through the finger is detected by a photodetector on the other arm of the clip. The absorption of light by blood constituents can be determined from changes in the quantity of transmitted light occurring during fluctuations in arterial volume with each cardiac cycle. The ratio of red and infrared light absorbed during these pulsatile fluctuations is used to calculate the oxygen saturation of haemoglobin. The oxygen saturation is then displayed as a moving time average as shown in **Fig 2.3**.

2.2.5 Measurement of heart rate using ECG

Heart rate was measured by placing ECG electrodes on the skin below each clavicle and below the lower left ribcage.

2.2.6 Measurement of leg movement using EMG

During sleep phasic limb movements (called *Periodic limb movements [PLM]*} can disrupt sleep in a similar way to OSA. Therefore, to differentiate between sleep disruption caused by occlusion of the upper airway, and that caused by PLM, EMG of the anterior tibialis muscle is monitored, and phasic

bursts of activity are scored. If more than 4 consecutive limb movements are noted, separated by not less than 5 seconds, but not more than 90 seconds the patients is said to have PLM. Limb movements can also occur during an arousal from sleep at the end of an apnoea/hypopnoea. Therefore, if the increase in anterior tibialis EMG activity occurs within 0.5 seconds of a respiratory event it is NOT scored as a limb movement. The PLM index is calculated as the mean number of PLMs per hour of sleep.

2.3 ASSESSMENT OF SLEEPINESS

One of the most common symptoms of OSA is excessive daytime sleepiness. The measurement of sleepiness is difficult. Subjective measurements, such as the commonly used ESS, are influenced by factors such as motivation, and objective measurements are often complex and time consuming. In this thesis, therefore, sleepiness has been measured subjectively using the ESS.

2.3.1 Epworth Sleepiness Scale

The Epworth Sleepiness Scale (Johns, 1991) is a widely used, validated questionnaire that assesses daytime sleepiness. The Epworth scale asks subjects to rank how likely, on average, "in recent times" they are to doze in 8 different situations. Each situation is given a mark out of 3 depending on how likely the subject is to doze in that situation (0 = never 1 = slight chance, 2 = moderate chance, 3 = high chance). The score for each situation is added to give a total out of 24. A total score of 10 or more indicates excessive daytime sleepiness.

Although it was designed to be self-administered, a recent study found that 33% of patients made errors and 23% required assistance when completing the Epworth Sleepiness Scale for the first time. Even among patient who had used the Epworth scale previously, 16% made errors. Furthermore 4% of patients were unable to complete the form because of poor reading skills or because they did not have their glasses with them (Ghiassi *et al.*, 2011). Another criticism is that ESS scores do not correlate (Furuta *et al.*, 1999) or correlate only weakly with objective measures of sleepiness such as the multiple sleep latency test (MSLT).

Despite these criticisms the ESS is widely used around the world. As well as the English language version, it has been translated into and validated in numerous other languages including: Croatian, French, German, Greek, Italian, Japanese, Korean, Mandarin, Norwegian, Portuguese, Serbian, Spanish, Thai and Turkish and a pictorial version has recently been validated (Drakatos *et al.*, 2015). It

has even been argued that it is a better discriminator of excessive daytime sleepiness than the MSLT or the maintenance of wakefulness test (MWT) (Johns, 1991).

2.4 ASSESSMENT OF COGNITIVE FUNCTION

In Chapter 1 the relationship between OSA and cognitive function was reviewed, with a discussion of the possible mechanisms that may cause cognitive dysfunction in patients with OSA. In Chapter 2, I will review the cognitive tests used in the experimental Chapters 3-5. The tests were selected based on the results of previous studies carried out by the Sleep Group at the Royal Brompton Hospital (**Fig 2.4**). A further discussion of the changes in brain morphology and function that may lead to the cognitive impairment is given in Chapter 3, Section 3.2.

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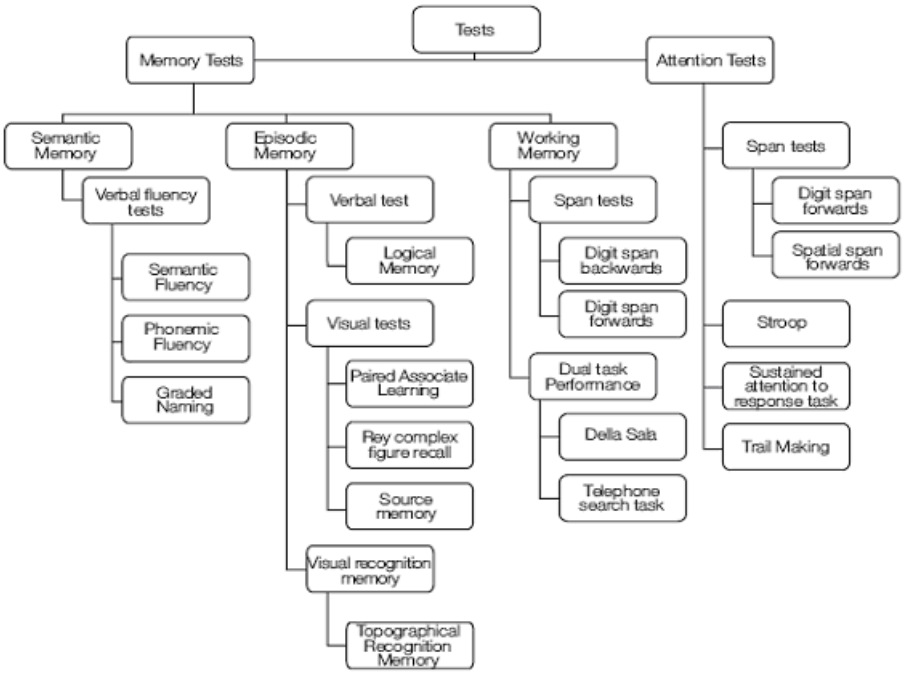

Figure 2.4: This diagram shows the cognitive testing battery examining different aspects of memory and attention used in the studies of (Twigg *et al.*, 2010). The battery was designed in consultation with Dr Kim Graham, MRC Cognition and Brain Sciences Unit, Cambridge. Each test was selected to examine aspects of long-term memory (semantic and episodic) and working memory. Episodic memory tests was further divided into verbal and visual tests. Two aspects of working memory were included in the battery, specifically maintenance of working memory and dual task performance. Several tests of attention were included in order to cover the different aspects of attention.

2.4.1 Logical Memory

The Logical Memory test (WMS III, Harcourt, UK) is a validated test of verbal episodic memory. The different components of the test allow assessment of different subsets of episodic memory such as recognition memory, immediate recall and delayed recall. Two short stories are read to the subject. After each story is read the subject is asked to repeat the story, using as close to the same words as he can remember (immediate recall). One point is awarded for each unit of the story correctly repeated according to criteria supplied with the test. The subject is asked to repeat the 2 stories a second time 35 minutes after they were read (delayed recall). The same scoring system is used. Immediately following this second repetition the subject is asked a series of true-false questions about the stories (recognition memory). One mark is scored for each correct answer.

2.4.2 Trail Making

This is one of the most widely used neuropsychological tests and is included in most neuropsychological panels. TMT provides information on visual conceptual and visuo-motor tracking, motor speed, attention and executive functions. It consists of two parts, A and B. TMT – A is a dot-to-dot in which circles containing the numbers 1 to 25 must be joined in numerical order by a line. In TMT – B the circles contain either a number (1 to 12) or a letter (A to L). The aim is to join the circles in numerical and alphabetical order, alternating between letters and numbers (e.g. 1, A, 2, B, 3, C, 4, D etc.). The time taken to complete the tests is measured. Younger subjects and those with more years of education complete the test in less time than older subjects and those with fewer years of education, although normative data is available (Tombaugh, 2004). Most subjects complete the test in less than 300 seconds (Reitan, 1979).

2.4.3 Spatial Span

Spatial Span Forward and Backwards is a discriminator of visual-spatial processing and working memory. The examiner takes a standardised board with 10 cubes in set positions on the board and points to a predetermined series of cubes. The subject is required to repeat the series in the same order (forward span) or in reverse order (backwards span). The sequences gradually increase in length as the test proceeds, from a minimum of 2 cubes, to a maximum of 9 cubes. One point is scored for each correct series, resulting in a maximum score of 16 in both parts of the test. Both tests are validated discriminators of cognitive impairment but spatial span backwards is more sensitive (Wiechmann *et al.*, 2011). Spatial Span forward mainly tests attention. Spatial Span backward tests working memory and executive function as information must be held in the memory and processed.

2.4.4 Digit Span

Digit Span is similar to spatial span, but instead of a having to repeat a series of tapping cubes, the subject has to repeat a series of digits that are read to him. Again there is a forwards and a backwards component of the test and the series grows in length as the test proceeds. Digit Span forwards mainly tests auditory attention. Digit Span backwards mainly tests working memory and executive function.

2.4.5 ACER

This test (not listed in **Fig 2.4**) is an assessment of global cognitive function. It has been validated as a discriminant of even mild cognitive dysfunction (Mioshi *et al.*, 2006). It incorporates the Mini Mental State Examination (MMSE), the most widely used screening tool for a global assessment of cognitive function (Folstein *et al.*, 1983), but also encompasses cognitive domains not assessed in the MMSE including attention, orientation, memory, verbal fluency, language and visuospatial skills.

The test comprises a series of questions and simple tasks such as memorising an address, obeying a 3 stage command and drawing a clock face. ACE-R is available and has been validated in many languages (including English, Japanese, Cantonese, Italian and Slovak. It takes approximately 15 minutes to complete the test. There are 26 tasks in total with a maximum possible score of 100, with individual scores for each domain. There are age and education dependent norms for the total score as well as for the individual domains. A cut-off score of 88 is 94% sensitive and 89% specific for the diagnosis of dementia. A cut-off of 82 is 84% sensitive and 100% specific.

2.5 ASSESSMENT OF BRAIN STRUCTRE

In this Thesis Magnetic resonance imaging has been used to assess the impact of OSA on brain structure. The principle of MRI is outlined below with a brief description of the methods of image analysis. The details of the scanners and the scanning protocols used in each study will be given in Chapters 3, 4 and 5.

2.5.1. Magnetic resonance imaging

MRI utilises the magnetic properties of hydrogen ions within the body to produce detailed images. Each hydrogen ion consists of a single proton. This proton spins on its axis. In normal circumstances

the direction of spin is random, but, because a proton has positive electrical charge, when a strong magnetic field is applied to the body, the protons' spins align.

A radiofrequency pulse produced at the same frequency as the protons are spinning, will cause the protons to absorb the energy from the pulse and change the direction of their spins. When the radiofrequency is removed, the protons will release these quanta of energy and return to their relaxed state. These quanta are detected by the MR receiver and allow the position of hydrogen ions within the body to be determined.

In order to produce contrast between different tissue types, MRI relies on the different speeds at which different tissues lose their energy after the RF pulse has been turned off. This occurs through two processes: T1 recovery refers to transfer of energy from nuclei to the surrounding environment. T2 recovery refers to the loss of energy through inhomogeneities in the intrinsic magnetic field. This recovery occurs at different rates in different tissues. Scans can be either T1 or T2 weighted, referring to the process by which contrast is achieved.

2.5.2. Image analysis

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/).

The description given here is written by the software's authors and is how they request that the analysis is described.

> *"The technical details of these procedures are described in prior publications (Dale et al., 1999; Dale and Sereno, 1993; Fischl and Dale, 2000; Fischl et al., 2001; Fischl et al., 2002; Fischl et al., 2004a; Fischl et al., 1999a; Fischl et al., 1999b; Fischl et al., 2004b; Han et al., 2006; Jovicich et al., 2006; Segonne et al., 2004, Reuter et al. 2010, Reuter et al. 2012). Briefly, this processing includes motion correction and averaging (Reuter et al. 2010) of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al., 2002; Fischl et al., 2004a) intensity normalization (Sled et al., 1998), tessellation of the gray matter white matter boundary, automated topology correction (Fischl*

et al., 2001; Segonne et al., 2007), and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale et al., 1999; Dale and Sereno, 1993; Fischl and Dale, 2000). Once the cortical models are complete, a number of deformable procedures can be performed for further data processing and analysis including surface inflation (Fischl et al., 1999a), registration to a spherical atlas which is based on individual cortical folding patterns to match cortical geometry across subjects (Fischl et al., 1999b), parcellation of the cerebral cortex into units with respect to gyral and sulcal structure (Desikan et al., 2006; Fischl et al., 2004b), and creation of a variety of surface based data including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting submillimeter differences between groups. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004). Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al., 2006; Reuter et al., 2012)".

To extract reliable volume and thickness estimates, images where automatically processed with the longitudinal stream (Reuter *et al.*, 2012) in FreeSurfer. Specifically an unbiased within-subject template space and image is created using robust, inverse consistent registration (Reuter *et al.*, 2010). Several processing steps, such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations are then initialized with common information from the within-subject template, significantly increasing reliability and statistical power (Reuter *et al*., 2012).
One of the advantages of using FreeSurfer is that the longitudinal stream (LS) has specifically been designed to analyse longitudinal data, unlike other analysis techniques, such as voxel based morphometry and deformation based morphometry (DBM), which were designed specifically to analyse cross sectional data. DBM has been shown to overestimate the rate of hippocampal degeneration in Alzheimer's disease (AD) (Yushkevich *et al.*, 2010). Thompson and Holland recognised that the majority of brain degeneration in AD demonstrated using tensor based morphometry occurred in the first six months. By using the same techniques, they were able to demonstrate similar

changes using serial images acquired from healthy controls (Thompson & Holland, 2011). This reduction in test-retest reliability is likely to be due to several factors, including motion artefact, change of head and jaw position and variability in image processing .(Reuter & Fishel 2011).

The longitudinal stream has been validated, and found to reduce within-subject variability compared to the cross-sectional (CS) analysis techniques, when used to analyse two sets of MR images obtained from the same healthy subjects on the same day, as well as four sets of images of healthy controls obtained over a period of 14 days (Reuter *et al.*, 2012). MR images were obtained twice in the same session, using the same protocol, from 115 healthy subjects and analysed using both LS and CS. There was significantly less volume change in all brain regions, including thalamus and hippocampus when using LS [mean dice coefficient (SD): LS, 0.942 (0.030) vs CS, 0.859 (0.058); $p<0.001$] (Reuter *et al.*, 2012). LS was also shown to have better test retest agreement in all brain structures than CS when analysing images obtained from healthy subjects multiple times over several weeks at 8 different sites [e.g. hippocampus dice coefficient (SD): LS, 0.92 (0.02) vs CS, 0.87 (0.02); $p<0.001$, thalamus dice coefficient (SD): LS, 0.94 (0.03) vs CS, 0.89 (0.04); $p<0.001$]. This study also showed that LS gives higher spatial consistency than CS across multiple sites ($p<0.01$) (Jovicich *et al.*, 2013) i

FreeSurfer analysis can detect the increased cortical volume loss over 2.5 years seen in patients with early AD relative to age matched healthy controls [mean % volume loss (SD): AD, 3.45 (1.51) vs controls 0.64 (1.06); $p<0.05$] (Adriaanse *et al.*, 2014). Furthermore, this volume loss was not seen in the motor or sensory cortices . This suggests that the increased volume loss seen in AD patients is not a result of systematic measuring errors and is consistent with evidence from other imaging modalities that these areas are relatively preserved in early AD (Svarer *et al.*, 2005). LS analysis has been shown to be more sensitive than CS to the different brain volume loss between healthy controls and patients with AD and also between healthy controls and patients with Parkinson's disease (Reuter *et al.*, 2012).

Chapter 3

THE ASSOCIATION BETWEEN OF OSA AND HIPPOCAMPAL HYPERTROPHY

3.1 SUMMARY

The impact of OSA on neural structure and function is unclear. Preclinical studies suggest that intermittent hypoxia (IH) and sleep fragmentation, both common in OSA, can affect the brain by disrupting hippocampal neurogenesis. Previous studies from the Brompton Sleep Group have shown grey matter loss in patients with OSA, compared to healthy controls. However, in animal models the notion of a biphasic response to chronic IH has been proposed. Prior to the study presented in this thesis there was little evidence for increased 'compensatory' neurogenesis in humans with OSA.

The objective of this study was to carry out a re-analysis of previously collected data to determine the effect of OSA on hippocampal, thalamic and cerebellar volumes: in prior studies these structures are most consistently shown to be affected. A cross-sectional case-control study was completed using structural MRI.

MRI scans from thirty two patients with mixed severity OSA (mean [SD]: AHI, 42.3 [23.81] events/h; 3 women; age 48.5 [12.51] years) and 33 non-apnoeic controls (AHI: 2.1 [1.61]) events/h; 5 women; age 49.7 [11.30] years) were analysed to determine hippocampal, thalamic and cerebellar volumes. Inferential analysis showed that the hippocampi were bilaterally larger in the OSA group than in the healthy control group; on the non-dominant (right) side this reached significance (p=.029). No significant difference in cerebellar volume was noted between the OSA patients and healthy controls. Although a trend towards smaller thalami in the OSA group was also noted, with larger reductions on the non-dominant side, this did not reach significance (p=.09). Inter-regional correlation analysis suggested aberrant connectivity between hippocampal and thalamic structures and the cerebellum in the OSA group.

The finding presented in this study suggests that hypertrophy of hippocampus can occur in young and middle-aged OSA patients with mixed disease severity. The possible role of hypoxia preconditioning in the neuropathology of OSA is discussed, and the clinical functional implications of these changes are investigated in Chapter 4. The effects of aging on neuroplasticity in older OSA patients are discussed in Chapter 5.

Contributions: The study was designed by Rosenzweig, Morrell, Glasser and Kempton. The data were previously acquired by Morrell and Corfield. Analysis and interpretation of data were carried out by Glasser, Rosenzweig, Milosevic, Beniczky and Williams. Data have now been published in a manuscript with Rosenzweig, Glasser, Kempton, Crum, Beniczky, Williams and Morrell as authors. Statistical analysis was carried out by Milosevic and Beniczky.

3.2 INTRODUCTION

OSA is a prevalent multisystem disease affecting up to 13% of the population (See Chapter 1, Section 1.2.2). It is predicted that it will become an even greater health problem in the future because rates of two of its most important risk factors, obesity and older age, are increasing.(Durgan & Bryan, 2012; Li & Veasey, 2012). This means that it is important to understand the consequences of OSA, and to ensure that treatment strategies are optimal.

OSA patients experience recurrent collapse of their upper airway that produces apnoeas and hypopnoeas, resulting in intermittent hypoxia (IH). Each event is terminated by an arousal from sleep, resulting in reoxygenation, and producing fragmented sleep patterns.(Gozal, 2013a; Rosenzweig *et al.*, 2013b) It has been suggested that in older people the intermittent hypoxia and fragmented sleep may accelerate brain atrophy, cognitive decline, and may even increase the severity of dementia.(Fotuhi *et al.*, 2009; Ayalon *et al.*, 2010; Durgan & Bryan, 2012; Gozal, 2013a). The mechanism of this cognitive impairment may be mediated by the impact of the intermittent hypoxia and / or sleep fragmentation on neurogenesis and brain structure, particularly in the hippocampus (see **Chapter 1, Section 1.4**). However, a review of the changes in brain morphology associated with OSA shows that multiple regions are influenced, and that the overall picture is confusing (see **Table 3.1**).

The differences shown in **Table 3.1** are likely to be caused by the use of different image analysis methods in the reported studies. Over the years the techniques for analysis of MRI scans have improved; in addition the statistical thresholds used in the analysis varied (Morrell & Glasser, 2011). Nevertheless, this explanation does not take into account the between-subject variability to a given hypoxic stimulus (Rosenzweig *et al.*, 2013b, d) or the within-subject differences across multiple brain regions. Additionally, it does not account for any cardiovascular and cerebrovascular protection conferred by ischaemic preconditioning, resulting from the nocturnal cycles of hypoxia-reoxygenation (Lavie & Lavie, 2006; Rosenzweig *et al.*, 2013b). The role of ischaemic preconditioning, which represents a generalized adaptation to ischaemia is described in detail in Chapter 1, Section 1.4.1. (Lavie & Lavie, 2006; Dirnagl *et al.*, 2009)

Table 3.1: A review of studies that have used MRI (voxal based morphomatry) in OSA (Morrell & Glasser, 2011)

Study	Sample Size	AHI Mean (SD)	Statistical Analysis and Threshold	Changes in Gray Matter Concentration
Macey 2002 (1)	21 OSA* 21 Control subjects	34 (20)	SMP V99 $P < 0.001$, uncorrected for multiple comparisons	Diffuse changes across the entire brain: frontal and parietal cortices, temporal lobe, anterior cingulate, hippocampus, and cerebellum
Morrell 2003 (15)	7 OSA 7 Control subjects	Median 28 (range, 25–40)	SMP V99 $P < 0.01$, corrected for multiple comparisons within a small volume	No significant reductions across the entire brain Focal reduction within the left hippocampus
O'Donoghue 2005 (4)	**27 OSA** **24 Control subjects**	**71 (17)**	**SMP V2.0** **$P < 0.05$, corrected for multiple comparisons using FDR**	**No significant reductions**
Yaouhi 2009 (16)	*16 OSA* *14 Control subjects*	*38 (14)*	*SMP V2.0* *$P < 0.005$, uncorrected for multiple comparisons, but with a minimum cluster size*	*Bilateral prefrontal cortex, inferior parietal gryrus, right temporal cortex, occipital cortex, right thalamus, some basal ganglia, right hippocampus, para-hippocampus, and cerebellum*
Morrell 2010 (17)	60 OSA 60 Control subjects	55 (95% CI, 48–61)	SMP V8.0 $P < 0.05$ corrected for multiple comparisons using FDR	Left cerebellum, right temporal gyrus
Joo 2010 (18)	36 OSA 31 Control subjects	53 (range, 32–106)	SMP V2.0 $P < 0.05$ corrected for multiple comparisons using FDR	Left gyrus rectus, bilateral superior frontal gyrus, left precentral gyrus, bilateral cingulated gyrus, right insular gyrus, bilateral caudate nucleus, bilateral thalamus, bilateral amygdale, bilateral hippocampus, bilateral temporal gyrus, bilateral quadrangular lobe, bilateral biventer lobe
Canessa 2011 (3)	***17 OSA*** ***15 Control subjects***	***56 (19)***	***SMP V5.0*** ***$P < 0.05$ corrected for multiple comparisons using FDR***	***Left posterior-parietal cortex, right superior-frontal gyrus, left hippocampus***
Torelli (2011) (19)	*13 OSA* *9 Control subjects*	*53 (26)*	*SMP V8.0* *$P < 0.05$ corrected for multiple comparisons using FWE*	*Left hippocampus, bilateral temporal lobe*

Definition of abbreviations: FDR = false discovery rate; FWE = family wise error; SPM = statistical parametric map.
Rows in bold are studies that measured both gray matter changes in brain pre and post CPAP; rows in italics measured both cognitive function and changes in gray matter.
* 20 patients with co-morbidities.

3.2.1. Neurogenesis and the role of intermittent hypoxia in OSA

New neurons are produced on a continuous basis in the healthy adult human brain, with neural stem/progenitor cells residing in two major neurogenic regions: the subventricular zone lining the lateral ventricles and the dentate gyrus (DG) of the hippocampal formation (Seki, 2011). Additional sites of neurogenesis, such as the temporal horn, thalamus, striatum, cerebellum and cortex have been reported in some animal models (Seki, 2011). Hypoxic/ischaemic insults in rodent models are powerful stimuli of adult neuronal cell production in both neurogenic areas and otherwise dormant regions such as the striatum and hippocampal pyramidal cell layer CA1 (Lichtenwalner & Parent, 2006) – Also see **Fig 3.1** Top panel.

In the classic study of (Gozal *et al.*, 2001), chronic intermittent hypoxia in animal models of OSA was associated with impaired spatial learning that coincided with the increased structural change in the cortex and CA1 region of the hippocampus (Gozal *et al.*, 2003b; Gozal *et al.*, 2003c; Haddad & Yu, 2009). Moreover, these researchers demonstrated increased cell proliferation in the dentate gyrus at

a later stage of this process, which was present in spite of the ongoing intermittent hypoxia. Therefore the biphasic, temporal change in dentate gyrus proliferation may account for the partial recovery of clinical function in the later stages of IH exposure (Gozal *et al.*, 2003b). Several other preclinical studies have also reported a 'protective' response to moderate intermittent hypoxia(Jung *et al.*, 2008; Haddad & Yu, 2009) suggesting that ischaemic preconditioning-like processes may occur. For example, in one rodent model, the intermittent hypoxia intervention after the ischaemic event led to increased expression of brain derived neurotrophic factor (BDNF), increased hippocampal neurogenesis and functional synaptogenesis, as well as in improvement in spatial learning and long-term memory impairment (Tsai *et al.*, 2011; Tsai *et al.*, 2013). In another study, intermittent hypoxia in adult rats was also shown to promote hippocampal neurogenesis, and to mimic antidepressant-like effects (Zhu *et al.*, 2010).

Despite the animal data suggesting the association between chronic intermittent hypoxia, ischaemic preconditioning and the subsequent adaptive increase in adult neurogenesis in several affected brain regions, this putative association has not so far been shown conclusively in clinical studies of brain changes in OSA (**Table 3.1**) (Macey *et al.*, 2002; Morrell *et al.*, 2003; Macey *et al.*, 2008; Yaouhi *et al.*, 2009; Joo *et al.*, 2010; Morrell *et al.*, 2010; Torelli *et al.*, 2011). To date, volumetric, predominantly voxel based morphology (VBM), studies of brain changes in OSA patients indicate a predominantly hypotrophic effect in a number of cortical regions and subcortical structures (Macey *et al.*, 2002; Morrell *et al.*, 2003; O'Donoghue *et al.*, 2005; Macey *et al.*, 2008; Yaouhi *et al.*, 2009; Morrell *et al.*, 2010; Torelli *et al.*, 2011). This includes the findings of studies carried out by my own group at the Brompton Hospital. However, there is high variability in results across clinical studies of OSA and the neuroimaging methods used in earlier studies might not be sufficiently sensitive to capture the subtle and spatially diffuse changes in regions such as the hippocampal formation. The connectivity between these regions is also important in describing the neurocircuitry of OSA neuropathology (Gozal, 2013b; Rosenzweig *et al.*, 2013b).

In order to address some of these issues, I decided to re-analysis the magnetic resonance imaging scans, previously obtained by my group, using a fully automated volumetric analysis method, FreeSurfer (FS). Specifically I wished to study the changes in several subcortical structures in OSA patients with different severity of disease, and age-matched healthy controls (Doring *et al.*, 2011). The FS method is more fully described in Chapter 2, Section 2.3.6. It has also now been validated in a number of clinical studies where it was shown to be efficient in quantifying subcortical volumes in dementia (Pengas *et al.*, 2009), epilepsy (McDonald *et al.*, 2008), depressive disorders (Tae *et al.*, 2008) and aging (Walhovd *et al.*, 2005). The hypothesis investigated in the study presented in this thesis was

that volumes in the hippocampus, thalamus and cerebellum would differ across diagnostic groups. Analysis of the MR scans used in this study has been previously published as a sub-set of a wider study (Morrell *et al.*, 2010); the hypothesis and techniques used in this chapter are different.

3.3 METHODS

Patients were recruited from Royal Brompton and Charing Cross Hospitals sleep clinics. Inclusion criteria were: AHI >15 events/h. Exclusion criteria were: a history of other respiratory disease, cerebrovascular disease or ischaemic heart disease, diabetes mellitus, a neurological or psychiatric disorder, alcohol or illicit drug abuse, or current intake of psychoactive medications.

Healthy controls were recruited through a database of healthy volunteers. Exclusion were: a history of habitual snoring or any other sleep complaint. All control subjects were screened with polysomnography and questionnaires to exclude OSA (AHI <5 events/h).

The study was approved by the ethics committee of the Royal Brompton Hospital (Ref: 01-160), and all patients and healthy volunteers gave informed written consent.

3.3.1 Measurements and Analysis

The protocol and measurements made during nocturnal sleep studies are described in Chapter 2, Section 2.2. Apnoeas were defined as >80% drop in airflow for 10s and hypopneas were defined as >50% reduction in airflow from baseline with a >4% dip in saturation, or an arousal from sleep.

3.3.2 Magnetic resonance imaging and analysis

The principle of MRI is described in Chapter 2, Section 2.5.1. In the study presented in this chapter all participants underwent MR imaging at the Charing Cross Hospital. T1-weighted MR images were acquired using a 1.5T scanner (Magnetom Vision, Siemens Healthcare, Camberley, Surrey, UK) and a 3D MP-RAGE sequence (TI 300 ms, TE 4 ms, in-plane resolution 1.031.0 mm) with contiguous 2 mm coronal slices.

The T1-weighted images were processed and volumetry performed using an automated method unbiased by non-linear registration, FS, as previously described in Chapter 2, Section 2.5.2. (Fischl *et al.*, 2002; Walhovd *et al.*, 2005; Tae *et al.*, 2008; Cerasa *et al.*, 2009; Pengas *et al.*, 2009; Doring *et al.*,

2011). In summary during this fully automated process removal of non-brain tissue, automated Talairach transformation, segmentation of the subcortical white matter (including segmentation of the corpus callosum to five parts) (Webb *et al.*, 2012) and deep grey-matter volumetric structures, intensity normalization, and cortical reconstruction were performed. A neuroanatomical label to every voxel in the MR image volume was assigned and the Markov Random Field Theory was applied, where the probability of a label at a given voxel was computed not just in terms of the grey-scale intensities and prior probabilities at that voxel, but also as a function of the labels in a neighbourhood of the voxel in question. Given the *a priori* hypothesis regarding the differences in hippocampal volumes, this step was particularly pertinent as it enabled correct separation of the hippocampus and amygdala, which have similar grey-scale values (Doring *et al.*, 2011).

The analysis was performed for all scans on the same workstation using parallel running streams with no variability to the data processing conditions (Gronenschild *et al.*, 2012). The segmented 3D images of structures of interest were inspected for gross errors through visualization with 3D slicer (Version 3.2 1.0, NIH, USA) (IR), and the volume values were extracted by implemented Unix scripts by Dr Crumm and Dr Kempton.

3.3.3 Statistical Testing

The Kolmogorov-Smirnov test was used to test the normality of distributions. To analyse differences in variety of demographic parameters between controls and OSA patients, a student's t-test was initially applied. All statistical analyses had a 2-tailed α level of <.05 for defining significance and were performed by a biostatistician on the statistical software "STATISTICA 10.0" (http://www.statsoft.com).

The gender differences between the two groups were found to be non-significant (Pearson Chi-Square test, p=.479). The intracranial volume (ICV) calculated by the FS did not differ significantly between groups (**Appendix 3.1**) and a one-way analysis of covariance (ANCOVA) was conducted (using age as a covariant) on the ICV controlled data to assess between-group differences. Finally, the presumed inter-regional connectivity between the hippocampi and thalami and cerebellar cortices was explored using Pearson correlations; controlled for ICV and age.(Plessen *et al.*, 2006)

3.4 RESULTS

Sixty five participants were studied with MR neuroimaging. Demographic data is given in **Table 3.2**.

Table 3.2. Demographic Information

	OSA **n= 32; mean [SD]**	**Control** **n= 33; mean [SD]**
Age [years]	48.50 [12.51]	49.73 [11.30]
BMI [kg/m^2]*	31.48 [4.34]	24.76 [3.66]
AHI [events/h]*	42.3 [23.81]	2.1 [1.61]
ODI [events/h]*	31.4[19.43]	1.2 [1.33]
ESS*	13.2 [4.64]	4.7 [3.69]
Right-handedness [%]	100	100

*Significant difference between OSA patients and healthy controls (P<.001). There was no significant difference in the age between the two groups (P=.68). Normality was checked using Kolmogorov-Smirnov test. The plots appeared approximately normally distributed so independent sample *t-test* statistics were used to compare patients and controls. **Abbreviations**: **AHI**, apnoea/hypopnoea index; **BMI**, body mass index; **ESS**, Epworth sleepiness scale; **n**, number; **ODI**, oxygen desaturation index; **OSA**, obstructive sleep apnoea; **SD**, standard deviation.

The *a priori* hypothesis investigation concentrated on analysis of group differences for three neuroanatomical structures previously shown to be affected in OSA; the hippocampus, thalamus and cerebellum. The hippocampus was found to be larger bilaterally in the OSA group and hypertrophy in the right hippocampus was statistically significant (absolute mean values: OSA, 4336.5mm^3 vs control, 4090.6mm^3; F (1,62)=5.02, P=.029). A statistically non-significant trend for smaller thalami in the OSA group was also noted, more so on the right (absolute mean values: OSA, 6718.9mm^3 vs control, 7056.4mm^3; F (1,62)= 2.9, P=.092). No statistically significant differences were noted between cerebellar cortical and white matter volumes of the two studied groups (**Table 3.3**).

Amongst the values for several other subcortical structures automatically calculated by FS, only two

more group differences in volume reached statistical significance; those of choroid plexus and the middle anterior portion of the corpus callosum. Both left (F (1,62)=4.39, P=.040) and right choroid plexus (F (1,62)=6.13, P=.016) (**Table 3.3**) were found to be hypertrophic in the OSA group. Conversely, the volume of the mid-anterior portion of the corpus callosum was significantly decreased in OSA patients (F (1,62)=4.26, P=.043). The hypertrophy of the right choroid plexus along with the hypertrophy of the right hippocampus in the OSA group was found to be statistically significant even when controlled for age (ICV controlled t-test; P<.05; **Table 3.3**).

The results for the remaining subcortical structures by FS are summarised in **Appendix 3.1.**

Table 3.3. Subcortical Volumes as determined by analysis of brain MRI using FreeSurfer.

Region	Study Group	n	Mean [SD] mm^3	ANCOVA[a] *P* values	*t* – test[a] *P* values
Right Hippocampus	Control OSA	33 32	4090.58 [663.41] 4336.47 [461.99]	***.029****	***.025****
Left Hippocampus	Control OSA	33 32	4321.61 [483.03] 4453.81 [477.37]	.096	.084
Right Thalamus	Control OSA	33 32	7056.39 [1037.81] 6718.88 [827.24]	.092	.212
Left Thalamus	Control OSA	33 32	7190.27 [1042.69] 6965.72 [960.79]	.327	.547
Right Cerebellum (Cortex)	Control OSA	33 32	54018.21 [5284.82] 52464.13 [4974.43]	.461	.533
Left Cerebellum (Cortex)	Control OSA	33 32	52611.33 [5081.25] 51001.41 [5119.22]	.401	.486
Right Cerebellum (White Matter)	Control OSA	33 32	15275.03 [2460.36] 14658.78 [1776.50]	.399	.442
Left Cerebellum (White Matter)	Control OSA	33 32	15059.58 [2397.52] 14772.03 [1985.43]	.849	.929
Right Choroid Plexus	Control OSA	33 32	1982.91 [446.91] 2172.81 [476.45]	***.016****	***.035****
Left Choroid Plexus	Control OSA	33 32	1733.67 [373.39] 1839.22 [371.26]	***.040****	.087
Corpus Callosum *(Middle Anterior Portion)*	Control OSA	33 32	459.55 [139.62] 406.53 [72.13]	***.043****	.078

In the table, for each neuroanatomical structure statistical analysis of group differences for ICV controlled volumes was performed; t-test and ANCOVA test (covariate with age),were done. [a]Bonferroni corrected P values. *****Significant difference between OSA patients and healthy controls (p<.05). **Abbreviations**: **ANCOVA**, Analysis of covariance; **ICV**, intracranial volume; **OSA**, obstructive sleep apnoea; **SD**, standard deviation.

Post hoc interregional volumes correlation analysis revealed an increased number of significant correlations between the cerebellar cortex and hippocampal and thalamic structures in the OSA group when compared to controls (**Fig 3.1 bottom panel**). On the non-dominant side, in the OSA group significant correlation was found between the right hippocampus and thalamus with the ipsilateral (hippocampus, r=.363; P=.041; thalamus, r=.407; P=.021) and the contralateral cerebellar cortex (hippocampus, r=.379; P=.032; thalamus, r=.455; P=.009).

In the control group, the only significant correlation was found between right thalamus and the

contralateral cerebellar cortex (r=.349; P=.047). On the dominant side, in the OSA group, there was a significant positive correlation between left thalamus with both ipsilateral (r=.383; P=.030) and contralateral (r=.381; P=.031) cerebellar cortex. Only ipsilateral correlation was found significant for left hippocampus (r=.357; P=.045). Conversely, control subjects had no significant correlations.

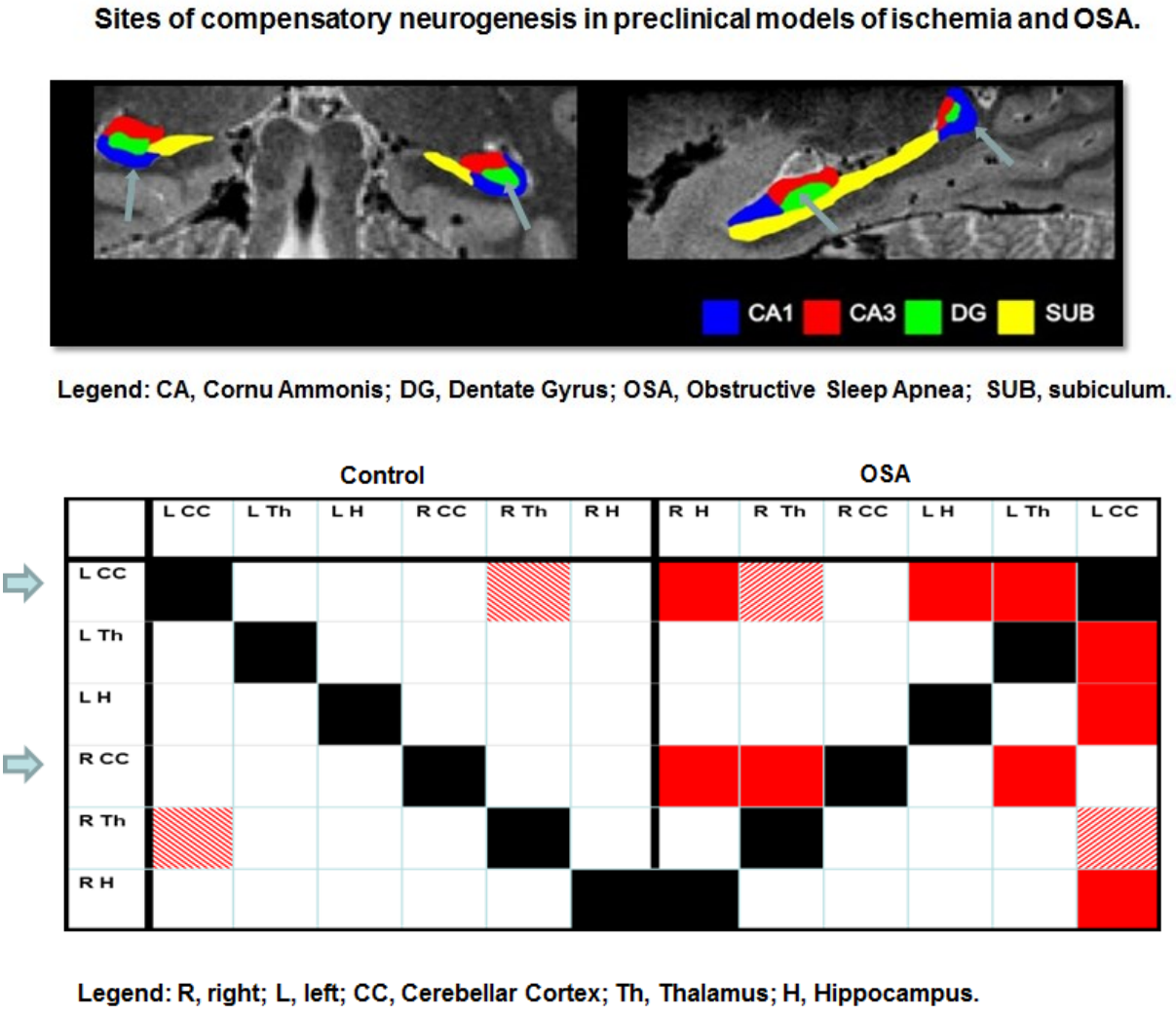

Figure 3.1: Dentate gyrus (DG) and CA1 (*arrows*) support neurogenesis in animal models of ischemia/OSA (**Top left panel**). Human hippocampal subfields are shown (coronal/sagittal planes), adapted from[69] (**Top right panel**) Potential aberrant/additional connectivity between cerebellar cortices (*arrows*) and thalamus and hippocampus in OSA patients **(Bottom panel)**.

3.5 DISCUSSION

The main finding of this study was the coexistence of both hyper- and hypotrophic changes in the OSA group compared to healthy controls. To my knowledge these data provide some of the first experimental evidence that patients with OSA are exposed not only to noxious factors that cause brain injury, but also to mechanisms that act to repair brain tissue. This may be mediated by ischaemic preconditioning and enhanced neurogenesis. (Ferriero, 2005; Dirnagl *et al.*, 2009; Seki, 2011)

3.5.1 Hypertrophic changes and relation to previous studies

The role for the altered neurogenesis and possible conditioning effect of OSA in the OSA patients was suggested by the significant increase of hippocampal volumes, which were up to 6% greater than in the control group. Additionally, the hypertrophy of the choroid plexus, an important source of adult neurogenic factors and signalling molecules for the migration of cells in the subventricular zone (Seki, 2011), was also noted. These findings are compatible with reported hypertrophic change of these structures under ischaemic conditions (Comi, 2003).

Previous clinical studies of the neural changes in OSA, including those from the Royal Brompton Group, have predominantly reported hypotrophic changes in OSA patients (see **Table 3.1**). Although the cross-sectional design of this study only permits associations to be described, and does not provide evidence of cause and effect, it is possible that both hyper- and hypotrophic changes could occur in OSA patients, as there are a range of plausible physiological explanations: Firstly, I studied patients with mixed disease severity and hence they potentially differed from other investigated cohorts that incorporated patients at the more severe end of the OSA spectrum. Indeed, the duration of the exposure to IH and the intensity of the bouts of hypoxia are important determinants of whether IH is protective or harmful (Jung *et al.*, 2008; Haddad & Yu, 2009; Aviles-Reyes *et al.*, 2010). Secondly, the patients were relatively young, mostly in their forties with no obvious major co-morbidities, and it is possible that the onset of OSA was relatively recent. The age-dependent decline in adult neurogenesis is an accepted phenomenon, although it appears to be mediated more by the age-related alterations in the cellular environment than impaired responsiveness of progenitor cells to neurogenic stimuli (Lichtenwalner & Parent, 2006).

3.5.2 Automated FS analysis

Unlike the earlier studies that utilized the optimized VBM method, this study used the fully automated FS analysis that has been proven particularly effective for analysis of subcortical structures (Fischl *et al.*, 2002; Cerasa *et al.*, 2009; Doring *et al.*, 2011). Conversely, the whole-brain VBM method is

generally regarded as less sensitive than the other methods when it comes to detecting abnormalities in small subcortical structures (Cerasa *et al.*, 2009).

In a recent magnetic resonance spectroscopy study of OSA patients, decreased frontal lobe neuronal viability and integrity was shown, as was decreased hippocampal membrane turnover. However, when the structural MRI images were analysed using the VBM method no lesions in those regions were shown in the same patients (O'Donoghue *et al.*, 2012). It should be noted that in animal studies, subregions of the hippocampus were shown to be differentially sensitive to chronic IH (Gozal *et al.*, 2003c; Aviles-Reyes *et al.*, 2010; Papadakis *et al.*, 2013). For example CA1 was particularly IH-sensitive and prone to increased levels of change in structure whilst CA3 and DG were significantly less so (Papadakis *et al.*, 2013). DG was additionally able to undergo compensatory neurogenesis (Gozal *et al.*, 2003b). Further enhancements of cognitive vulnerability to IH exposures occurred in CA1 in rats fed on an obesity-inducing diet (Goldbart *et al.*, 2006). It is, hence, possible that depending on the balance of these changes and their overall offset, the whole volume of the hippocampus might be ultimately noted as either hyper- or hypotrophic; whereas in the current study I have been able to segment these effects, which represents a step forward and differs from previous papers from the Brompton group. Finally, volume increase in the hippocampus could represent an epiphenomenal, or downstream, effect of the connectivity with other brain regions, which include prefrontal cortex, amygdala and thalamus.

3.5.3 Hypotrophic changes

In the current study, hypotrophic changes were noted in OSA patients in the mid-anterior portion of the corpus callosum and the thalami. A trend towards smaller thalamic volumes in OSA group in comparison to controls was observed, but it did not reach statistical significance. This is in accordance with previous clinical studies and possibly supports the hypothesis that a disturbed thalamocortical oscillator underlies some of the neurocognitive deficits seen in OSA (Schonwald *et al.*, 2012; Rosenzweig *et al.*, 2013b). One of the major sources of thalamic afferents to the hippocampus (e.g. CA1 and subiculum) is the nucleus reuniens, the largest of the midline nuclei of the thalamus, the region known to be strongly activated by chronic IH (Sica *et al.*, 2000; McKenna & Vertes, 2004). Nucleus reuniens has been implicated in associative learning and object recognition and it is proposed to gate information flow between the hippocampus and the medial prefrontal cortex (Aggleton *et al.*, 2010; Eleore *et al.*, 2011). Similarly, the noted volume reduction of the mid-anterior portion of the corpus callosum in OSA patients in the current study is in agreement with previous diffusion tractography (DTI) studies of white matter tract changes; it likely represents the effects of IH on the later myelinating part of this tract (Kumar *et al.*, 2012; Rosenzweig *et al.*, 2012).

3.5.4 Correlations with cerebellar cortex volumes

The Royal Brompton group has previously shown hypotrophic changes in the cerebellar cortices of OSA patients (Morrell *et al.*, 2010). It is possible that the functional deficits noted in OSA, could be seen as the by-product of being at the milder end of a spectrum of cerebellar cognitive affective syndrome (Schmahmann & Sherman, 1998; Rosenzweig *et al.*, 2013b). In the study presented in this chapter, no significant difference in cerebellar volumes was recorded, although aberrant connectivity with hippocampal and thalamic structures was suggested by the interregional volume correlations analysis (**Figure 3.1 – bottom panel**). This suggestion is in broad agreement with previous VBM and DTI studies (Kumar *et al.*, 2012; Macey, 2012). Whilst these volumetric correlations can be only very tentatively taken to suggest a true aberrant connectivity (Plessen *et al.*, 2006). in the OSA group, they nonetheless circumstantially intimate that 'compensatory' entraining of the cerebellum by a hypertrophic hippocampus may occur.

3.5.5 Limitations

Limitations of this study include the moderate sample size and cross-sectional design, which can suggest only an association, rather than a causal relationship between the neural changes noted in OSA patients and the mechanisms of intermittent hypoxia and sleepiness.

This study did not incorporate neuropsychological testing and the lack of the related correlational data with the noted volumetric changes means that no conjecture about the compensatory role of the prominent enlargement of hippocampi can be made. Furthermore, correlations between regional volumes were exploratory and hypothesis-generating and therefore should be interpreted with caution. They will need to be confirmed in future studies.

It should be noted that the ultra-structural determinants of group differences in morphology of the hippocampus and thalamus are unknown. Addressing these limitations would require detailed post-mortem and other in vivo (adult neurogenesis) imaging methods in order to determine those ultra-structural underpinnings.

Finally, the strict exclusion criteria used in this study does not allow any judgments to be made regarding interactions between OSA and its comorbidities such as hypertension and diabetes, both strongly associated with OSA and known to also cause brain injury (Devisser *et al.*, 2011; Li & Veasey, 2012; Gozal, 2013a).

3.5.6. Conclusions

In summary, these findings demonstrate for the first time to my knowledge hypertrophy of the hippocampus in OSA patients with mixed disease severity. It is proposed that these enlargements represent the end product of neuroglial ischaemic preconditioning (Ferriero, 2005; Lichtenwalner & Parent, 2006; Nanduri *et al.*, 2008; Dirnagl *et al.*, 2009; Aviles-Reyes *et al.*, 2010). This interpretation is consistent with extensive preclinical evidence that increased hippocampal neurogenesis occurs in response to IH, which consequently leads to increases in hippocampal volume and thickness (Gozal *et al.*, 2003b; Lichtenwalner & Parent, 2006; Zhu *et al.*, 2010; Bonnici *et al.*, 2012). Aberrant connectivity between limbic regions, the prefrontal cortex and the cerebellum was also inferred by the study.

It has been previously suggested that increasing age and OSA work additively (or even synchronistically) to overwhelm the brain's capacity to respond to cognitive challenges with compensatory recruitment, and to maintain performance (Ayalon *et al.*, 2010; Sforza & Roche, 2012; Gozal, 2013a). It would, therefore, be important to recognise which compensatory mechanisms evoked by OSA are functionally viable and which may be further detrimental, especially in older people. I will begin to explore these issues in an MRI study carried out in older people, presented in Chapter 5.

Appendix 3.1: Table Shows the Percentage Ratios of Subcortical Volumes to the ICV as determined by the FreeSurfer

Structures		N	Mean	Std. Deviation	Student *t-test*[a] P values	ANCOVA[a] *P* values
L-Cerebellum-White-Matter	OSA	32	0.972	0.130	0.929	0.849
	Control	33	0.975	0.153		
L-Cerebellum-Cortex	OSA	32	3.353	0.289	0.486	0.401
	Control	33	3.408	0.339		
L-Thalamus-Proper	OSA	32	0.457	0.046	0.547	0.327
	Control	33	0.464	0.055		
L-Caudate	OSA	32	0.229	0.028	0.214	0.235
	Control	33	0.221	0.027		
L-Putamen	OSA	32	0.373	0.045	0.276	0.316
	Control	33	0.361	0.048		
L-Pallidum	OSA	32	0.111	0.012	0.827	0.732
	Control	33	0.112	0.014		
L-Hippocampus	OSA	32	0.293	0.032	0.084	0.096
	Control	33	0.280	0.030		
L-Amygdala	OSA	32	0.108	0.012	0.289	0.327
	Control	33	0.104	0.016		
L-choroid-plexus	OSA	32	0.120	0.020	0.087	0.040
	Control	33	0.112	0.020		
R-Cerebellum-White-Matter	OSA	32	0.964	0.104	0.442	0.399
	Control	33	0.990	0.163		
R-Cerebellum-Cortex	OSA	32	3.450	0.281	0.533	0.461
	Control	33	3.498	0.340		
R-Thalamus-Proper	OSA	32	0.441	0.038	0.212	0.092
	Control	33	0.456	0.056		
R-Caudate	OSA	32	0.230	0.026	0.609	0.661
	Control	33	0.227	0.024		
R-Putamen	OSA	32	0.353	0.042	0.263	0.302
	Control	33	0.340	0.047		
R-Pallidum	OSA	32	0.104	0.012	0.554	0.619
	Control	33	0.101	0.016		
R-Hippocampus	OSA	32	0.285	0.030	0.025	0.029
	Control	33	0.265	0.042		
R-Amygdala	OSA	32	0.105	0.013	0.114	0.129
	Control	33	0.099	0.015		
R-choroid-plexus	OSA	32	0.142	0.026	0.035	0.016
	Control	33	0.128	0.026		
CC_Posterior	OSA	32	0.060	0.007	0.514	0.522
	Control	33	0.058	0.010		
CC_Mid_Posterior	OSA	32	0.027	0.005	0.330	0.381
	Control	33	0.026	0.006		
CC_Central	OSA	32	0.027	0.007	0.505	0.433

	Control	33	0.028	0.007		
CC_Mid_Anterior	OSA	32	0.027	0.004	0.078	0.043
	Control	33	0.030	0.009		
CC_Anterior	OSA	32	0.057	0.007	0.954	0.969
	Control	33	0.057	0.011		
SubCortGrayVol	OSA	32	11.917	0.820	0.843	0.704
	Control	33	11.962	0.992		

In the table, for each neuroanatomical structure statistical analysis of group differences for ICV controlled volumes was performed; t-test and ANCOVA test (covariate with age), were performed. [a]Bonferroni corrected P values. *****Significant difference between OSA patients and healthy controls (p<.05). **Abbreviations**: **ANCOVA**, Analysis of covariance; **CC**, corpus callosum; **ICV**, intracranial volume; **OSA**, obstructive sleep apnoea; **SD**, standard deviation. L: Left, R: Right

Chapter 4

CHANGES IN NEUROCOGNITIVE FUNCTION AND BRAIN MORPHOLOGY IN OSA PATIENTS TREATED WITH CPAP

4.1 SUMMARY

Treatment with CPAP offers some protection from the effects of OSA, although it is still unclear which populations should be targeted, for how long, and what the effects of treatment are on different organ systems. The focus of this Thesis is specifically neurocognitive and brain function.The objective of this study was to investigate whether cognitive improvements could be achieved as early as one month into CPAP treatment in patients with OSA.

Fifty-five patients (mean (SD) age: 47·6 (11·1) years) with newly diagnosed moderate-severe OSA (ODI: 36·6 (25·2) events/hour; ESS: 12·8 (4·9) units) and 35 matched healthy volunteers were studied. All participants underwent neurocognitive testing, neuroimaging and polysomnography. Patients were randomised into parallel groups: CPAP with BSC, or BSC alone for one month, after which they were re-tested. I found that one month of CPAP with BSC resulted in a hypertrophic trend in the right thalamus [mean difference (%): 4·04, 95%CI 1·47 to 6·61], which was absent in the BSC group [-2·29, 95%CI -4·34 to -0·24]. Significant improvement was also recorded in ESS, in the CPAP plus BSC group, following treatment [mean difference (%): -27·97, 95%CI -36·75 to -19·19 *vs* 2.46 95%CI -5·23 to 10·15; P=0·012], correlated to neuroplastic changes in brainstem (r=-0·37; P=0·05), and improvements in delayed logical memory scores [57·20, 95%CI 42·94 to 71·46 *vs* 23·41 95%CI 17·17 to 29·65; P=0·037]. The finding presented in this study suggest that one month of CPAP treatment can lead to adaptive alterations in the neurocognitive architecture that underlies the reduced sleepiness, and improved

verbal episodic memory in patients with OSA. Therefore even short treatment periods can lead to partial neural recovery and this information can be used to encourage patients to use CPAP therapy.

Contributions: The study was designed by myself, Prof Morrell and Dr Rosenzweig. I carried out all the data collection and analysis except the operation of the MRI scanner, and the manipulation of the FreeSurfer MRI. Statistical analysis was carried out with the help of Drs Rosenzweig and Milosevic. Interpretation of data was carried out by Glasser, Rosenzweig and Morrell.

4.2 INTRODUCTION

OSA is a chronic sleep disorder that arises from recurrent partial or complete pharyngeal obstruction during sleep (see Chapter 1, Section 1.2.1). The close association of OSA with early onset of cognitive decline, by as much as a decade, has been reviewed in Chapter 1, whilst a growing body of clinical and animal work advocates that OSA should be recognized as one of the rare modifiable risks for accelerated cognitive decline is discussed in Chapter 1, and illustrated by the findings of Chapter 3. In addition, treatment with CPAP, the main treatment for OSA, has also been shown to halt the onset, decelerate the progression, or offer a better prognosis in patients with co-morbid dementia, epilepsy and stroke (Campos-Rodriguez *et al.*, 2014; Pornsriniyom *et al.*, 2014; Yaffe *et al.*, 2014; McMillan *et al.*, 2015; Osorio *et al.*, 2015).

The additive impact of progressive changes in sleep quality and structure, changes in cerebral blood flow, neurovascular and neurotransmitters, plus the cellular redox status are all likely to contribute to the cognitive deficits reported in up to one in four newly diagnosed OSA patients (Lavie, 2015; Rosenzweig et al., 2015), Despite concerted efforts, OSA remains widely underdiagnosed in the general population, with its prevalence predicted to increase sharply over the coming years due to ageing and the epidemic of obesity (Heinzer *et al.*, 2015; Lévy *et al.*, 2015). The important questions of what, who and when to treat, are far from clear (Djonlagic *et al.*, 2015; Rosenzweig *et al.*, 2015). Persistent deficits, even after prolonged treatment with CPAP in some patients, suggest that early detection of the central nervous system (CNS) sequelae in OSA could be crucial (Castronovo *et al.*, 2014; Rosenzweig *et al.*, 2015).

In a recent study of patients with OSA, augmentation of subjective experience, attention and vigilance has been demonstrated after only one night of CPAP (Djonlagic *et al.*, 2015). However, no appreciable impact on procedural memory consolidation was noted, suggesting differential impact on brain structures underlying these processes (Djonlagic *et al.*, 2015). On the other hand, in a seminal study, three months of CPAP treatment led to a significant recovery of cognitive and morphometric deficits (Canessa *et al.*, 2011). Taken together, empirical clinical experience and early research findings suggest that subjective memory improvements are reported as early as one month following the commencement of CPAP treatment (McMillan *et al.*, 2015).

In the present study, I aimed to investigate the timeframe over which CPAP treatment could produce changes in neurocognitive function and brain morphology. I tested the hypothesis that one month of CPAP treatment would lead to cognitive improvements, and that any changes would be associated with neuroplastic changes in patients with OSA.

4.3 METHODS

4.3.1 Participants and Design

Patients with newly diagnosed OSA (18-65 years old) were recruited from Royal Brompton and Harefield Hospitals sleep clinics. Inclusion criterion was an AHI >10 events/h. Exclusion criteria were a history of respiratory, cerebrovascular and/or ischaemic heart disease, diabetes mellitus, neuropsychiatric or neurological disorder, alcohol or drug abuse, or psychoactive medications.

The same exclusion criteria were used for healthy controls (age- and education-matched) recruited from a database of healthy volunteers. Those with a history of sleep problems on questionnaires, or evidence of OSA on pulse-oximetry (ODI >5 events/h) were excluded.

The study was approved by the UK central research ethics committee (10/H0706/51). All patients gave written informed consent.

4.3.2 Measurements and Analysis

All enrolled patients were randomly allocated [stratified by age, OSA severity (ODI & AHI) and years of education], by an independent study coordinator, to receive CPAP with BSC, or BSC alone, for one month and then re-assessed (**Fig 4.1**).

CPAP treatment was initiated using standard clinical practice at each centre (ResMed S9; with humidification as required). BSC consisted of advice on minimising daytime sleepiness through sleep hygiene, naps, caffeine, exercise and weight loss as appropriate to each patient. Both groups were provided with BSC and asked to continue with their usual medical care during the trial.

Structured assessments were performed at baseline (patients and controls) and at follow-up after one month (patients only). In addition, all patients received a telephone call at 1 week to record symptoms, side-effects, and to optimise CPAP adherence. All OSA patients completed an inpatient polysomnography (SOMNOscreen PSG, S-Med, UK – see Chapter 2, Section 2.2.3 for full details) prior to CPAP initiation. Domiciliary overnight pulse-oximetry (Konica-Minolta Inc,) was performed at one month. Treatment compliance was measured objectively by download of the CPAP smart card at the one-month visit.

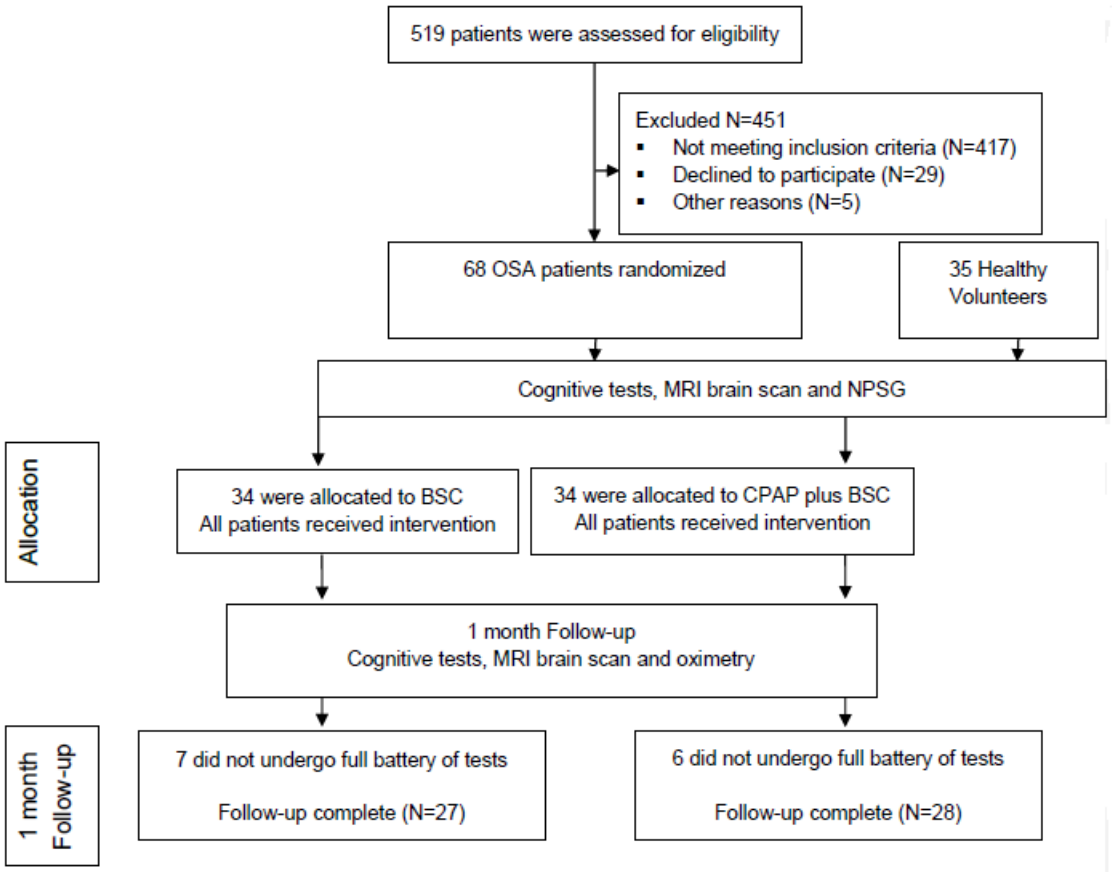

Figure 4.1: CONSORT diagram

Cognitive Tests Battery: A full description of the cognitive tests used in this chapter is given in Chapter 2, Section 2.4. In brief, the cognitive function of all participants was assessed using a battery of tests comprising the Addenbrooke's Cognitive Examination-Revised (ACE-R) (Graham *et al.*, 2004; Mioshi *et al.*, 2006), Trail Making Test A and B (Reitan, 1979) (TMA, TMB), Logical Memory (LM) Test: immediate and delayed LM with alternate stories used at baseline and follow up, subtests from the Wechsler Memory Scales (D, 1987), Digit Span Test: forward (DSF) and backward (DSB) (D, 1987), Spatial Span subtest forward (SSF) and backward (SSB) (the visual-spatial version of Digit Span) (Wechsler, 1997). The tests used were chosen to target cognitive domains that have been previously shown to be affected by OSA from results of the Brompton Sleep group and other (Twigg *et al.*, 2010; Rosenzweig *et al.*, 2015).

Magnetic Resonance Imaging: All participants underwent MR imaging at the Royal Brompton Hospital, and T1-weighted MR-images were acquired using a 1.5T scanner (Magnetom Vision, Siemens

Healthcare, Camberley, Surrey, UK) and a 3D MP-RAGE sequence (TI 300ms, TE 4ms, in-plane resolution 1·0x1·0mm) with contiguous 2mm coronal slices. The T1-weighted images were processed, and volumetry was performed using the automated method FreeSurfer, as previously described in Chapter 2, Section 2.5.2.

4.3.3. Statistical Analyses

The Kolmogorov-Smirnov test was used to test the normality of distributions. To analyse differences in a variety of demographic parameters between healthy controls and OSA patients, Student's t-test was applied (**Table 4.1**). All statistical analyses had a 2-tailed α level of <0.05 for defining significance and were performed with the help of a biostatistician on the statistical software "STATISTICA 10·0" (http://www.statsoft.com).

Investigation of *a priori* hypotheses was focused on group differences for several neuroanatomical structures that had been previously highlighted by my data (see Chapter 3) and that of other groups, to present network hubs of OSA-affected neurocircuitry: the hippocampus, amygdala, basal ganglia, thalamus, brainstem, corpus callosum (and its subdivisions) and cerebellum.(Gozal, 2013a; Rosenzweig *et al.*, 2015) In addition, several previously reported affected cognitive domains were assessed.(Twigg *et al.*, 2010)

The intracranial volume (ICV) calculated by FreeSurfer did not differ significantly between groups (t-test, P=·514) and student's t-test was done on the ICV normalised data (i.e. volume/ICV) to assess between-group differences. Hierarchical cluster analysis was further undertaken to identify homogeneous groups of variables amongst differential (diff Δ) changes in brain volumes following the two interventions.

During *post-hoc* analyses interregional connectivity and inter-domain relationships between the right thalami, somnolence (ESS test scores) and verbal episodic memory (delayed LM test scores) were explored with Pearson correlations; controlled for ICV and normalised for differential changes (diffΔ%) from the baseline ($diff\Delta=[V/domain_{pre}-V/domain_{post}]/V/domain_{pre}$ x100). All results were Bonferroni corrected for multiple comparisons.

4.4 RESULTS

Fifty five OSA patients (81%) completed the study, in addition to 35 age- and education- matched healthy volunteers (**Fig 4.1**). Attrition was due to incomplete investigations in 10% (7/68) of the BSC group, and 9% (6/68) of the CPAP group. Mean (SD) age of OSA patients was 47·6(11·1) years, ODI 36·6 (25·2) events/hour, and ESS 12·8 (4·9). Baseline demographic characteristics of participants are shown in **Table 4.1**. Baseline sleep characteristics are shown in **Table 4.2**. The baseline characteristics were similar between the CPAP with BSC group and BSC only group. Median CPAP use over one month was 5·0 (range 1·9-6·9) hours/night (**Table 4.1**).

Table 4.1: Baseline characteristics of participants.

	Controls	**$BSC_{baseline}$**	**$CPAP_{baseline}$**
Age (years)	43·5 (2·1)	46·8 (2·3)	49·9 (2·0)
BMI (kg/m^2)	n/a	34·4 (5·1)	35·2 (2·9)
Education (years)	15·0 (0·4)	14·6 (0·5)	14·7 (0·5)
AHI (events/h)	n/a	36·4 (4·0)	36·6 (5·0)
ODI (events /h)	2·31 (0·2)	37·8 (4·5)	35·4 (5·2)
ESS	6·18 (0·49)	12·44 (0·88)	13·14 (1·0)
Median CPAP use (hours/night)	n/a	n/a	5 (1·9-6·9)
Dexterity (right-handed) (%)	100	100	100

Data presented as mean (SEM), median (25^{th}-75^{th} percentiles) or number of patients N (%). *Abbreviations*: Controls: healthy volunteers (N=35); BSC: baseline values for patients treated with best supportive care (BSC) (N=27); CPAP: baseline values for patients treated with continuous positive airway pressure (CPAP) with BSC (N=28); BMI: Body mass index, AHI: apopnea/hypopnea index. ODI: oxygen desaturation index;

Table 4.2: Sleep baseline characteristics of two groups of OSA patients

Additional Baseline Characteristics		N	Mean	SD	P[a]
TST	BSC	27	369·39	71·86	0·042
	CPAP	28	419·31	102·18	
Total Deep sleep	BSC	27	52·32	31·69	0·684
	CPAP	28	56·17	37·64	
total REM	BSC	27	64·10	37·91	0·611
	CPAP	28	69·38	38·58	
% deep sleep	BSC	27	13·27	7·23	0·937
	CPAP	28	13·11	7·78	
% REM	BSC	27	17·49	9·84	0·537
	CPAP	28	15·99	7·98	
Desats (events)	BSC	27	235·19	140·77	0·786
	CPAP	28	248·11	203·96	
ODI (events/hour)	BSC	27	37·83	23·24	0·726
	CPAP	28	35·42	27·41	
apnoeas/hypopnoeas (events)	BSC	27	227·78	134·42	0·802
	CPAP	28	239·75	208·90	
AHI (events/hour)	BSC	27	36·40	20·91	0·979
	CPAP	28	36·58	27·15	
AI (events/hour)	BSC	27	15·00	16·61	0·418
	CPAP	28	19·73	25·31	
Time SaO2 <90%	BSC	27	55·65	56·37	0·503
	CPAP	28	69·28	89·10	

[a]Bonferroni corrected P values. Apart from the total sleep time, the baseline characteristics were broadly similar between the two groups.

Abbreviations: TST: total sleep time; REM: rapid eye movement sleep; desats: deaturations; ODI: oxygen desaturation index; AHI: apnoea hyponea index; AI: arousal index; SD: standard deviation; C: BSC: best supportive care at baseline; CPAP: patients to be treated with continuous positive airway pressure (CPAP) at baseline.

4.4.1. Changes in brain morphology

At baseline, hypotrophic changes were recorded in the left hippocampus, in the bilateral pallidus and the mid-posterior part of the corpus callosum of OSA patients (n=55) in comparison to those recorded in healthy controls (n=35) (**Fig 4.2:** pre-CPAP). Following one month of interventions, the between-group comparison (patient groups vs healthy controls) no longer showed statistically significant hypotrophic changes in either of these structures, suggesting that adaptive neuroplastic changes have occurred (**Table 4.3**). The hierarchical cluster analysis further demonstrated the distinct alteration pattern for each intervention during this period **(Fig 4.3**), with the thalamic alterations in the CPAP group closely linked to wider core neurocircuitry adaptations, compared to the BSC group. Of particular interest is the close clustering of the left and right hippocampi in the CPAP group, but not in the OSA group. The left and right cerebellum also form a cluster with the right and left thalamus, and right and left hippocampus in the CPAP group but not the BSC group.

Table 4.3: The effect of one month of interventions on normalised volumes of structures that were registered hypotrophic at baseline.

Structures	Control	OSA	BSC	CPAP	P^a	P^aBSC	P^aCPAP
L Pallidum	0·1094 (0·015)	0·1026 (0·015)	0·1045 (0·024)	0·1067 (0·017)	0·039*	0·177	0·504
L Hippocampus	0·2355 (0·025)	0·2244 (0·03)	0·2290 (0·051)	0·2381 (0·027)	0·071*	0·334	0·697
R Pallidum	0·1028 (0·015)	0·0960 (0·014)	0·0974 (0·020)	0·0980 (0·013)	0·031*	0·177	0·172
CC_Mid_Posterior	0·0296 (0·006)	0·0269 (0·005)	0·0279 (0·006)	0·0283 (0·006)	0·033*	0·270	0·400

Data presented mean (SD), number of patients N. Mean values: percentage ratios of subcortical volumes to the ICV as determined by FreeSurfer. *P* columns: *P*= OSA at baseline vs controls; *P* BSC= BSC vs controls; *P* CPAP= CPAP with BSC group vs controls. [a]Bonferroni corrected *P* values. *Abbreviations*: R: right; L: left; CC: corpus callosum; ICV: intracranial volume; ODI: oxygen desaturation index; SD: standard deviation; C: Controls: healthy volunteers (N=35). OSA: baseline values for all obstructive sleep apnoea (OSA) patients before any intervention (N=55); BSC: patients treated with best supportive care (BSC) for one month (N=27); CPAP: patients treated with continuous positive airway pressure (CPAP) with BSC for one month (N=28).

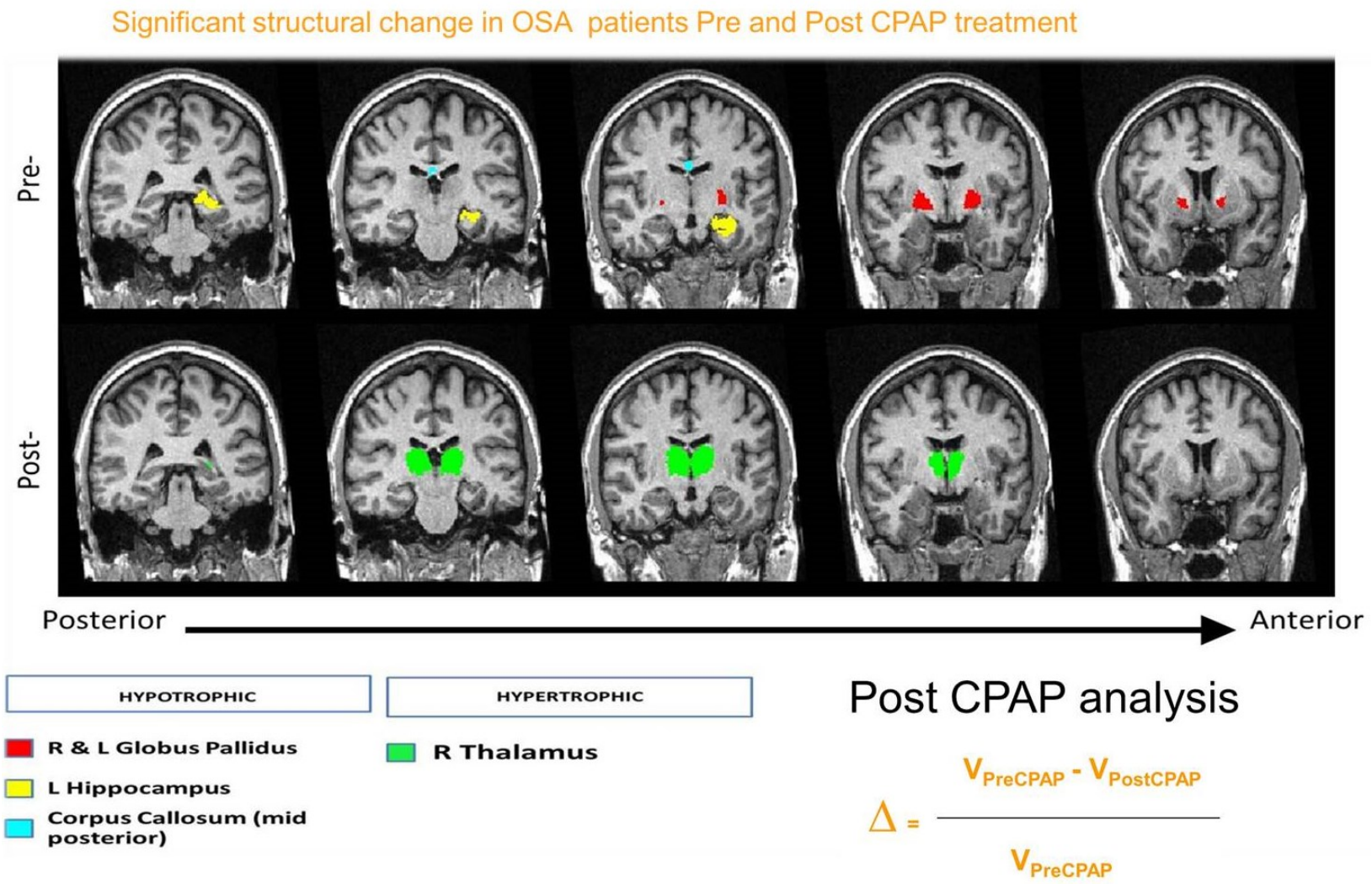

Figure 4.2: Significant structural change in OSA patients Pre and Post CPAP treatment. The first row shows neuroanatomical structures that were noted as hypotrophic in OSA patients (N= 55) before any intervention, by comparison to matched healthy volunteers (N=35); bilateral globus pallidi, left hippocampi and mid posterior section of corpus callosum. The second row depicts hypertrophic changes noted in bilateral thalami following one month of treatment with CPAP; the longitudinal structural changes were calculated by normalization to the individual baseline volumes (*refer to the equation*).

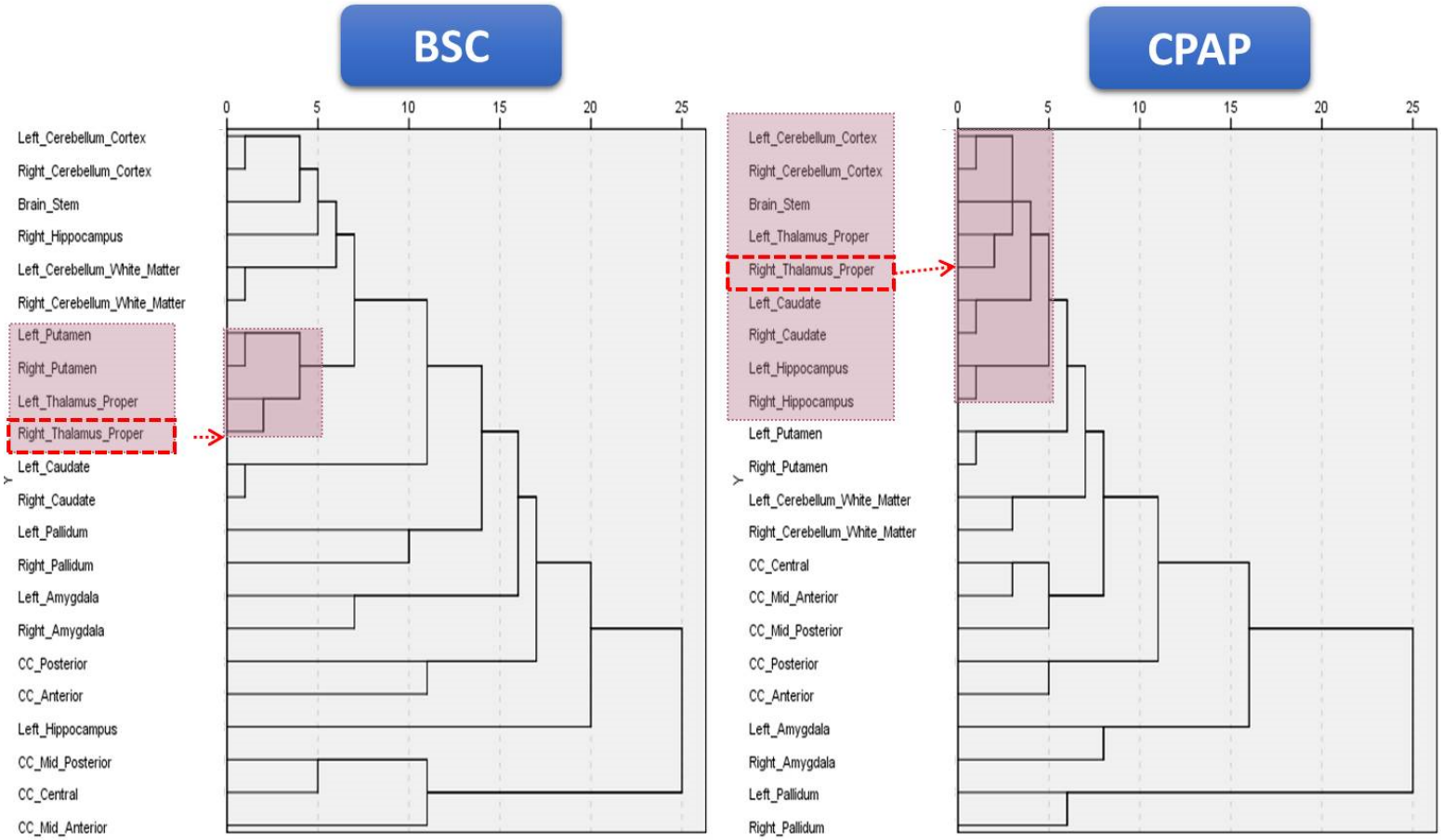

Figure 4.3:

Dendrograms depict relationships between structural changes in brain volumes following one month of intervention with the Continuous Positive Airway Pressure or Best Supportive Care treatment. Dendrograms were constructed using the results of Hierarchical Cluster Analysis (HCA) of the diff Δ structural changes, using Average Linkage with squared Euclidean distance as a measure of distance (x-axis values). HCA resulted in the formation of clusters that were iteratively joined in a descending order of similarity. Resulting dendrograms show different patterns of neuroanatomical changes in patients with OSA following interventions with BSC alone (N=27) from those resulting post CPAP with BSC (N=28) treatment. The HCA findings also suggest that the thalamic

alterations (arrows) in the CPAP group are closely linked to a wider core of neurocircuitry adaptations and particularly that there is increased linkage between the left and right brain, including hippocampi (pink boxes).

The differential neuroanatomical structural changes at one month between the CPAP group and the BSC group were subsequently explored. In the CPAP group, a significant hypertrophic trend (**Fig 4.2**) for the right thalamus was recorded [mean diffΔ% (SD): CPAP,4·04 (13·61) vs BSC,-2·29 (10·66); P=0·06]. No other statistically significant differences between groups were found (**Table 4.4**).

Table 4.4: The effect of the Continuous Positive Airway Pressure or Best Supportive Care regimes at one month on changes in volumes of structures.

Structure	BSC N=27	SEM	CPAP N=28	SEM	P[a]
L CerebellumWhiteMatter	2·87%	(2·44%)	4·27%	2·93%	0·716
L CerebellumCortex	-1·13%	1·65%	1·37%	2·62%	0·428
L Thalamus	-0·76%	2·30%	3·04%	2·55%	0·274
L Caudate	5·08%	2·82%	1·26%	2·12%	0·281
L Putamen	1·20%	2·65%	7·90%	3·23%	0·116
L Pallidum	3·10%	3·10%	7·52%	5·49%	0·490
BrainStem	0·49%	2·10%	-0·11%	2·07%	0·840
L Hippocampus	6·73%	3·97%	4·84%	2·91%	0·701
L Amygdala	-0·42%	3·70%	6·62%	3·79%	0·190
R CerebellumWhiteMatter	3·36%	2·42%	3·48%	3·41%	0·977
R CerebellumCortex	-0·09%	2·13%	2·59%	2·71%	0·443
RThalamus	-2·29%	2·05%	4·04%	2·57%	0·061*
R Caudate	2·92%	2·86%	0·98%	2·73%	0·626
R Putamen	2·47%	2·83%	8·53%	3·35%	0·174
R Pallidum	1·10%	3·02%	8·69%	6·92%	0·325
R Hippocampus	2·60%	2·66%	3·51%	3·25%	0·831
R Amygdala	0·33%	3·27%	8·83%	5·25%	0·179
CC_Posterior	3·77%	3·44%	-0·16%	3·18%	0·404
CC_Mid_Posterior	7·38%	4·81%	5·07%	3·75%	0·705
CC_Central	7·86%	3·90%	3·72%	2·80%	0·390
CC_Mid_Anterior	7·50%	3·52%	3·27%	3·35%	0·388
CC_Anterior	5·52%	3·04%	0·41%	3·45%	0·273

Data presented mean (SEM), controlled for ICV and normalised for differential changes (diff%) from the baseline (diff=[V/domainpre-V/domainpost]/V/domain pre x100)· [a]Bonferroni corrected P values· Significant difference between changes in two groups of patients after one month of CPAP (with BSC) or BSC alone ·

Abbreviations: R: right; L: left; CC: corpus callosum; ICV: intracranial volume; SEM: standard error of mean; C: BSC: best supportive care; CPAP: patients treated with continuous positive airway pressure (CPAP) for one month·

4.4.2. Changes in cognitive function

At baseline, OSA patients showed significantly impaired cognitive processing in several domains in comparison to healthy controls (**Table 4.5**). Initially I used the analysis technique employed by Canessa (Canessa *et al.*, 2011) and was able to reproduce after one month of interventions the improvementthat she recorded in the majority of tested domains after three months, compared to performance at baseline (**Table 4.5**). However, using more stringent analysis by compariing differential cognitive changes between interventions, only changes in sleepiness [ESS mean diffΔ% (SD): CPAP, 2.46 (39·97) vs BSC,-27·97 (46·46); P=0·012] and verbal episodic memory test scores [delayed LM mean diffΔ% (SD): 23·41 (32·45) vs 57·20 (75·46); P=0·037] were statistically significant (**Table 4.6**).

The NICE/HTA assessment of CPAP (McDaid 2009) found that CPAP therapy led to a mean reduction in ESS of 1.07 points in middle aged OSA patients. This was found to be clinically significant and CPAP therapy was found to be cost effective. In my study of young and middle aged patients the mean reduction in ESS after CPAP was 4.62 points.

A recent analysis of the results of delayed LM testing in the American National Alzheimer's Coordinating Centre database of over 20,000 subjects found that there was a mean difference of 5.2 points between subjects diagnosed with mild cognitive impairment and those with normal cognition. The difference between patients diagnosed with mild cognitive impairment and those with Alzheimer's dementia was 5.1 points (Chapman *et al.*, 2016). In my study, the CPAP group showed an 8.1 point increase after one month of CPAP.

Table 4.5: Changes in neurocognitive function using the analysis employed by Canessa (Canessa *et al.*, 2011)

Cognitive Tests	Controls	$OSA_{baseline}$	BSC_{1month}	$CPAP_{1month}$	P^a	P^a *BSC*	P^a*CPAP*
immediate LM	47·06 (1·84)	36·36 (1·32)	41·70 (2·28)	44·43 (1·99)	<0·001*	0·070	0·338
delayed LM	29·79 (1·37)	22·25 (0·97)	27·33 (1·55)	29·93 (1·42)	<0·001*	0·238	0·946
ACE-R	94·91 (0·99)	90·55 (1·11)	90·70 (1·85)	91·86 (2·44)	0·008*	0·038	0·220
Memory	24·18 (0·41)	22·05 (0·57)	23·07 (0·65)	23·79 (0·37)	0·009*	0·143	0·493
Fluency	12·50 (0·3)	11·25 (0·3)	11·41 (0·49)	11·89 (0·45)	0·006*	0·050*	0·248
Language	24·12 (0·71)	24·18 (0·37)	23·63 (0·68)	25·11 (0·24)	0·930	0·628	0·230
SSF	9·00 (0·31)	8·33 (0·23)	7·93 (0·38)	8·18 (0·4)	0·079	0·032*	0·104
SSB	8·65 (0·26)	7·65 (0·24)	7·37 (0·39)	8·18 (0·31)	0·009*	0·007*	0·250
DSF	12·06 (0·33)	10·22 (0·31)	10·7 (0·4)	10·32 (0·51)	<0·001*	0·011*	0·005*
DSB	8·18 (0·39)	6·73 (0·31)	7·41 (0·47)	7·26 (0·52)	0·005*	0·21	0·157
TMA	24·12 (1·18)	27·34 (1·04)	28·02 (1·56)	24·26 (1·28)	0·049*	0·047*	0·937
TMB	41·23 (2·0)	62·02 (3·65)	61·53 (4·81)	51·05 (3·68)	<0·001*	<0·001*	0·017*
ESS	6·18 (0·49)	12·80 (0·67)	12·11 (0·93)	8·18 (0·85)	<0·001*	<0·001*	0·038*

Data presented as mean (SEM). Memory, fluency and language test scores were calculated from respective subtest scores of the Addenbrooke's Cognitive Examination-Revised (ACE-R) test. Normality was checked using the Kolmogorov-Smirnov test. The plots were normally distributed so independent sample t-test statistics were used to compare patient groups and controls. P columns: *P*= baseline OSA scores vs controls; *P* BSC= BSC vs controls; *P* CPAP= CPAP with BSC group vs controls. [a]Bonferroni corrected P values. *Abbreviations*: Controls: baseline values for healthy volunteers (N=35). OSA: baseline values for all obstructive sleep apnoea (OSA) patients before any intervention (N=55); BSC: patients treated with best supportive care (BSC) for one month (N=27); CPAP: patients treated with continuous positive airway pressure (CPAP) with BSC for one month (N=28). ESS: Epworth sleepiness scale. TMB: Trail Making Test B; TMA: Trail Making Test A; DSF: Digit-span Forward Task; DSB: Digit-Span Backward Task; SSF: Spatial-span Forward Test; SSB: Spatial-span Backward Test; ACE-R: Addenbrookes Cognitive Examination- Revised ; LM: logical memory test.

Table 4.6: The effect of the Continuous Positive Airway Pressure or Best Supportive Care regimes at one month on changes in cognitive domains·

Cognitive test	BSC N=27		CPAP N=28		P[a]
	Mean	SD	Mean	SD	
Immediate LM	14·28%	39·00%	34·78%	48·43%	0·090
Delayed LM	23·41%	32·45%	57·20%	75·46%	0·037*
ACE-R	2·63%	3·67%	-0·34%	14·52%	0·306
Memory (ACE)	11·93%	16·49%	5·63%	14·79%	0·141
Fluency (ACE)	1·17%	10·81%	8·14%	25·49%	0·195
Language (ACE)	0·45%	6·71%	1·45%	4·75%	0·523
SSF	-1·87%	21·75%	0·46%	30·66%	0·748
SSB	1·03%	26·62%	8·46%	27·94%	0·317
DSF	7·40%	23·88%	5·50%	33·34%	0·810
DSB	12·43%	37·78%	16·22%	53·19%	0·762
TMA	-3·11%	18·97%	-1·35%	24·89%	0·771
TMB	-6·99%	21·13%	3·17%	53·42%	0·361
ESS	2·46%	39·97%	-27·97%	46·46%	0·012*

Data presented mean (SD), normalised for differential changes [diffΔ(%)] from the baseline (diff=[score/domainpre-score/domainpost]/score/domainpre x100). [a]Bonferroni corrected P values. Significant difference between changes in two groups of patients after one month of CPAP (with BSC) or BSC alone. *Abbreviations*: ESS: Epworth sleepiness scale· TMB: Trail Making Test B ;TMA: Trail Making Test A; DSF: Digit-span Forward Task; DSB: Digit-Span Backward Task; SSF: Spatial-span Forward Test; SSB: Spatial-span Backward Test; ACE-R: Addenbrookes Cognitive Examination- Revised ; LM: logical memory test;·SD: standard deviation; C: BSC: best supportive care; CPAP: patients treated with continuous positive airway pressure (with BSC) for one month·

In summary: the findings of *a priori* analyses between-interventions (CPAP vs BSC) demonstrated the differential neuroplastic cognitive and morphometric adaptations already after only one month of treatment (**Figs 4.2 & 4.3**; **Tables: 4.4 & 4.6**).

4.4.3. Post hoc (Secondary) Analyses

Secondary correlational analyses were dictated by the three significant findings of *a priori* investigations. I explored the pathomechanisms behind improvements in episodic memory, somnolence and the hypertrophic trend for the thalamus in the CPAP group (**Figs 4.2 & 4.4**). Significant correlation between improvement in sleepiness and volumetric changes in the brainstem (r= -0·37; P=0·05) was found (**Fig 4.4**), whilst improvements in verbal episodic memory were not obviously correlated to any singular

morphometric change. Similarly, hypertrophic changes in the right thalamus appeared strongly correlated to changes in bilateral cortical cerebellar (CC) and hippocampal (H) structures (left CC r= 0·77; $P=1{\cdot}96x10^{-6}$; right CC r= 0·75; $P= 3{\cdot}78x10^{-6}$; left H r= 0·49;P<0·01; right H r= 0·51; P<0·01), which was not the case for OSA patients who received only BSC intervention. In the CPAP group, thalamic changes were also found to be correlated with changes in overall cognitive functioning (ACE-R) scores (r=-0·554; P=0·002), attentional span changes (DSF, r=-0·499; P=0·007) and with changes in visuospatial working memory (SSB, r=-0·370; P=0·05).

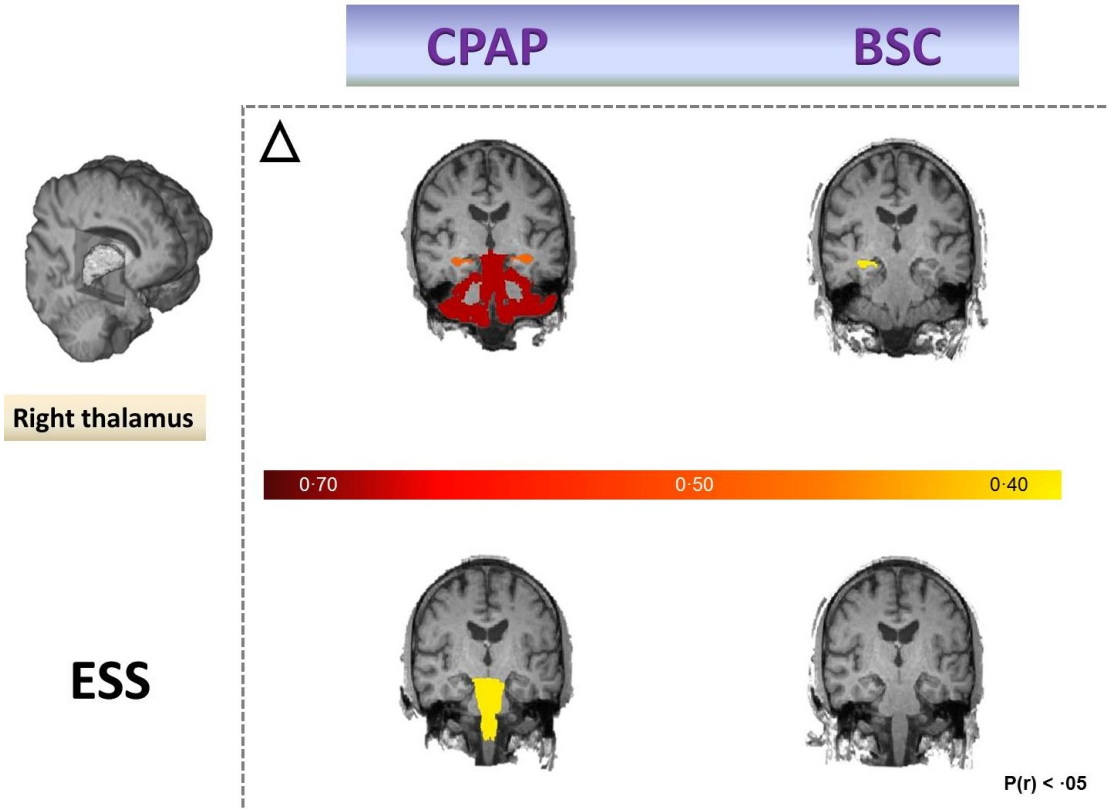

Figure 4.4 : Neuroplastic structural changes following one month of intervention with the Continuous Positive Airway Pressure or Best Supportive Care treatment. Potential strengthening in interregional connectivity between the non-dominant thalamus with hippocampus and (or) cerebellar cortex in OSA patients was noted only in the CPAP treated group (*first row)*. In addition, in the same group of patients, improvement in sleepiness (as shown by the ESS), was strongly positively correlated to changes occurring in brainstem during this period (*second row*).

Positive correlations between improvements in scores of verbal episodic memory and semantic subdomain of verbal fluency (fluency ACE-R) (r=0·406;P=0·032) that were otherwise negatively correlated in the BSC group (r=-0·190,P=0·343), were also shown.

Episodic memory showed significant positive correlation with span changes in the CPAP (r=0·451, P=0·02) vs BSC group (r=-0·081, P=0·69). ESS scores were not significantly correlated to changes in working memory in the CPAP (r=0·295, P=0·127), although were significant in the BSC group (r=0·591, P=0·001). The results of other neuroplastic adaptations and corresponding changes in cognitive domains are summarised in Table 4.7.

Table 4.7: Post Hoc targeted Interregional and Cognitive Domains Differential Correlations·

CPAP N=28	**r (P value)**		
	R Thalamus	**ESS**	**Delayed LM**
Brainstem	**·741 (·000)**	**-0·37 (·05)**	·052(·791)
L Cerebellum Cortex	**0·77 (1·96 10^{-6})**	·010(·961)	-·023(·909)
R Cerebellum Cortex	**0·75 (3·78 10^{-6})**	·027(·89)	·026(·897)
L Hippocampus	**0·49 (·01)**	-·224(·25)	·096(·628)
R Hippocampus	**0·51 (·01)**	-·233(·234)	·123(·53)
ACE-R	**-·554(·002)**	·062(·76)	·134(·496)
DSB	-·230(·24)	0·295 (·127)	**0·451(·02)**
DSF	**-0·499(·007)**	·224(·253)	·230(·239)
SSB	**-0·370(·05)**	-·279(·150)	·289(·136)
fluency ACE-R	**-0·554(·002)**	·002(·992)	**0·406 (·032)**
BSC N=27	**r (P value)**		
Interregional and Cognitive Domains Differential Correlations	**R Thalamus**	**ESS**	**Delayed LM**
Brainstem	·387 (·05)	·100(·618)	·105(·60)
L Cerebellum Cortex	·384 (·05)	·320(·104)	-·059(·77)
R Cerebellum Cortex	·339 (·08)	·119(·55)	-·192(·34)
L Hippocampus	·366 (·06)	·188(·349)	-·242(·22)
R Hippocampus	**·402 (·04)**	·107(·59)	-·120(·55)
ACE-R	-·022(·91)	·260(·19)	·370 (·06)
DSF	·113(·57)	**·499(·008)**	·280(·16)
DSB	·275(·16)	**0·591(·001)**	-0·081(·69)
SSB	-·096(·63)	·245(·22)	·301(·13)
fluency ACE-R	·181(·37)	·031(·88)	-0·19 (·34)

[a]Bonferroni corrected P values. Significant difference between changes in two groups of patients after one month of CPAP (with BSC) or BSC use. *Abbreviations*: ESS: Epworth sleepiness scale· TMB: Trail Making Test B ;TMA: Trail Making Test A; DSF: Digit-span Forward Task; DSB: Digit-Span Backward Task; SSF: Spatial-span Forward Test; SSB: Spatial-span Backward Test; ACE-R: Addenbrookes Cognitive Examination- Revised ; LM: logical memory test;·SD: standard deviation; C: BSC: best supportive care; CPAP: patients treated with continuous positive airway pressure (with BSC) for one month.

4.5 DISCUSSION

The main findings of this study were that just one month of CPAP treatment, combined with lifestyle modifications, can redress several cognitive and morphometric deficits in patients with moderate-severe

OSA. These data are consistent with the recovery of grey matter regions and cognition in patients with OSA reported by Canessa and colleagues (2011) to occur following three months of CPAP treatment (Canessa *et al.*, 2011). Moreover, the same group has recently shown that appreciable recovery of cognition and white matter, including that in corpus callosum, occurs over the course of 12-month treatment with CPAP (Castronovo *et al.*, 2014). My study is, to the best of my knowledge, the first time these findings have been replicated in much shorter timeframe (Gozal, 2013a).

The baseline neuroanatomical and neurocognitive impairments recorded in OSA patients were comparable to previously reported neurocognitive deficits (Yaffe *et al.*, 2007) and involved the neurocircuitry hubs formerly shown as specifically vulnerable to chronic sleep fragmentation and associated nocturnal cycles of intermittent hypoxia in clinical and preclinical studies (Gozal, 2013a; Rosenzweig *et al.*, 2013a; Rosenzweig *et al.*, 2013c; Yaffe *et al.*, 2014; Rosenzweig *et al.*, 2015). Specifically, hypotrophic changes in regions corresponding to hippocampal formation, basal ganglia and parts of the corpus callosum were suggested by neuroimaging investigations. Similarly, excessive daytime somnolence and significant impairments in cognitive domains of attention, working memory, verbal episodic memory and semantic memory were recorded in the OSA patients at baseline (**Table 4.5**).

Of note is that a minimal improvement, both in cognitive and neuroanatomical measures, was demonstrated in the OSA patients randomized to BSC over one month (**Table 4.4 & 4.5**). Similar improvement in patients with OSA has been previously associated with the improved sleep quality and argues for the beneficial impact of diet, exercise and lifestyle modifications (Araghi *et al.*, 2013). In addition to any intervention effects or natural neuro-homeostatic fluctuations, it is also possible that some of the cognitive changes may have represented practice effects.

In order to distinguish the beneficial impact of CPAP from that of any other interventional modality, the between-groups comparison of normalized changes was performed. Consequently, three major constructs were highlighted. Specifically, neuroplastic hypertrophic changes in thalamus, decreased daytime somnolence and improvements in verbal episodic memory were shown as more likely to result from adaptive processes driven by treatment with CPAP, and as such merit further discussion.

4.5.1. Excessive Daytime Somnolence

Somnolence is a major complaint in OSA, and whilst its exact neurological substrate is incompletely understood (Guilleminault & Brooks, 2001), it is likely that chronic low-level neuro-inflammation affects brain structures that participate in the initiation and maintenance of sleep and alertness (Rosenzweig *et al.*, 2015). In agreement with this notion, improvement in daytime somnolence following one month of treatment with CPAP was significantly correlated with neuroplastic alterations in brainstem (**Fig 4.4**) in my study. In addition, brainstem alterations were associated with hypertrophic thalamic changes, which were in turn closely correlated with alterations in bilateral cerebellar cortices and hippocampi (**Fig 4.3**). The neuroplastic stream of these associations correlated closely with changes in scores of tests of attentional regulation and working memory capacity (**Fig 4.5**).

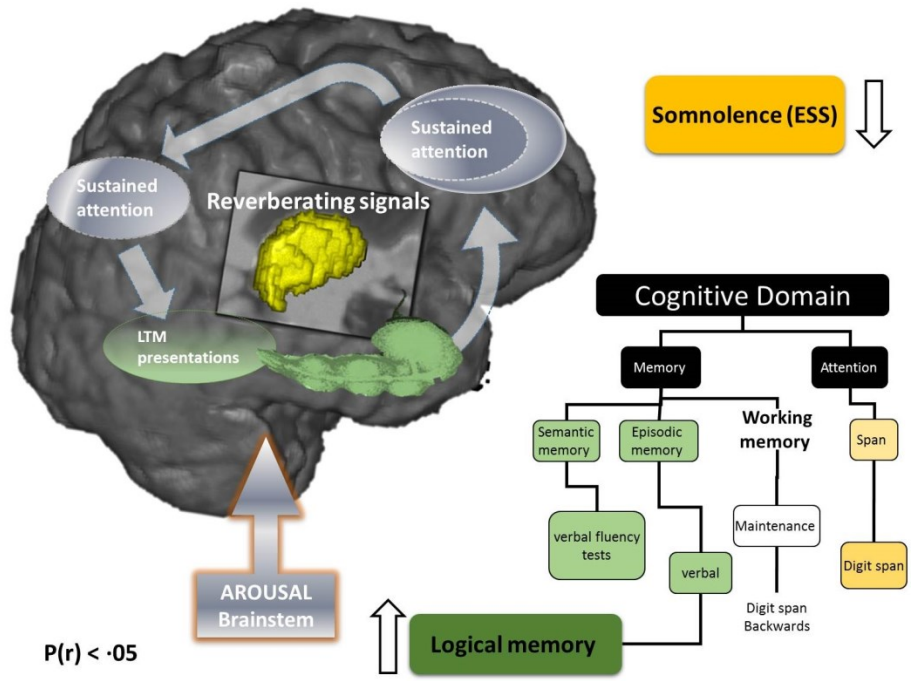

Figure 4.5: Schematic presentation of the neuroarchitecture behind working memory maintenance processes that might be implicated in improved daytime somnolence and verbal episodic memory by the CPAP treatment. Distributed nature of processes and representations involved to solve working-memory tasks is shown, with thalamus (central structure) acting as a functional interface between arousal and attentional regulation. The hippocampus (elongated green), structure most frequently reported affected by neuroimaging in OSA, is here proposed to bind aspects of working and episodic memory. The list of cognitive tests used in my study to investigate the impact of CPAP treatment on associated cognitive domains is also depicted. CPAP treatment leads to improvement in verbal episodic memory (green box), which may be due to the interplay of a cascade of subtle gradual changes in the semantic and verbal working memory. In addition, CPAP regime leads to the improvement of excessive daytime somnolence (yellow box), which in turn appears to be significantly correlated to ensuing brainstem alterations. The results of this study also suggest that comparable subjective tiredness has significantly less impact on the attention and maintenance of working memory capacity in patients treated with CPAP. *Abbreviations*: CPAP: continuous positive airway pressure; BSC: best supportive care; LTM: long term memory; ESS: Epworth Sleepiness Scale.

It is possible that the findings of the study reported in this Chapter represent changes in consciousness and arousal regulation that can occur within one month, perhaps primarily driven by increased activity/plasticity of brainstem pathways. Many of these pathways are known to underlie rapidly generated brief shifts of arousal at times of increased cognitive and cortico-thalamic demand (Schiff, 2008). It follows that the findings of the present study might also suggest specific vulnerability of a thalamo-cortical oscillator to sleep fragmentation and nocturnal cycles of intermittent hypoxia, something that has been long suggested by neurophysiological and neuroimaging data in OSA (Rosenzweig *et al.*, 2013a; Rosenzweig *et al.*, 2013c). In cases of a faulty oscillator, distributed network activity and associated memory may suffer across long-range cortico–cortical pathways, and within cortico–striatopallidal–thalamo-cortical loop volleys (Schiff, 2008).

It is notable that the findings also show that, whilst in untreated OSA patients changes in scores of tests of attention and working memory capacity were strongly affected by severity of their sleepiness, this significant association was all but negated by the effects of treatment with CPAP over one month (**Fig 4.5**). As patients with OSA have been shown to be more likely to experience a driving-related traffic accident, and given that such accidents have been shown as more likely in those who manifest greater daytime sleepiness, it can be argued that this finding implies strong clinical rationale for CPAP treatment even for a short duration (Gozal, 2013a; Malhotra *et al.*, 2015; Rosenzweig *et al.*, 2015).

4.5.2. Thalamo-cortical Circuitry in OSA

As already suggested, recruitment of central thalamic neurons via the brainstem occurs in response to increasing cognitive demand, stress and fatigue that reduce behavioral performance (Schiff, 2008). Through thalamic activation, neurons across the cerebral cortex and striatum can be depolarized, and their activity selectively gated by descending or ascending signals related to premotor attention and alerting stimuli (Schiff, 2008).

In Chapter 1 I outlined how OSA could directly injure the thalamo-cortical neurons, or their prominent deafferentation as a result of multifocal, neuro-inflammatory brain processes, could lead to severe impairment of prefrontal functional integration and arousal regulation (Portas *et al.*, 1998; McNab & Klingberg, 2008; Schiff, 2008; Rosenzweig *et al.*, 2013c). In keeping with this suggestion, I now speculate that these findings show neuroplastic compensatory thalamic changes, instigated by one month of CPAP treatment, leading to a wider circuitry reorganization involving brainstem, cerebellum and hippocampal connectivity. This connectivity is seen both between these structures and between the left and right sides of the brain in the CPAP group but not the BSC group (**Figs 4.2 & 4.4**). I also propose that CPAP-driven adaptive processes further optimize activation of the thalamic system that acts as functional interface

between arousal and attentional regulation (Portas *et al.*, 1998; Eriksson *et al.*, 2015). This might then underlie positive subjective experience of decreased mental effort required in order to solve tasks following the CPAP treatment.

4.5.3. Neurocognitive Architecture of Working and Episodic Memory in OSA

One month of CPAP treatment resulted in a partial recovery of episodic- and working-memory capacity. A crucial role for working-memory in temporary information processing and guidance of complex behavior, as well as its impairment in OSA (Twigg *et al.*, 2010), has been recognized (Eriksson *et al.*, 2015). There is emerging consensus that working-memory maintenance results from the interactions among associative memory representations and basic processes, including attention, that are instantiated as reentrant loops between frontal and posterior cortical areas, as well as subcortical structures (McNab & Klingberg, 2008; Eriksson *et al.*, 2015). In the present study, over one month, wider reorganization in a distributed memory system for associative learning occurred, e.g. thalamo-cortical changes were associated with changes in bilateral hippocampi and cerebellar cortices. None of these alterations were observed in the BSC group.

It is of further interest that the hippocampus, the structure most frequently reported as affected by neuroimaging in OSA (Rosenzweig *et al.*, 2013a; Rosenzweig *et al.*, 2015) has recently been proposed to act as a major shared substrate for working and episodic memory (Eriksson *et al.*, 2015). Axmacher and colleagues have suggested that the hippocampus plays a role in working-memory maintenance of multiple items by neural assemblies synchronized in the gamma frequency range, locked to consecutive phase ranges of oscillatory activity in the hippocampal theta range (Axmacher *et al.*, 2010). Of note, a decrease in theta band has been shown to occur after apnoea and hypopnoea events in some patients with OSA, and CPAP has been shown to normalise EEG changes (Rosenzweig *et al.*, 2014).

4.5.4. Limitations

Several limitations should be considered when interpreting the findings of this study; the cross-sectional design and strict exclusion criteria used disallow conclusions to be made regarding causality or interactions between OSA, its treatment, and various comorbidities such as hypertension and diabetes. Also, one cannot infer which, if any, interventional aspects of the lifestyle modifications might have contributed to observed changes. Furthermore, correlations between changes in regional volumes and neuropsychological scores were exploratory and hypothesis-generating. Their interpretation of presumed complex and diverse causalities remains a challenging issue for the future. Nonetheless, these findings indicate an important clinical message about the benefits of CPAP treatment.

4.5.5. Conclusions

The results of this study show that one month of CPAP treatment provides a sufficient timeframe for rudimentary neuroplastic changes to occur within targeted brain structures of patients with moderate to severe OSA. These data lead to speculation that these structural changes provide a basic neurocognitive architectural scaffold for further reorganization, which underlies some of the observed functional recovery in working and episodic memory.

This work is important and will impact on clinical practice. Currently there exists a cohort of people who have OSA but are not sleepy. NICE guidelines do not recommend offering these patients CPAP therapy as there is no evidence that it improves outcomes. However, these subjects are exposed to the same intermittent hypoxia and sleep fragmentation as sleepy OSA patients. If changes in brain structure and cognitive function are sequelae of IH and/or sleep fragmentation, and these changes are at least partially reversible with CPAP therapy, then the guidelines will need to be updated.

Chapter 5

CHANGES IN BRAIN MORPHOLOGY IN OLDER PEOPLE WITH OSA FOLLOWING CPAP TREATMENT

5.1 SUMMARY

Published studies of brain structure in OSA have involved only young and middle-aged patients. However the population is aging and OSA is at least twice as common in older adults than the in the general population. Cognitive dysfunction and changes in brain structure are recognised phenomena in older people independent of sleep apnoea status. My previous studies had shown brain hypertrophy that I hypothesised was a result of dendritic sprouting and increased connectivity induced by ischaemic preconditioning. Animal models of neuroplasticity show that neurogenesis cannot take place beyond a certain age. Prior to the study presented in this chapter it was not known whether ischaemic preconditioning would lead to dendritic sprouting and brain hypertrophy in older patients with OSA.

The objective of this study was to investigate whether the change in brain structure seen in young and middle aged patients after CPAP treatment would be seen in older OSA patients.

MRI scans from 17 older people with OSA randomised to CPAP (mean [SD]: AHI, 34.1[20.2] events/h; 1 woman; age 70.8 [4.1] years) and 17 randomised to BSC (mean [SD]: AHI, 19.3[15.4] events/h; 5 women; age 70.8 [3.3] years) were analysed to determine hippocampal subfield volume at baseline and at 6 months. A significant decrease in left fimbria volume was seen in the CPAP group ($p=0.01$). A significant increase in the left presubiculum volume was seen in the BSC group ($p=0.028$). No hippocampal hypertrophy was seen in the CPAP group.

The findings presented in this study suggest that ischaemic preconditioning does not result in increased connectivity in older OSA patients in the same way as it does in young and middle-aged patients in Chapters 3 and 4. This may be because dendritic sprouting has been switched off due to age, or because of prolonged exposure to IH. Alternatively it may be the result of poor CPAP compliance.

Contributions: The study was designed by Glasser, McMillan, Rosenzweig and Kempton. The data were acquired by McMillan and Glasser. Analysis of the data wascarried out Glasser, Rosenzweig, Chih Cheng Tsai and Williams. Statistical analysis was carried out by Chih Cheng Tsai, Glasser and Rosenzweig.

5.2 INTRODUCTION

As has been discussed in Chapters 1, 3 and 4, cognitive impairment is a well-recognised and important sequela of OSA. Chapters 3 and 4 focused exclusively on younger and middle aged people with OSA; however the role of aging in cognitive decline is well recognised. Therefore in the last experimental chapter presented in this thesis I have taken the opportunity to investigate the possible changes in brain morphology associated with OSA in older people aged > 65 years.

5.2.1 The aging population

The world's population is ageing and by 2045 the number of older people (≥60 years) will be more than the number of children (<15 years) (United Nations, 2007). In Europe this historical crossover occurred in 1995 due to long term reductions in fertility and mortality, and by 2050 the ratio of older people to children will be 2:1 (**Fig 5.1**). This situation poses major social and economic challenges across Europe. For example in the UK, by 2040 the predicted requirement for long-term care will increase by 60%, at an additional cost of approximately £4 billion per annum (Karlsson *et al.*, 2006). Therefore strategies to reduce age-related health care costs are vital. One approach could be to promote health care that maintains the independence of older people and OSA is a potential therapeutic target (Tarasiuk *et al.*, 2008).

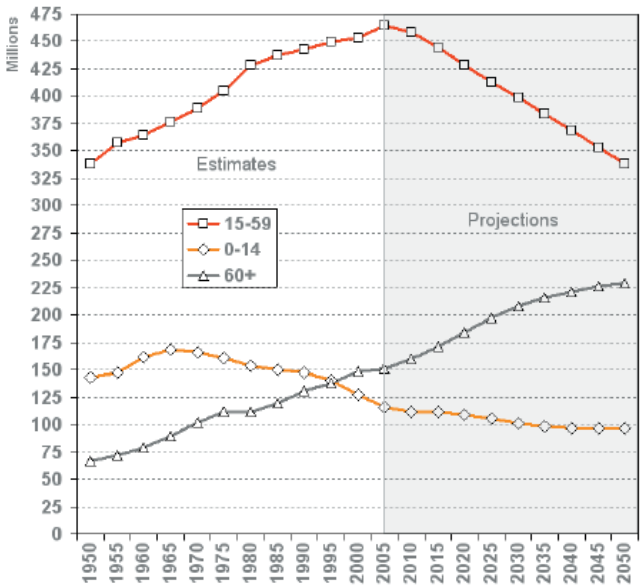

Figure 5.1: The European population from the 2006 revision of the world population prospectors. The older population is expected to increase in the future, whereas the population under age 60 is expected to decrease. (United Nations, Department of Economic and Social Affairs, Population Division, Population Estimates and Projections Section. Fact Sheet, Series A, 7 March 2007)

5.2.2 The prevalence of obstructive sleep apnoea in older people

The prevalence of OSA in younger people has been discussed in Chapter 1, section 1.2.2. In older people the prevalence of OSA is at least two fold greater, with estimates ranging between 13 and 32% (Hoch & Reynolds, 1990; Ancoli-Israel *et al.*, 1995; Bixler *et al.*, 1998; Young *et al.*, 2002a; Hader *et al.*, 2005a; Hass *et al.*, 2005). The wide variation in these estimates is likely to reflect the different health status of the older populations studied and the definitions of the disease. A detailed review of the prevalence of OSA in older people is shown in **Table 5.1**.

Table 5.1: Prevalence of obstructive sleep apnoea in older people

Reference	n	Female (%)	Age (yrs)	Population	Prevalence of OSA (%)	
					AHI ≥5	AHI ≥10 / ≥15
Carskadon et al 1981	40	55	62-86	Community	36	
Coleman et al 1981	83	28	66±5	Sleep Clinic	39	
McGinty et al 1982	26	0	64.4±4.4	Community		62
Roehrs et al 1983	97	–	61-81	Sleep Clinic	27	
Smallwood et al 1983	30	20	50-80	Community	37	
Yesavage et al 1985	41	0	69.5±6.5	Both	73	
Hoch et al 1986	56	52	69.3±5.4	Community	5	4
Knight et al 1987	27	NG	75.8±5.9	Primary Care	37	
Mosko et al 1988	46	65	68.7±6.7	Community	28	16
Ancoli-Israel et al 1989	233	65	65-101	Nursing Home	70	
Hoch et al 1990	105	53	60-91	Community	26	13
Philips et al1992	92	52	64.2±8.6	Community	15	
Ancoli-Israel et al 1995	346	53	72.8±6.1	Community		30
	54	57	70.8±6.2	Community		32
Bixler et al 1998	75	0	65-100	Community	31	24
Young et al 2002	3448	–	60-99	Community	54	20
Endeshaw et al 2004	58	76	77.7±6.7	Community	56	19
Haas et al 2005	3643	52	70.2±6.9	Community	46	20
Hader et al 2005	80	50	74.1±6.3	General Clinic	43	19

OSA: obstructive sleep apnoea, AHI: apnoea-hypopnoea index. *Table adapted from Glasser et al 2011.*

5.2.3 Aetiology of sleep apnoea in older people

The high prevalence of OSA in older people has led to debate regarding its causes and consequences in the elderly (Lévy *et al.*, 1996; Lavie *et al.*, 2005). Although the prevalence of CSA is increased in older people the majority of sleep disordered events are obstructive. The increase in OSA may be due to a generalised decrease in the size of the upper airway lumen in older people, specifically in males (Martin *et al.*, 1997). Structural changes to the dimensions of the upper airway include a lengthening of the pharyngeal airway in both men (Shigeta *et al.*, 2008) and women (Malhotra *et al.*, 2006) and the descent of the hyoid bone (Kollias & Krogstad, 1999), particularly in individuals with long faces (Pae *et al.*, 2008); leading to an increase in pharyngeal resistance. Even in healthy elderly people pharyngeal resistance is increased, compared to that in younger people; indicating a predisposition to airway collapse (Browne *et al.*, 2001).

The collapsibility of the pharyngeal airway is determined by the transmural pressure across the airway, which is in turn influenced by the extraluminal pressure (Chapter 1; Section 1.2.1). Fat tissue around the airway is a key factor in the development of OSA in middle aged people (Horner *et al.*, 1989; Schwab *et al.*, 2003) and neck circumference has been shown to be a significant risk factor (Davies *et al.*, 1992). Although, older people with OSA typically have a lower body mass index and neck circumference compared to younger patients with similar disease severity (Chung *et al.*, 2009).

Functionally, the response of the genioglossus muscle to negative pressure applied during wakefulness (Malhotra *et al.*, 2006) and sleep (Eikermann *et al.*, 2007) is reduced in older people, compared to younger people (Erskine *et al.*, 1993). The response to hypoxia is also reduced (Klawe & Tafil-Klawe, 2003). Overall these changes result in reduced respiratory pump and upper airway muscle function at sleep onset (Worsnop *et al.*, 2000a), and a more collapsible upper airway (Kirkness *et al.*, 2008a) with the critical closing pressure being -8.3 (2.3) cmH_2O in older people, compared to -16.0 (6.9) cmH_2O younger people, independent of body mass index (Eikermann *et al.*, 2007). This increased collapsibility of the pharyngeal airway in older people, may be the explanation for the reduced CPAP requirements in older people. The therapeutic level of CPAP being on average 2.5 cmH_2O less than younger OSA patients with matched disease severity - mean (sd) CPAP level: Older patients 6.9 (1.9) cmH_2O; Younger patients 9.4 (3.5) cmH_2O) (Kostikas *et al.*, 2006).

5.2.4 Cognitive function in older people with OSA

A decline in cognitive function is considered part of the aging process, and the ability to encode new memories is particularly impaired (Hedden & Gabrieli, 2004). OSA has also been shown to be associated with cognitive dysfunction (Redline *et al.*, 1997; Adams *et al.*, 2001; Ferini-Strambi *et al.*, 2003; Naegele *et*

al., 2006; Quan *et al.*, 2006; Twigg *et al.*, 2010) –see Chapter 1, Section 1.3; however few of these studies have included older OSA patients. Mathieu *et al* found cognitive dysfunction was independently related to both OSA severity and increasing age, but the coexistence of both factors did not result in increased cognitive dysfunction (Mathieu *et al.*, 2008). Ayalon *et al* showed cognitive dysfunction, on performance of the Go-No-Go cognitive task, in older OSA people, but no significant impairment when OSA and increasing age were considered separately (Ayalon *et al.*, 2010). Finally, Cohen-Zion *et al* used the mini-mental state examination to study the effects of OSA on cognitive function in older patients and found a significant association between the severity of OSA and the self-reported severity of daytime somnolence. Again, however, once other variables (including total sleep time) were included in the model, only the relationship between cognitive impairment and excessive somnolence remained significant (Cohen-Zion *et al.*, 2001; Cohen-Zion *et al.*, 2004).

When cognitive function is preserved in OSA patients, functional brain imaging has revealed that brain activation is increased, compared to the activation that occurs in healthy controls performing the same task. The association between preserved cognitive function, and greater activation in OSA patients suggests that increased cerebral recruitment is required to maintain cognitive performance (Ayalon *et al.*, 2006). Similar preservation of cognitive function, with compensatory increased cerebral activation has been found in older people; however older patients with coexistent OSA show decreased cerebral activation and cognitive dysfunction (Ayalon *et al.*, 2010). This suggests that age and OSA could have synergistic effects on cerebral activation and consequently cognitive function.

5.2.5 Sleepiness in older people with OSA

Sleep becomes more fragmented with age, independent of sleep apnoea (Mathur & Douglas, 1995; Boselli *et al.*, 1998; Browne *et al.*, 2003a), and there is a well documented age-related reduction in sleep quality (Bixler *et al.*, 1984; Redline *et al.*, 2004). These features of sleep in older people have led to the suggestion that older OSA patients may be habituated to the added disease-related sleep disruption of OSA, and therefore do not suffer symptoms of daytime sleepiness in the same way as younger patients. The large epidemiological study (Sleep Heart Health Study; n=5,407) of community dwelling adults (mean age 63, range 45-99 years) showed that age was associated with a reduction in subjective sleepiness, measured using the Epworth Sleepiness Scale (ESS) in females, but not in males (ESS, young (39-40) years vs older $\geq$ 80 yrs. Females 7.4 (4.4) vs 6.5 (4.0); Males 7.3 (4.3) vs 7.7 (4.7) (Unruh *et al.*, 2008)]). Assuming age was associated with an increased prevalence of OSA, these data could be interpreted as support for the notion that older OSA patients are less sleepy than younger patients. Although in another study comparing older people with and without OSA (defined as an apnoea / hypopnoea index (AHI) >10 to 15 events per hour)

patients with OSA were more sleepy than those without OSA (Endeshaw, 2006). In older OSA patients, recruited from sleep clinics; subjective daytime sleepiness was found to be similar to that experienced by younger patients matched for disease severity and body mass index in one study (Browne *et al.*, 2003b) and less in another (Chung *et al.*, 2009). Thus the current information on the symptoms of sleepiness in older people with OSA is unclear.

5.2.6 Changes in brain morphology in older people with OSA

As discussed in Chapter 1, section x the majority of studies of brain structure in OSA patients show hippocampal changes (see Table 3.1), likely due to intermittent hypoxia. This is supported by animal models of intermittent hypoxia which have resulted in cognitive impairment and hippocampal hypotrophy (Chapter 3, Section 3.2.1). However, there is increasing evidence that ischemic preconditioning may protect against some of the effects of hypoxaemia as discussed in Chapters 3 and 4. Again, this is supported by animal models. Rats with spatial memory deficits caused by intermittent hypoxia, demonstrated improvement cognitive performance associated with increased cell proliferation in the dentate gyrus (DG) of the hippocampus (Gozal *et al.*, 2003a; Gozal *et al.*, 2003b). Furthermore, it has been shown that mild intermittent hypoxia is neuroprotective. The results presented in Chapter 3 of the Brain MRI in middle-aged people with OSA, compared to healthy people appear to support this suggestion. Likewise the results in Chapter 4, where young and middle-aged patients with OSA have been compared to healthy people, before and after CPAP treatment also support the notion that OSA can cause both hyper and hypotrophy in different brain structures. The effects of OSA in older patients, who may have had undiagnosed OSA for a long period of time, and who have the normal changes in cognitive function associated with aging are not known.

5.3 AIMS

The aim of the study presented in this Chapter was to investigate the impact of CPAP treatment on hippocampal structure and cognitive function in older people with OSA, testing the hypothesis that treating intermittent hypoxia secondary to OSA with CPAP would be associated with changes in hippocampus morphology. To address this aim a pre-defined hippocampal sub-fields analysis was carried out on the brain MRI scans of older patients with OSA, before and after 6 months of CPAP treatment.

5.4 METHODS

5.4.1. Trial design

This study was a single-blinded (investigator blinded), parallel group, single centre, randomised controlled trial (RCT) of 6 months duration. Patients were randomised to receive either CPAP and Best Supportive Care or Best Supportive Care only. The trial design and recruitment is shown in **Fig 5.2**..

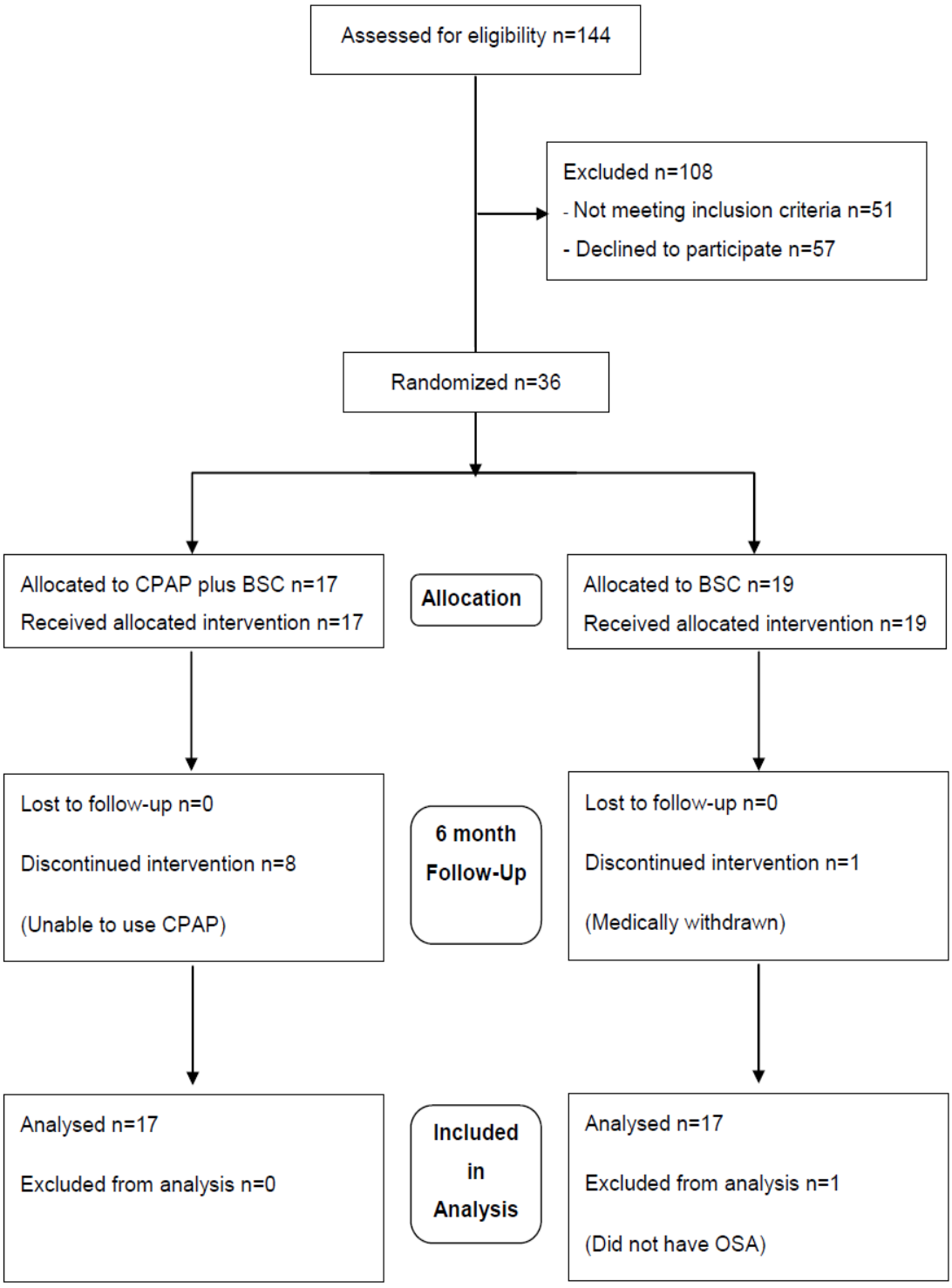

Figure 5.2 CONSORT diagram of patient recruitment, and progression through the study

5.4.2. Recruitment and Screening

Patients were recruited from those referred to the Royal Brompton Hospital sleep clinics with possible OSA. Patients who had completed the control arm of a large multicentre randomised study to investigate the treatment of OSA in older people (The PREDICT study - (McMillan *et al.*, 2014)), were also approached to take part in the study. Cognitive testing and sleep studies were performed in the Royal Brompton Hospital Clinical Research Facility. The brain MRI scans were completed in the Radiological Sciences Unit at Charing Cross Hospital, Imperial College Healthcare NHS trust. The study was approved by the National Research Ethics Committee (ref: 10/H0711/101) and patients gave written informed consent at the enrolment visit. The sponsor insurance and indemnity was provided by Imperial College London.

Patients were eligible to participate if they were aged 65 years or older at the enrolment visit, with a diagnosis of OSA (defined as ≥ 4% SpO_2; ODI > 7.5 events/hour). Patients were not eligible for the study if had any of the following: arterial awake oxygen saturation <90% on room air, FEV_1 / FVC ratio <60%, substantial problems with sleepiness driving (in those who are still driving), currently using heavy good vehicle or professional service vehicle driving license, shift work, any very severe complication of OSA such that CPAP therapy was mandatory, inability to give informed consent or comply with the protocol, previous or current psychiatric disorders (including alcohol or drug abuse), psychotropic medications (including all sedating medications, anxiolytics, major tranquilizers, antipsychotics, hypnotics, anticonvulsants), neurological disorders, and a previous head injury resulting in loss of consciousness or notable cognitive deficit.

Contraindication to undergoing the MRI scan, in particular metal within the body was also an exclusion criteria. The exclusion criteria for MRI were: pacemaker or pacing wires, aneurysm clips in the head, artificial heart valves or coronary stents, metal implants, cochlear implants, programmable shunts, deep brain stimulator or neurostimulator, and shrapnel or metal fragments in the body or eyes

All patients potentially eligible to participate in the study were identified from sleep and respiratory clinics by myself or Dr McMillan. Once the diagnosis of OSA was confirmed they were contacted and offered an opportunity to take part in the study. Screening logs were kept documenting the number of patients assessed for eligibility and, if applicable, the reason(s) for non-inclusion.

5.4.3. Data Collection

Patients were randomized to receive either CPAP plus Best Supportive Care (see Chapter 4 for a full description) or Best Supportive Care alone. As discussed earlier in Chapter 4, Section 4.8.1 this was a

physical device trial, and hence the treatment allocation for the individual patients could not be concealed. Randomised occurred after patients had completed their enrolment visit, the morning after the sleep study. Patients were allocated on a one to one basis, using block randomisation stratified by disease severity (enrolment ESS > 9 v ≤ 9) and ODI (ODI >15 v ≤15).

Both groups had identical schedules (see **Table 5.2)**. Structured clinical assessments were performed at baseline and 6 months. All patients received a telephone call at 1 week to optimize CPAP adherence and offer additional support. Domiciliary overnight, pulse-oximetry was completed at 6 months. **Table 5.3** summarizes the assessment completed at each time point. Assessment visits were carried out in the Royal Brompton Hospital research laboratory and clinical research facility. Occasionally, research staff agreed to see a patient in their own home when the patient was unable to attend the hospital.

The brain MRI scans were completed on a 3T scanner (Siemens, Germany) in the Radiological Sciences Unit at Charing Cross Hospital, Imperial College Healthcare NHS trust. The brain scan sequence was approximately 60 minutes and included T1 and T2 weighted and diffusion tension imaging. All MRI scans were done on the afternoon prior to the NPSG and randomization. The cognitive test panel was conducted in a quiet laboratory at 19.00 before their NPSG. Patients were asked to abstain from caffeinated beverages for the duration of their stay.

Table 5.2 Visit schedule

Time	Event
Day 1	
13.00	Patient arrival Royal Brompton Hospital: Consent and enrolment
14.00	The Oxford Sleep Resistance Test
15.00	Taxi to Charing Cross Hospital
16.00	Brain MRI scan
18.00	Patient returns to Royal Brompton Hospital Enrolment completed
18.30	Evening meal
19.00	Cognitive tests
20.00	Set up for Nocturnal Polysomnogram
Day 2	
08.00	Fasting bloods
08.30	Breakfast
09.00	Randomised and intervention delivered

Table 5.3 Summary of assessments by time point

Assessments and Measurements	Screening	Baseline	Week	Month
			1	6
Eligibility and exclusions	*			
Informed consent and enrolment	*	*		
Nocturnal polysomnogram		*		
Overnight pulse oximetry				*
Overnight respiratory polygraphy	*			
Clinical assessment visit including sleep history		*		*
Demographics		*		
Caffeine and alcohol intake		*		*
Exercise history		*		*
Anthropometry		*		*
Blood pressure and resting pulse		*		*
Fasting bloods		*		*
Subjective sleepiness (Epworth Sleepiness Scale)		*		*
Objective sleepiness (Oxford Sleep Resistance test)		*		*
Disease specific quality of life (SAQLI)		*		*
Mood (Hospital Anxiety and Depression Scale)		*		*
Cognitive function		*		*
Telephone call			*	
Continuous positive airway pressure adherence			*	*
Continuous positive airway pressure side effects			*	*
Adverse events			*	*

5.4.3. Brain MRI analysis

The change from baseline in brain morphology was measured in older OSA patients treated with CPAP and those treated with Best Supportive Care only. Images were processed using the FreeSurfer software package (version 5.3.0, available at http://surfer.nmr.mgh.harvard.edu/), which is fully automated (See Chapter 2, Section 2.5.2 for a full description).

A hybrid watershed algorithm was used to segement the image, removing non-brain tissue and transforming the images into Talairach space. GM and WM were segmented. Once the hippocampus had been identified, it was segmented using Bayesian inference and a probabilistic atlas of hippocampal formation. This was based on manual segmentation of ultra-high resolution T1-weighted images of several participants (Van Leemput *et al.*, 2009). Volumes from 7 subfields were generated; CA1, CA2-3, CA4-DG, subiculum, presubiculum (GM), fimbria (WM) and hippocampal fissure (CSF).

5.4.4. Additional analysis

The change in cognitive function from baseline to 6 months as measured in older OSA patients treated with CPAP and Best Supportive Care. The cognitive test panel is described in Chapter 2, Section 2.4. Changes in subjective sleepiness, measured by the ESS score (Chapter 2, Section 2.3.1) were also analysed. Treatment compliance was measured objectively by downloading a smart card in the CPAP machine at the 6 month visits.

5.4.5. Sample Size

The sample size calculations for this study were based on the cognitive data previously collected in our group (Twigg *et al* 2010). As well as the most recently published data at the time the protocol was devised (Canessa *et al.*, 2011). These data (**Table 5.4**) indicated that between group differences could be calculated, at an alpha error level of 5% (corresponding to a 95% confidence interval) and a beta error level or Statistical Power [1 - Beta] of 80%, assuming CPAP treatment reverses any reduction in cognitive function in older patients with OSA to levels comparable to those seen in these healthy people. Therefore, 20 Patients in each group would demonstrate a difference in spatial span forwards and backwards test.

The Stroop data had shown that the mean time to complete the test is 39 seconds in OSA patients, and 23 seconds in healthy controls (SD 8.1). Assuming a more conservative difference of 8 seconds after CPAP, using an alpha error levels of 5% and a Beta error or 80%, it was calculated that 17 patients were required in each group to detect this difference.

Table 5.4 Cognitive tests with sample sizes

Test	Sample 1		Sample 2		Sample Size in both groups
	Mean	Sd	Mean	Sd	
Spatial Span Forwards	8	2	6	1	10
Spatial Span Backwards	8	2	5	1	5
Logical Memory (immediate)	43	10	36	22	94
Logical Memory (delayed)	27	7	22	7	31

Previous MRI brain studies in OSA had shown a differences in brain structure between OSA patients and controls using sample size 7-21 (see Table 3.1) and changes in hippocampal structure had been found after 3 months CPAP in a study of 17 patients (Canessa *et al.*, 2011). Therefore a sample size of 44 (22 in each group) was assumed to be needed for the brain MRI analysis. This allowed for a 10% drop out (e.g. loss to follow-up, claustrophobia preventing MRI acquisition, inability to sleep in the laboratory).

5.5 STATISTICAL METHODS

Hippocampal sub-field volume produced by FreeSurfer were dividing by total intracranial volume to normalise the data. A student's t-test was used to compare BSC and CPAP groups both at baseline and at 6 months. Within group differences were analysed using paired t-tests. The 4 predefined groups (Best Supportive Care:baseline, BSC:6 months, CPAP:baseline, CPAP:6 months) were compared using ANOVA. Tukey's Honest Significan Difference was employed for post-hoc analysis which compared CPAP:6 months with the other 3 groups independently. ANCOVA was used when an additional covariate (OSA severity) was added. All statistical analysis was performed using SPSS statistical software (version 21.0, SPSS Inc., Chicago, Illinois, USA).

5.6 RESULTS

The screening and recruitment for this study were completed between January and December 2012. During this time there were 150 new patients with a potential diagnosis of OSA who were aged over 65. The CONSORT diagram shows recruitment (**Fig 5.2**). 144 individuals were screened, of whom 93 (65%) met the inclusion criteria. 57 eligible patients did not consent to entering the study and 36 (39%) patients were randomised. This was fewer than the 44 patients I had intended to study. One patient withdrew from the study for medical reasons and 1 patients was excluded from the analysis as he was found not to have OSA (AHI <5 events/h).

The 6 month visits occurred between July 2012 and August 2013 with 34 patients completing the protocol. The main reasons for non inclusion were: not wishing to enter the study n=57 (53%), inability to have MRI n=18 (17%), unable to make contact after initial contact and providing a patient information sheets n=14 (13%) and an inability to complete cognitive battery due to limited English n=8 (7%).

5.6.1 Baseline data

Seventeen patients were randomised to CPAP plus BEST SUPPORTIVE CARE; 19 to BEST SUPPORTIVE CARE alone. 28 (83%) were male with a mean age of 71 ranging from 65 to 77 years. 28 (82%) were white. Patients had a mean BMI of 30.8 (S.D. 4.9) Kgm^2. No significant difference was found between the groups for age, years of education, BMI, neck circumference or BP. Baseline demographic and clinical data are shown in **Table 5.5** and **5.6** respectively.

By chance the CPAP group had more severe OSA: Arousal Index (events/TST) (p=0.01), 4% Apnoea /hypopnoea Index (p=0.02), Obstructive events/h (p=0.04) and 4% ODI (p=0.01) although subjective sleepiness, objective sleepiness and symptoms were similar between groups. The sleep data are shown in **Table 5.7**.

Table 5.5: Baselines demographics

		Best Supportive Care N=17	Continuous positive airway pressure N=17	Independent T test p value
Age (years)		70.8 (3.3)	70.8 (4.1)	0.96
Male sex N (%)		12 (71%)	16 (94%)	Not tested
Education (years)		15 (6)	14 (4)	0.36
White N		15	14	Not tested
Asian N		1	1	Not tested
Other N		1	2	Not tested
Body mass index (kg/m^2)		31.4 (3.8)	30.1 (6.0)	0.46
Neck circumference (cm)		42 (4)	43 (4)	0.63
Caffeinated drinks /day		4 (3)	5 (3)	Not tested
Alcoholic drinks/week		4 (0-12)	3 (0-6)	Not tested
Smoking status N	Never	6	10	Not tested
	Ex	11	5	Not tested
	Current	0	2	Not tested

Data are mean (SD), median (25th-75th percentiles) or N number (%) as appropriate. As patients were randomised any differences that occurred between groups at baseline will be the result of chance. Statistical analysis by group was only completed after data collection was completed in parameters that may influence cognitive function. Parameters with small numbers were not tested.

Table 5.6 Baseline clinical characteristics

	Best Supportive Care N=17	Continuous positive airway pressure N=17	Independent T test p value
Asthma/COPD N	4	1	Not tested
Ischaemic Heart Disease N	7	7	Not tested
Hypertension N	11	13	Not tested
Diabetes N	4	5	Not tested
Cerebral vascular Disease N	1	2	Not tested
Systolic blood pressure (mmHg)	140.9 (22.4)	134.1 (17.5)	0.33
Diastolic blood pressure (mmHg)	81.7 (11.6)	81.5 (15.1)	0.97

COPD: Chronic Obstructive Pulmonary Disease. Data are mean (SD), median (25^{th}-75^{th} percentiles) or N number (%) as appropriate. As patients were randomised any differences that occurred between groups at baseline will be the result of chance. Statistical analysis by group was only completed after data collection was completed in parameters that may influence cognitive function. Parameters with small numbers were not tested.

Table 5.7 Baseline sleep measurements

	N	Best Supportive Care	N	Continuous positive airway pressure	Independent T test p value
Epworth sleepiness score	17	9.4 (4.8)	17	9.4 (3.9)	1.0
Oxford Sleep Resistance Test (minutes)	16	26.6 (14.5)	16	26.6 (15.8)	0.99
Sleep Apnoea Quality of Life Index	17	4.8 (1.5)	17	5.2 (0.9)	0.30
Total sleep time (TST) (minutes)	14	291.5 (99.5)	15	352.7 (91.8)	0.097
Sleep efficiency (%)	14	55.8 (16.6)	15	66.8 (13.1)	0.06
Arousal index (events/TST)	15	17.1 (9.4)	15	30.9 (16.8)	0.01
4% Apnoea /hypopnoea index (events/TST)	17	19.3 (15.4)	17	34.1 (20.2)	0.02
Apnoea index	17	6.6 (2.3 - 12.9)	17	23.9 (8.3 - 44)	0.06
Obstructive events/h	17	4.7 (1.2-9.3)	17	14.9 (4.8 – 31.6)	0.04
Central events/h	17	0.5 (0.05 – 1.7)	17	1.1 (0.2 -6.4)	0.5
4% Oxygen Desaturation index	17	18.3 (14.6)	17	35.1 (21.5)	0.01
SpO_2 Time < 90% (minutes)	17	62.6 (12.7-87.7)	17	73.5 (6.4-148.7)	0.72

Data are mean (SD), median (25^{th}-75^{th} percentiles) or N number (%) as appropriate. As patients were randomised any differences that occurred between groups at baseline will be the result of chance. Statistical analysis by group was only completed after data collection was completed in parameters that may influence cognitive function.

5.6.2. Brain MRI: Hippocampal volume analysis

Hippocampal volumes as absolute values and percentage of whole brain are shown in **Table 5.8** along with hippocampal sub-fields volumes. There was a significant reduction in left fimbria volume after 6 months in the CPAP group (p=0.01), A significant increase in left presubiculum volume was seen after 6 months in the Best Supportive Care group (p=0.028) (**Table 5.9**). No other significant differences were seen between or among groups either before or after treatment, whether using OSA severity as a covariate or not (**Table 5.9**).

5.6.3. CPAP Adherence

The overall CPAP adherence was measured at 6 months and results are shown in **Table 5.10**. Of the 17 patients randomised to CPAP 7 patients had stopped using CPAP or had low CPAP adherence, although at the end of the trial 12 patients (including 2 with low use) requested they continue using CPAP. Only 5 patients had over 4 hours a night of CPAP use. The median daily use was 3.4 (2.2) hours.

The oxygen desaturation index was statistically different between groups at 6 months in favour of CPAP 3.0 (10.7) vs. Best Supportive Care 20 (18.3) events/hour p<0.01. This is likely to be because pulse oximetry was only performed on one night at the end of the study, when the subjects knew their oxygen saturations were being measured, whereas compliance data was recorded by the device every night and downloaded at the end of the trial. Subjects were likely to be more compliant with CPAP on the night that they knew oximetry was being recorded than on other nights.

Table 5.8 Normalised mean hippocampal volume (mm^3) and percentage of whole brain and hippocampal sub-fields volume (mm^3) and percentage of hippocampus.

	BEST SUPPORTIVE CARE					
	Pre-treatment		Post-treatment		Pre-treatment	
(Left)						
Whole hippocampus	3822.0	(0.254%)	3878.8	(0.259%)	3785.8	(0.24
Fimbria	55.8	(1.463%)	55.7	(1.432%)	67.0	(1.77
CA1	338.3	(8.876%)	340.9	(8.811%)	326.3	(8.70
CA2-3	950.2	(24.881%)	962.3	(24.861%)	916.5	(24.30
CA4-DG	537.5	(14.057%)	550.0	(14.210%)	508.7	(13.48
Subiculum	606.9	(15.922%)	611.0	(15.784%)	583.1	(15.41
Presubiculum	419.6	(10.971%)	448.6	(11.576%)	416.3	(10.97
Hippocampal fissure	40.5	(1.044%)	43.2	(1.104%)	45.4	(1.16
(Right)						
Whole hippocampus	3973.3	(0.264%)	3904.5	(0.261%)	3877.0	(0.24
Fimbria	34.7	(0.875%)	37.2	(0.975%)	42.4	(1.09
CA1	339.8	(8.551%)	345.1	(8.864%)	338.9	(8.81
CA2-3	993.7	(25.029%)	996.0	(25.604%)	964.78	(24.94
CA4-DG	563.2	(14.162%)	568.5	(14.589%)	534.3	(13.82
Subiculum	624.4	(15.700%)	632.4	(16.285%)	601.9	(15.56
Presubiculum	414.7	(10.449%)	427.7	(11.048%)	410.1	(10.66
Hippocampal fissure	49.9	(1.256%)	49.2	(1.254%)	41.8	(1.08

Table 5.9 Student and paired t-tests.

	Student t-test		
	Pre-CPAP vs. Pre-BEST SUPPORTIVE CARE	**Post-CPAP vs. Post-BEST SUPPORTIVE CARE**	**Pre- vs. Post-BEST SUPPORTIVE CARE**
(Left)			
Whole hippocampus	0.437	0.429	0.094
Fimbria	0.207	0.432	0.785
CA1	0.659	0.662	0.674
CA2-3	0.404	0.647	0.956
CA4-DG	0.148	0.271	0.394
Subiculum	0.332	0.780	0.496
Presubiculum	0.991	0.233	**0.028***
Hippocampal fissure	0.362	0.593	0.282
(Right)			
Whole hippocampus	0.257	0.552	0.427
Fimbria	0.256	0.571	0.323
CA1	0.485	0.464	0.219
CA2-3	0.894	0.551	0.145
CA4-DG	0.332	0.140	0.079
Subiculum	0.771	0.487	0.180
Presubiculum	0.639	0.381	0.054
Hippocampal fissure	0.338	0.299	0.975

(* = P-value < 0.05)

Table 5.10 CPAP Adherence

	Over 6 months
Number randomised	17
Number (%) analysed	17 (100%)
Number of patient who used CPAP less than 1 months or stopped	7 (41%)
Number of patients who requested to continue using CPAP	12 (71%)
Number using CPAP >4 hours/night N (%)	5 (29%)
Median daily use (mean hours/night)	3.4 (2.2)

5.7 DISCUSSION

The main findings of this study were a decrease in left fimbrial volume after CPAP, and an increase in the left presubiculum volume. There was no demonstrate the increase in the hippocampal sub-field volumes in older patients with OSA, this was somewhat surprising as previous studies in younger and middle-aged patients with OSA have seen these differences (Chapters 3 and 4). Possible reasons including the poor adherence with CPAP treatment, loss of plasticity in brain tissue and limitations of the scans and their analysis are discussed in this Section of the Thesis. I will also try to explain these findings in the context of the improvements in attention and executive function. The cognitive data has previously presented by Dr McMillan in her PhD Thesis.

5.7.1. Left fimbria volume decrease

After 6 months of CPAP treatment, the only change in hippocampal sub-field volume seen was a decrease in the left fimbria. The fimbria is a collection of white matter that extends from the ventricular surface of the hippocampus into the fornix, which forms the largest efferent projection of subiculum into the hypothalamus (O'Mara *et al.*, 2001). In animal models the fimbria is involved in working memory. If the fimbria are surgically damaged rats will perform poorly at tasks of working memory (Walker & Olton, 1984), (Sutherland & Rodriguez, 1989). It is of note that the patients in the

present study showed improvements in tasks of attention and executive function but not in tasks of working memory.

5.7.2. Left presubiculum volume increase

The presubiculum volume increased after 6 months of Best Supportive Care. The presubiculum lies between the hippocampus and the cortex and as such is an important site for both afferent and efferent pathways. It receives cortical input from the cingulate gyrus, and projects indirectly into the hippocampus via the entorhinal cortex (Cragg, 1965; Shipley, 1975; Vanhoesen & Pandya, 1975; Pandya *et al.*, 1979 cited from Seltzer, 1979). It receives afferent projections from the hippocampus that ultimately lead to the cortex (Meibach & Siegel, 1975; Swanson & Cowan, 1975; Meibach & Siegel, 1977; Rosene & Van Hoesen, 1977). It is known to be involved in spatial memory (Simonnet *et al.*, 2013).

The volume increase does was not matched by an improvement in spatial memory or any other cognitive domain in patients in the BEST SUPPORTIVE CARE group but it could still represent real brain hypertrophy. Alternatively it could represent oedema as a result of changes in intracellular sodium and potassium concentrations induced by ischaemia (Kreisman & LaManna, 1999). Finally it could be a statistical quirk and not represent a real change.

5.7.3. Absence of brain hypertrophy after CPAP

There are several possible reasons for the absence of brain hypertrophy found this study in older people, compared to those in younger and middle-aged people presented in **Chapters 3 and 4**. These include:

Poor adherence to CPAP treatment: CPAP only prevents apnoeas when it is actually being worn. Thus if the patient sleeps with CPAP for some of the night and without it for some of the night, he/she will still be exposed to intermittent hypoxia for the period CPAP is not worn. Although there is no formal definition of CPAP adherence, most studies use a cut-off of 4 hours/night. Using this definition between 29 and 83% of patients were not adhering with CPAP (Weaver & Grunstein, 2008). Factors that are associated with poor adherence include depression, increased nasal resistance, mild or severe OSA, mild symptomatology of OSA and poor education (Sawyer *et al.*, 2011). Older men with nocturia as a result of prostate disease also use CPAP less, as they need to reapply the mask several times during the night (Wolkove *et al.*, 2008). Additionally older people may be able to nap during the day, therefore poor nightime adherence may not present such a problem compared to younger people who have more demanding schedules.

After 3 months of CPAP, adherent patients (mean CPAP use 8.5 hours/night) showed significantly more improvement in multiple cognitive domains than non- adherent patients (mean CPAP use 3.9 hours/night) (Aloia *et al.*, 2003). A subsequent study showed that 21% of poor users (<2 hous/night), 44% of moderate users (2-6 hours/night), and 68% of optimal users (>6 hours/night) exhibited memory performance in the clinically normal range following 3 months of CPAP (Zimmerman *et al.*, 2006). In the present study only 5 patients (29%) achieved good adherent and 7 (41%) used CPAP for less than a month in total over the 6 months. If CPAP leads to improved cognitive function because the removal of intermittent hypoxia leads to hippocampal hypertrophy, then this level of adherent may be ineffective. However although my patients did not show improved memory, they did demonstrate improvements in attention and executive function.

Loss of neuroplasticity: Rodent studies suggest that neuroplasticity is maximal in the first 2 weeks of life and had significantly reduced after 6-12 months (Kuhn *et al.,* 1996). Expression of highly polysialyted neural cell adhesion molecule, expressed by new neural cells, falls steadily from 2 months of age until it is virtually undetectable at 18 months (Seki & Arai, 1995). I do not think there is corresponding data for human brains, but it is plausible that the neuroplasticity seen in younger individuals may not be present in those aged 65 years and older.

Daytime sleepiness: The older patients who took part in this study did not report being excessively sleepy (mean ESS 9.4). It is therefore possible that the relatively low burden of symptoms may mean that they had had undiagnosed OSA for many years. If this was the case they may also have had prolonged exposure to intermittent hypoxia and sleep fragmentation. There is some evidence that the effects of these insults are cumulative and that may mean that my older patients' prolonged exposure has resulted in irreversible damage. In animal models, prolonged exposure to sleep deprivation suppresses hippocampal neuronal proliferation, differentiation, maturation and survival (Roman *et al.*, 2005).

The CA1 hippocampal sub-field has been shown to be especially sensitive to hypoxia (H.M. & P., 1998; Harrison & Eastwood, 2001). Irreversible CA1 injury can be the result of even short periods of induced ischaemia (Nitatori *et al.*, 1995; Csernansky *et al.*, 1998). Although other sub-fields may be more resilient, prolonged exposure to intermittent hypoxia may result in irreversible damage in these regions. This explanation would be particularly interesting with regards to the results presented in this Chapter. Memory impairment, which did show some resolution in younger patients in Chapter 4, did not improve in older patients in this study because memory is mediated by the hippocampus, and the hippocampal damage in older OSA patients was irreversible, either because of prolonged exposure to intermittent hypoxia and sleep fragmentation or because of age-related loss of neuroplasticity. The

improvements in attention and executive function seen in older patients in this study could have to be mediated by a different mechanism, perhaps sleepiness: Although ESS reduction in the CPAP group (baseline mean 9.4, post-CPAP mean 7.3) was not statistically significant; it may have been clinically significant.

5.7.4. Limitations of the analysis

I have discussed the limitations of FreeSurfer in Chapters 3 and 4. The same limitations mentioned previously would apply to this study also. Furthermore, in this study I limited our analysis to the hippocampus and its sub-fields. This was based on the observation that almost all studies of brain structure in OSA patients have shown hippocampal hypotrophy (Morrell & Glasser, 2011). Additionally, the first study to show an increase in grey matter volume after CPAP treatment showed this in the hippocampus. I was therefore surprised by the results found in the study presented here. However, in Chapter 4 is should be noted that thalamic hypertrophy and hippocampal hypotrophy was found. Therefore, it is possible that by only studying hippocampal sub-fields I have missed hypertrophy in the thalamus or other brain regions. Further analysis will help to clarify this point.

5.7.5. Conclusion

The results of this study have shown that after 6 months of BEST SUPPORTIVE CARE there was an increase in left presubiculum volume, and that after 6 months of CPAP there was a decrease in left fimbria volume. There was no evidence of CPAP induced hippocampal hypertrophy.

CHAPTER 6: GENERAL DISCUSSION

6.1. SUMMARY OF FINDINGS

OSA leads to changes in brain structure that are associated with cognitive dysfunction. However it was unclear if these changes were reversible and whether any resolution of brain hypotrophy would result in resolution of cognitive dysfunction.

Study 1 (Chapter 3) was a cross-sectional study of brain structure in young and middle-aged patients with OSA. A new automated technique, FreeSurfer, was used to analyse MRI brain images of patients newly diagnosed with OSA compared to healthy controls. It is the first study to show that, as well as hypotrophy of brain tissue, OSA patients also have regions of hypertrophy within the hippocampus, an area of brain associated with memory. I propose that this hypertrophy was the result of ischaemic preconditioning, with IH leading to dendritic sprouting and increased connectivity and protection of brain function.

Study 2 (Chapter 4) was a randomised controlled trial of CPAP's effects on brain structure and cognitive function in young and middle-aged OSA patients. FreeSurfer was used to analyse MRI brain images of newly diagnosed OSA patients and healthy controls. In this study I also investigated a link between brain structure and brain function by analysing subjects' performance in a battery of cognitive tests. In order to assess the effects of CPAP, OSA patients were then randomised to receive either CPAP or best supportive care for one month following which brain MRI and cognitive testing were repeated. I found hippocampal hypotrophy in OSA patients compared to healthy controls at baseline. However after one month of CPAP treatment this was no longer significant. Furthermore there was significant thalamic hypertrophy in the CPAP group compared to the BSC group. Hypertrophic changes in the right thalamus were strongly correlated to changes in bilateral cortical cerebellar and hippocampal structures. CPAP was also shown to significantly improve performance in tests of working memory. The association of improvement in working memory with wider reorganization in a distributed memory system for associative learning (thalamocortical changes associated with changes in bilateral hippocampi and cerebellar cortices) suggests that ischaemic preconditioning may be neuroprotective in young and middle-aged OSA patients. This leads to resolution of brain hypotrophy following treatment with CPAP. This resolution of brain structure in a system known to be involved in working memory may be the cause of the improvements in working memory following CPAP treatment that I found.

Study 3 (Chapter 5) was a randomised controlled trial of CPAP's effect on brain structure in older people with OSA. This is the first time the combined effects of OSA and aging on brain structure have been reported. MRI brain images were collected at baseline and 6 months after randomisation to

either CPAP or BSC. These were analysed using FreeSurfer, this time focusing specifically on hippocampal subfields, as I had already shown hippocampal hypertrophy in younger patients in study 1 and the hippocampus is known to be a site of neurogeneration in ischaemic preconditioning in animal models. I found fimbial hypotrophy after 6 months of CPAP in older patients with OSA but no hippocampal hypertrophy. This may be because aging leads to a reduction in neurogenerative capabilities inhibiting the protective effects of ischaemic preconditioning. Alternatively older OSA patients, who are less sleepy than younger patients, have actually had undiagnosed OSA for a long period, and prolonged exposure to IH has resulted in cessation of neurogeneration, as it has been shown to do in animal models.

6.2. FUTURE RESEARCH

Further work is needed to investigate the effects of intermittent hypoxia on brain structure and function in humans, as well as the protective role of ischaemic preconditioning. There is a subset of patients with moderate and severe OSA who are not symptomatically sleepy. Current guidelines state that these patients do not need to be offered CPAP. However these patients are still exposed to IH and sleep fragmentation. If either of these stimuli cause neural hypotrophy and cognitive dysfunction then these guidelines may have to be reviewed. This would be especially important if, as my thesis suggest, the hypotrophy and memory impairment are reversible in younger people, but not in older people, who may have been exposed to the stimuli for a prolonged period.

We have shown an association between cognitive function and brain structure in OSA. It would be very difficult to prove a cause and effect mechanism, but more studies investigating both brain structure and cognitive function in large numbers of patients would provide strong circumstantial evidence. In order to achieve the required sample size, collaboration among multiple research teams would be required. This might lead to the development of a standard battery of cognitive tests, sensitive to the deficits seen in OSA patients, which would aid the comparison of results between groups and allow meta-analyses to be carried out.

Driving ability is one of the most important complex cognitive tasks known to be impaired in OSA. This has a high cost, both economic and in terms of mortality. Current guidelines suggest that OSA patients can continue to drive if they are not excessively sleepy. IH may be linked to brain hypotrophy and impaired driving ability in non-sleepy OSA patients. I have shown that some patients do not develop brain hypertrophy after CPAP treatment but in fact develop further hypotrophy. Further research is required to investigate if brain hypotrophy in these patients is associated with impaired driving performance. If it is then the guidelines would need to be reviewed.

In order to further explore the relative roles of intermittent hypoxia and sleep fragmentation on brain structure, cognitive function and driving ability in OSA patients, I would propose a three armed randomised controlled trial. The first arm would be allocated to best supportive care and would therefore be exposed to both IH and sleep fragmentation. The second arm would be allocated to CPAP therapy and would therefore be exposed to neither IH nor sleep fragmentation. The third arm would be allocated to nocturnal oxygen therapy. This would not prevent airway obstruction and sleep fragmentation, but would remove the intermittent hypoxia. I would obtain MR brain images and measure cognitive function and driving simulator performance at baseline and again after 3 months of treatment. I would also study matched healthy controls at baseline and at three months.

It would be intriguing to study brain structure and cognitive function in subjects with restless leg syndrome, whose sleep is fragmented but who are not exposed to intermittent hypoxia. However it would not be possible to determine whether any changes in brain structure in these subjects were the result of sleep fragmentation or whether they were caused by frequent leg movements, or were even the cause of the restless leg syndrome The effects of pure sleep fragmentation could also be studied by using external interruptions to provoke frequent arousals from sleep in healthy controls over a prolonged period. However this approach would be difficult both practically and ethically.

The effects of intermittent hypoxia during sleep could be studied by manipulating the atmospheric oxygen concentration while healthy controls slept. Again, to mimic OSA, this would need to be done over prolonged period and this would be difficult practically and ethically. However this approach could be used in animal models to determine at what point IH stops being beneficial.

New automated techniques for the analysis of brain imaging are being developed constantly. Studies are needed to ensure that these techniques are validated in human brains, perhaps in post-mortem studies. Once they are proven to be valid they should be used to analyse as much data as possible. Automated analysis allows for rapid and relatively inexpensive analysis of large volumes of data. It is imperative that it used effectively and to its full potential.

Poor CPAP compliance means that this treatment, which is highly efficacious at preventing apnoeic events if worn, cannot be considered the panacea of OSA treatment. Potential new techniques for preventing apnoeas may include nerve stimulation or less invasive devices to maintain airway patency. If the sequelae of OSA are mediated by IH then enriching inspired air with oxygen may be beneficial. Pharmacological cures must continue to be explored. Above all, preventative measures in the field of public health must succeed to prevent an epidemic of OSA and all the health problems it causes.

6.3 **CONCLUSION**

The overall conclusion of the studied presented in this Thesis is that OSA leads to brain hypotrophy and cognitive impairment. However ischaemic preconditioning by IH may also be protective and lead to increased connectivity through dendritic sprouting. In younger patients ongoing dendritic sprouting leads to restoration of functional cognitive neural pathways after treatment. Future work should focus on preventing brain hypotrophy and ensuring effective treatment of OSA before neurogeneration switches off.

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