Aus der Klinik für Kardiologie und Angiologie
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DISSERTATION

OBSTRUCTIVE SLEEP APNOEA/HYPOPNOEA SYNDROME: AROUSALS, AUTONOMIC ACTIVITY AND HOME-BASED DIAGNOSIS

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ABBREVIATIONS

AHI: Apnoea Hypopnoea Index

AF: Atrial Fibrillation

AI: Apnoea Index

AR: Autoregressive modelling

ArI: Arousal Index

ANN: Artificial Neural Network

ANOVA: Analysis of Variance

ANP: Atrial Natriuretic Peptide

APAP: Auto Continuous Positive Airway Pressure

ARAS: Ascending Reticular Activating System

ASDA: American Sleep Disorders Association

BL: Baseline

BP: Blood Pressure

BPAP: Bi-level Positive Airway Pressure

BRS: Baroreflex Sensitivity

BMI: Body Mass Index

BTS: British Thoracic Society

CAP: Cyclic Alternating Pattern

CAD: Coronary Artery Disease

CI: Cardiac Index

CO: Cardiac Output

COPD: Chronic Obstructive Pulmonary Disorder

CPAP: Continuous Positive Airway Pressure

CRPSG: CardioRespiratory PolySomnoGraphy

CVD: Cerebrovascular Disease

DC: Direct Current

ECG: Electrocardiography

EDRF: Endothelium Derived Relaxing Factor

EDS: Excessive Daytime Sleepiness

EEG: Electroencephalography

EMG: Electromyography

EOG: Electro-oculography

ESS: Epworth Sleepiness Scale

FFT: Fast Fourier Transform

FN: False Negative

FP: False Positive

FOSQ: Functional Outcomes of Sleep Questionnaire

GABA: Gamma Amino-Butyric Acid; an inhibitory synaptic transmitter

HF: Heart Failure

HF: High Frequency

HI: Hypopnoea Index

HR: Heart Rate

HRV: Heart Rate Variability

IQR: Interquartile Range

LAUP: Laser-Assisted Uvulo-Palatoplasty

LF: Low Frequency

MI: Myocardial Infarction

MLG: Midline Laser Glossectomy

MLP: Multilayer Perceptron

MMO: Maxillo-Mandibular Advancement Osteotomy

MRI: Magnet Resonance Imaging

MRS: Mandibular Repositioning Splint

MSLT: Multiple Sleep Latency Test

MSNA: Muscle Sympathetic Nerve Activity

MWT: Maintenance of Wakefulness Test

NHS: National Health Service

NREM: Non Rapid Eye Movement

NYHA: New York Heart Association

ODI: Oxygen Disturbance Index

OR: Odds Ratio

OSAHS: Obstructive Sleep Apnoea/Hypopnoea Syndrome

PAP: Pulmonary Artery Pressure

PET: Positron Emission Tomography

PGO waves: Ponto-Geniculo-Occipital waves

PH: Pulmonary Hypertension

PLMD: Periodic Limb Movement Disorder

PDS: Power Spectral Density

PSG: Polysomnography

PTT: Pulse Transit Time

RAI: Respiratory Arousal Index

RCT: Randomised Controlled Trial

RDI: Respiratory Disturbance Index

RE: Respiratory Events

REM: Rapid Eye Movement

RERA: Respiratory Effort Related Arousal

R&K: Rechtschaffen & Kales

ROC: Receiver Operator Characteristic Curve

RR: RR interval

RSS: Respiratory Arousal Index per Sleep Stage

RTA: Road Traffic Accidents

SAI: Spontaneous Arousal Index

SaO_2: Arterial Oxygen Saturation

SD: Standard Deviation

SDB: Sleep Disordered Breathing

SEM: Standard Error of the Mean

SF-36: Short Form 36 health status questionnaire

SHHS: Sleep Heart Health Study

SPT: Sleep Period Time

SV: Stroke Volume

SWS: Slow Wave Sleep

TF: Total Frequency

TIA: Transitory Ischeamic Attack

TN: True Negative

TP: True Positive

UARS: Upper Airway Resistance Syndrome

UPPP: Uvulo-Palatino-Pharyngo-Plasty

VEGF: Vascular Endothelial Growth Factor

VPF: Vascular Permeability Factor

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ABSTRACT

The Obstructive Sleep Apnoea Hypopnoea Syndrome (OSAHS) affects approximately 4% of the middle aged working population. It causes daytime sleepiness and cognitive impairment and affects the patients' professional and social performance. Causative associations have been demonstrated between the syndrome and cardiovascular disease, in particular arterial hypertension, affecting the patients' long-term outcomes.

Markers of disease severity such as the Apnoea/Hypopnoea Index (AHI) and sleepiness scores have been associated with the adverse effects in un- or undertreated patients.

The Arousal Index (AI), one of the markers of sleep disruption, correlates poorly with measures of sleepiness, one of the reasons being that apnoeas and hypopnoeas are not always associated with cortical arousals on the EEG.

The research within this thesis aimed to contribute towards the further understanding of the concept of the hierarchical arousal model, its interactions with abnormal breathing and sleep.

A further aim was to contribute towards the development of novel diagnostic modalities based on pathophysiological markers of the disease, enabling a timely, cost-effective, home-based diagnosis and treatment of the syndrome, limiting the number of patients who may suffer the adverse long-term consequences.

Four studies have been included in this thesis.

In the first study, to ascertain the influence of sleep stages on visible cortical arousal - as defined by the American Sleep Disorders Association in 1992 [9] - 2667 apnoeas and hypopnoeas were analysed across 15 OSAHS patients with a wide range of disease severity. The Apnoea-Hypopnoea-Index was 39/hr in NREM1&2, significantly higher compared to 17/hr REM or to 11/hr SWS sleep ($p<0.001$). During SWS, 34% of apnoeas/hypopnoeas were associated with arousal, significantly less than the 77% during NREM1&2 and 62% during REM sleep ($p<0.001$). Arousal induction was not affected by oxygen desaturation, event type, duration or time of the night. It is therefore concluded that apnoeas/hypopnoeas in SWS are associated with fewer cortically apparent, visually detected arousals.

To investigate whether non-visible changes in EEG were occurring in association with apnoeas and hypopnoeas, in the second study power spectral analysis of the EEG was performed centering around the restoration of flow in 2596 apnoeas and hypopnoeas across 15 patients.

Comparisons were made with periods of undisturbed sleep within the sleep periods of each patient. Normalised theta power (4-8Hz) decreased significantly at apnoea/hypopnoea termination ($p \leq 0.01$). During NREM sleep, changes were detected at event termination irrespective of arousal visibility. During REM sleep, apnoeas/hypopnoeas which were not accompanied by visible arousals showed no evidence of significant spectral power changes in the EEG. The patients' EEG recordings were also compared to the recordings of 7 healthy individuals of similar age. Theta power was significantly lower across patients compared to healthy subjects ($p=0.03$) and was correlated to the Apnoea-Hypopnoea-Index ($rho=0.6$, $p=0.008$). It is concluded that EEG spectral analysis improves detection of changes at apnoea/hypopnoea termination in non-REM sleep. Further validation is needed to determine whether it improves correlation between nocturnal measures and daytime symptoms.

The third study focused on the cardiovascular autonomic activity changes related to apnoeas and hypopnoeas. Analysis of the Heart Rate Variability [HRV] using the Fast Fourier Transform [FFT] algorithm was applied to detect autonomic activity changes during sleep in 14 OSAHS patients and compared to 7 healthy individuals. The main finding was the reduced HRV (increased LF power) around flow restoration compared to periods of stable breathing ($p<0.02$), irrespective of the presence of visible cortical arousal. Compared to healthy individuals, LF, HF and TF power were significantly higher across the OSAHS patients ($p<0.03$). Despite the inherent limitations of the algorithm used, sympathetic activity was found to increase at the end of apnoeas/hypopnoeas, irrespective of the presence of EEG arousals. This may represent part of the hierarchical arousal model. The increase in LF, HF and TF power found across patients compared to healthy controls ($p<0.03$), may represent the nocturnal autonomic dysfunction in OSAHS patients. Nocturnal sympathetic activation may contribute to the patients' long-term cardiovascular consequences.

In the last part of the work in this thesis, Embletta, a portable device for the home-based diagnosis of the OSAH, was validated. Initially, a synchronous comparison to polysomnography was performed in 40 patients. This was followed by a comparison of home Embletta studies with in-laboratory polysomnography in 61 patients. In the synchronous study, there was good correlation between the polysomnography and Embletta apnoeas and hypopnoeas per hour spent in bed ($rho=0.98$, $p<0.001$). These data were used to construct diagnostic categories in symptomatic patients from their Embletta results: "OSAHS" ($\geq$20 A+H/hr in bed), "possible OSAHS" (10-20 A+H /hr in bed) or "not OSAHS" (<10 A+H/hr in bed). Using this

classification, most patients were satisfactorily classified by home Embletta studies but 29 out of 61 patients required further investigation. The study suggested a 42% saving in diagnostic costs over polysomnography if this approach were adopted.

It is concluded that the application of non-conventional analysis methods may improve the understanding of the arousal cascade and the pathophysiological changes associated with apnoeas and hypopnoeas during sleep. Diagnostic modalities based on pathophysiological surrogate markers may contribute towards remote, decentralised, timely and cost effective diagnosis promoting early treatment of the disease in order to minimise the long-term cardiovascular effects.

ZUSAMMENFASSUNG

Das Obstruktive Schlafapnoe-Hypopnoe-Syndrom (OSAHS) betrifft ungefähr 4% der berufstätigen Bevölkerung zwischen 40 und 60 Jahren. Es verursacht Schläfrigkeit, beeinflusst die kognitive Leistung und beeinträchtigt die berufliche und soziale Leistung der Patienten. Die kausativen Assoziationen zwischen dem Syndrom und Herz-Kreislauf-Erkrankungen, insbesondere arterielle Hypertonie, mit Auswirkungen auf die Prognose der OSAHS Patienten, sind vielfaltig demonstriert worden. Der Apnoe/Hypopnoe Index (AHI) und die Auswertung der Schläfrigkeit kennzeichnen die Schwere des Syndroms und haben ungünstige Auswirkungen in unbehandelten OSAHS Patienten. Die Korrelation zwischen der objektiven Schlaefrigkeits-auswertung und dem Arousal-Index (AI) ist schwach. Ein wichtiger Grund ist die unregelmäßige Assoziation zwischen Apnoen und Hypopnoen und EEG Arousals.

Die in dieser Dissertation durchgeführte Forschung zielte darauf, dem weiteren Verständnis des Konzepts der hierarchischen Arousal Modells und den Wechselwirkungen zwischen Atemstörungen und Schlaf beizutragen. Ein weiteres Ziel war der Beitrag zur Entwicklung neuer diagnostischen Algorithmen, die auf die pathophysiologischen Merkmale des Syndroms basieren und die zeitnahe, kostengünstige Diagnose ermöglichen. Damit wird die Anzahl der Patienten, die unter den Konsequenzen des unbehandelten OSAHS leiden, begrenzt.
Die vorliegende Dissertation beinhaltet vier Forschungsprojekte.

Im ersten Projekt wurde der Einfluß der Schlafstadien auf die Entstehung der EEG Arousals [9] studiert - 2667 Apnoen und Hypopnoen in 15 OSAHS Patienten wurden analysiert. Der AHI war 39/hr in NREM1&2, erheblich höher im Vergleich zu 17/hr in REM oder 11/hr in SWS Schlaf ($p < 0{,}001$). Im SWS, Arousals begleiteten 34% der Apnoen/Hypopnoen, erheblich weniger im Vergleich zu 77% im NREM1&2 und 62% im REM Schlaf ($p < 0{,}001$). Die Anwesenheit von EEG Arousals war nicht beeinflußt von Sauerstoffentsättigungen, von dem Auftreten von Apnoen versus Hypopnoen, deren Dauer oder dem Zeitpunkt ihres Auftretens in der Nacht. Es wurde festgestellt, dass Apnoen/Hypopnoen im SWS seltener von EEG Arousal begleitet werden.

Im zweiten Projekt, EEG Spektralanalyse wurde eingesetzt um Unterschiede zwischen sichtbaren und unsichtbaren EEG Veränderungen in Bezug auf Apnoen/Hypopnoen, festzustellen. Insgesamt wurden 2596 Apnoen und Hypopnoen in 15 Patienten analysiert und verglichen mit Intervallen von physiologischem Schlaf gleicher Dauer. Wir haben eine statistisch signifikante Verminderung in der Power (Leistung) der Theta Wellenlaenge (4-8Hz) am Ende der Apnoen /Hypopnoen beobachtet ($p < 0,01$). Im REM Schlaf, Apnoen/Hypopnoen die nicht von sichtbaren EEG Arousals begleitet wurden, gaben keine Anzeichen für statistisch signifikante Veränderungen der spektralen Leistungsdichte. Die EEG Aufzeichnungen der Patienten wurden weiterhin verglichen gegen die Aufzeichnungen von 7 gesunden Probanden gleichen Alters. Es wurde eine statistisch signifikante Verminderung im Bereich der Theta-Power in den OSAHS Patienten festgestellt im Vergleich zu gesunden Probanden ($p = 0,03$). Die Verminderung korrelierte mit dem Apnoe-Hypopnoe-Index ($rho=0,6$, $p=0,008$). Es wird gefolgert dass im NREM-Schlaf, die EEG-Spektralanalyse die Erkennung von Apnoe-/ Hypopnoe-bezogenen Änderungen verbessert. Weitere Validierung dieser Methode ist erforderlich um festzustellen ob sich die Korrelation zwischen nächtlichen Veränderungen und Symptomen verbessert.

Im dritten Projekt, haben wir die Variabilität der autonomen Aktivität in Bezug auf Apnoen und Hypopnoen studiert. Die Analyse der Herzfrequenzvariabilität [HRV] mittels des Fast Fourier Transform [FFT] Algorithmus wurde angewandt, um Veränderungen der autonomen Aktivität während des Schlafes in 14 OSAHS Patienten zu erkennen. Die Änderungen in der Patientengruppe wurden verglichen mit 7 gesunden Probanden. Die wichtigste Beobachtung war die Reduktion der HRV (erhöhte Low Frequency Leistung) am Ende der Apnoen/Hypopnoen im Vergleich zur physiologischen Atmung ($p < 0,02$). Diese Beobachtung war unabhängig von der Anwesenheit sichtbarer EEG Arousals. Der Vergleich zwischen OSAHS Patienten und gesunden Individuen ergab ein signifikant höheres Leistungsspectrum in den Low (LF), High (HF) und Total (TF) Power in der Patientengruppe ($p < 0,03$). Es wird festgestellt, dass trotz den Begrenzungen des angewandten FFT Algorithmus, die Beendigung von Apnoen/Hypopnoen ist mit einem Anstieg der sympathetischen Aktivität assoziiert. Die sympathetische Aktivierung ist unabhängig von EEG Arousals und repräsentiert möglicherweise ein Teil des hierarchischen Arousal Modells. Der beobachtete Anstieg der LF, HF und TF Power (Leistung) in der Patientengruppe im Vergleich zu den gesunden Probanden ($p < 0,03$) repräsentiert die nächtliche Störung des autonomen Nervensystems in OSAHS Patienten. Die nächtliche Aktivierung des

sympathischen Nervensystems könnte zu den beobachteten langfristig kardiovaskulären Konsequenzen beitragen.

Im letzten Abschnitt dieser Dissertation wurde Embletta, ein ambulantes diagnostisches Gerät, validiert. Zunächst wurde ein synchroner Vergleich zur Polysomnographie, bei 40 Patienten durchgeführt. Anschliessend, die ambulanten Embletta-Aufzeichnungen von 61 Patienten wurden mit ihren Schlaflabor-polysomnographischen Daten verglichen. In der synchronen Studie wurde eine gute Korrelation zwischen der Polysomnographie und der Embletta Apnoen und Hypopnoen pro Stunde im Bett beobachtet (rho = 0,98 , p <0,001). Diese Embletta Ergebnisse wurden verwendet, um diagnostische Kategorien in symptomatischen OSAHS Patienten zu konstruieren: "OSAHS" (> 20 A + H /hr im Bett), " OSAHS möglich" (10-20 A + H /hr im Bett) und "kein OSAHS "(<10 A + H /hr im Bett). Die Mehrheit der Patienten wurde aufgrund dieser Einteilung durch die ambulanten Embletta Aufzeichnungen korrekt klassifiziert, wobei für 29 der 61 Patienten eine weitere Untersuchung erforderlich war. Diese Ergebnisse deuten auf eine 42% Minderung in diagnostischen Kosten im Vergleich zur Polysomnographie, wenn dieser Konzept eingesetzt würde.

Es wird festgestellt, dass der Einsatz unkonventionellen Methoden, zur Erläuterung des hierarchischen Arousal Models und den damit zusammenhängenden pathophysiologischen Veränderungen im Schlaf beitragen. Diagnostische Modelle die pathophysiologische Ersatzmarker benutzen, können zur Vereinfachung und Dezentralisierung von zeitgerechter und kostengünstiger Diagnose beitragen. Die frühzeitige Behandlung verhütet die Entwicklung von Herz- und Gefäßkrankheiten, die bei dem OSAHS beschrieben wurden.

CHAPTER 1
INTRODUCTION

1.1. HYPNOS

The attempts to understand sleep date back to ancient Egypt. It was believed that common people as well as royalties, could contact their gods through dreams. Ancient texts describe many dreams, used as a medium to understand the will of the gods. Some temples offered dormitories, where people could go to sleep in order to enhance their contact with the gods.

In Greek mythology, Hypnos was the personification of sleep (figure 1.1). He was the son of Nyx ("night") and Erebus, and the twin of Thanatos ("death"). According to some sources, both Hypnos and his brother lived in the underworld, though others say he lived on the island of Lemnos. Hypnos enters the sleep of mortals and, at the bidding of the Olympians, gives them dreams of foolishness or inspiration, depending on the individual and their divine protectors or enemies. During the Trojan War, Hera (eager to cast her influence on the side of the Greeks) persuaded Hypnos to lull Zeus to sleep so that her brother Poseidon (who detested the Trojans) could intervene on behalf of the Greeks. Hypnos gave Endymion the power of sleeping with open eyes so he could see his beloved, the moon goddess Selene.

The Romans had a similar deity, named Somnus.

Figure 1.1. This floor Mosaic shows Hypnos, the God of Sleep. It was found in a 3rd century A.D. in a villa in the town Kotor, on the Mediterranean coast of Montenegro.
This image of the pagan god Hypnos is the only known in the world.

1.2. BACKGROUND OF THIS THESIS AT ITS START

The Obstructive Sleep Apnoea Hypopnoea Syndrome (OSAHS), its pathophysiology and diagnostic modalities are the main focus of this thesis. The syndrome has been defined in the year 1972 during a landmark symposium. The estimated prevalence is around 4% among middle aged men and around 2% among middle aged women [378]. The most common symptom is daytime sleepiness. Sleepiness is becoming acknowledged as the most common cause of driving accidents on highways. As the OSAHS remains underdiagnosed, it represents a public health issue. The deployment of time- and cost effective diagnostic modalities is therefore of major importance for the prevention of short- and long-term consequences of the syndrome.

I started exploring the pathophysiology of the Obstructive Sleep Apnoea Hypopnoea Syndrome in the year 1997. I was fascinated by the physiology of sleep and motivated to understand the mechanisms and interactions between the state of vigilance, autonomic activity and breathing (figure 1.2). These had been important areas of research at the time and contributed significantly to the current knowledge of the syndrome and the understanding of the pathophysiology of sleep.

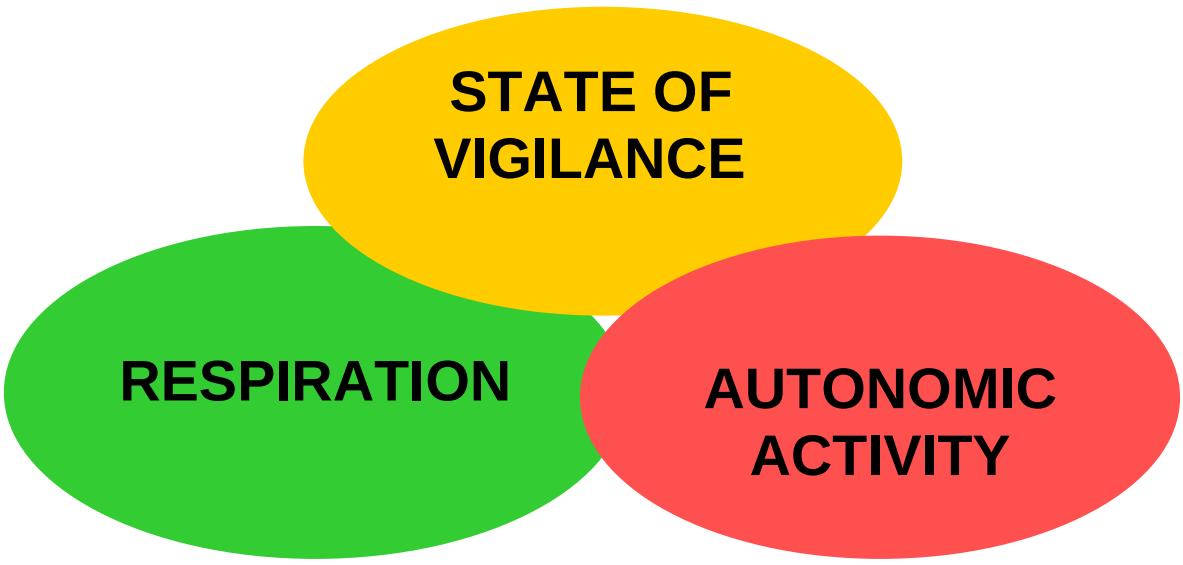


Figure 1.2. Functional and anatomical overlap between the central control of breathing, autonomic activity and the different sleep stages. Interactions and influences have been an important study objective within this thesis.

My research objectives were fuelled by a number of studies, the most important of which are briefly discussed here in chronological order.

In 1992, Cheshire et al reported the lack of significant correlation between objective measures of sleepiness - based on outcomes of the MSLT- and nocturnal measures of sleep disruption such as arousal frequency and time spent in the different sleep stages [60]. The authors suggested that non-conventional methods of sleep analysis and re-definition of disruption indices may improve detection of sleep disruption and predict daytime performance.

In the same year, Stoohs et al published the outcomes of haemodynamic measurements carried out in 5 OSA patients during sleep [332]. The observations confirmed periodic changes in heart rate, stroke volume, cardiac output and blood pressure during apnoeas/hypopnoeas and parallel to intrathoracic pressure fluctuations. Albeit based on a small number of patients and apnoeas studied (56 during REM and 72 during NREM sleep), the observations were suggestive of differences in the degree of cardiac output changes during NREM Stage 2 and REM sleep.

In 1993, the study outcomes by Davies et al confirmed consistent periodic increases in blood pressure parallel to the termination of apnoeas [75]. Based on these observations, the authors identified that changes in autonomic activity may represent a novel pathophysiological measure, whilst facilitating a non-invasive analysis method. In a further study, Davies et al applied auditory stimuli during sleep in healthy individuals [74]. The stimuli induced increase in BP and HR; often but not always was the increase in sympathetic activity associated with a cortical arousal response on the EEG. The findings confirmed that the rise in the markers of autonomic activity is a direct consequence of the stimulus and not a confounding factor of the cortical arousals.

During the same year, Somers et al published the differences found in sympathetic activity – based on the detection of blood pressure, heart rate, Muscle Sympathetic Nerve Activity - across the 5 stages of sleep in 14 healthy individuals [323]. The authors confirmed increased sympathetic activity during REM sleep under physiological conditions. Simulating the apnoea-related increase in intrathoracic pressures through the Mueller manoeuvre during wakefulness, Somers et al identified the interplay between centrally (BP) and peripherally mediated sympathetic activity (MSNA) with intrathoracic pressure changes [324]. Release of the manoeuvre was associated with suppression of MNSA and increase in BP, with potential cardiovascular consequences in sleep apnoea patients.

In 1994, Pitson et al used a different method to monitor autonomic activity, the Pulse Transit Time [281]. The authors exposed 8 healthy volunteers to auditory and vibratory stimuli during

sleep and noted that increases in autonomic activity can occur in the absence of visible cortical electrical activity changes on the EEG.

Consequently, the concept of a hierarchical arousal model was introduced: the “subcortical”, “autonomic” or “brainstem” arousal, were terms used to describe autonomic activation in the absence of cortical EEG activity.

In 1995, Rees et al studied the changes in EEG activity and blood pressure associated with apnoeas/hypopnoeas in 15 OSAHS patients and found that 28% of the detected breathing abnormalities were not associated with a visible change in the cortical electrical activity, whereas a rise in blood pressure was consistently present at apnoea/hypopnoea termination [291]. The authors concluded that visual arousal scoring may underestimate the changes associated with apnoeas/hypopnoeas and the severity of sleep disruption. The findings support the concept that the arousal response consists of a spectrum of pathophysiological changes and is therefore a continuous, variable rather than a discrete response.

During the same year Garpestad et al examined the influence of sleep state on the post-apneic rise in blood pressure in 12 OSA patients and found the haemodynamic response to be more pronounced during REM compared to NREM sleep [117].

In 1996, Svanborg et al applied analysis of the EEG power spectrum to detect changes related to the termination of apnoeas/hypopnoeas in 5 OSA patients [339]. Albeit based on a small number of patients and apnoeas/hypopnoeas studied (53 during NREM and 28 during REM sleep), the observations demonstrated a consistent increase in delta band power (0.5-3.5 Hz) towards the end of apnoeas and hypopnoeas during NREM sleep.

In the same year, Stradling et al challanged the contribution of conventional methods of sleep analysis towards the diagnosis of OSA and towards the differentiation of symptomatic patients who may benefit from and comply to treatment [334].

In 1997, Martin et al published the outcomes of a modelling study, during which 12 healthy volunteers were exposed to auditory stimuli that induced increases in HR and BP – autonomic arousals -, but no visible cortical arousals on the EEG [215]. During the study power spectral analysis of the EEG was used in order to improve detection of cortical electrical activity. Intermittent increases in nocturnal sympathetic activity resulted in less time spent in SWS affecting the sleep architecture and in daytime sleepiness as measured with the MSLT and the MWT. The authors concluded that autonomic arousals contribute to symptoms of sleepiness and are therefore an important measure of disruption of sleep physiology.

During the same year, Bonnet et al applied the concept of Heart Rate Variability (HRV) using spectral analysis of the RR-interval time series from the ECG to detect increases in sympathetic activity parallel to cortical arousals during sleep in healthy volunteers [42].
A further study published in 1997 by Keyl et al established that HRV analysis has a high sensitivity in the detection of changes related to apnoeas/hypopnoeas during sleep and may therefore represent a novel measure of sleep disruption [184].

Inspired by the above findings, I was motivated to explore further the concept of the hierarchical arousal model, the factors contributing towards brainstem versus cortical arousals and the importance of the different sleep stages on autonomic and cortical activity.
Nonconventional methods of analysis were applied in order to establish novel pathophysiological indices, which can potentially improve diagnostic sensitivity whilst facilitating the implementation of a remote, simplified, cost-effective diagnostic tool in order to reduce waiting lists and diagnostic costs.
In the following chapters I will provide an in-depth analysis of the physiology of sleep, autonomic activity and breathing and explore the pathophysiology of the OSAHS and the associated potential long-term consequences. The literature review will include publications up to the year 2008.

CHAPTER 2
SLEEP

2.1. ELECTROENCEPHALOGRAPHY

Before the invention of electroencephalography (EEG) by Hans Berger in 1924, sleep was thought to be a uniform state, with variations in waking threshold, motility and respiration which could not be explained at the time. Qualitative analysis of the EEG during sleep was introduced by Loomis in 1937 [208]. The letters A to E were assigned to stages: stage A corresponded to early drowsiness, stage E to slow wave sleep. In 1953, Aserinsky and Kleitman observed and differentiated Rapid Eye Movement (REM) sleep [12]. Based on their observations of healthy individuals, Dement and Kleitman introduced in 1957 the cyclic pattern of sleep: the 4 Non-REM (NREM) sleep stages and REM sleep formed the basis of qualitative sleep analysis [79]. Monroe's findings of a low inter-scorer agreement [235] led to the establishment of a committee chaired by Rechtschaffen and Kales (R&K), which aimed to standardise recording and scoring techniques. In 1968, the committee published a manual of rule-based sleep analysis [288], a significant part of which, resembles the criteria proposed by Dement and Kleitman.

According to the R&K rules, sleep states and wakefulness are detected by the setting of three physiological correlates:

- brain voltage activity, detected by the EEG
- eye movement, detected by the EOG
- muscle tone, detected by the EMG

The classification is based on the dominant EEG voltage pattern, which alternates between 20 and 200 microVolts, and the EOG and EMG activity during consecutive 30-second epochs. The differentiation between wakefulness and sleep, Rapid Eye Movement (REM) and non-REM (NREM) sleep and the classification into the 4 different stages of NREM sleep is mainly based on the frequency pattern of the cortical electrical activity (voltage) which form characteristic waves.

The waves are classified according to the number of cycles per second, as followed:

Delta: 0.5 - 4 Hz

Theta: 4 - 8 Hz

Alpha: 8 – 12 Hz

Sigma: 12 – 16 Hz

Beta: 16 – 20 Hz

According to the R&K rules, stage scoring of each epoch into NREM stages 1-4 and REM sleep is based on the degree of appearance of these frequency patterns on the cortex surface.

NREM sleep is characterised by a synchronised cortical electrical activity, REM sleep by a desynchronised pattern.

Sleep stage 1, the transition from wakefulness to sleep is characterised by alpha, few theta and vertex sharp waves, stage 2 mainly by theta waves, K-complexes – which are synchronised, amplified vertex potentials -, spindles (12-14Hz for 0.5-2s) and Slow Wave Sleep (SWS) stages 3 and 4 are dominated by high-voltage delta waves (amplitudes >75μV) (figure 2.2). During REM sleep, cortical activity fluctuates in a desynchronised, high frequent (>4Hz) pattern parallel to muscle atonia with intermittent phasic increase of muscle tone and bursts of rapid eye movements. Saw tooth waves have a notched shape and the frequency of theta waves; they are common but not required for staging REM sleep (figure 2.3). Voltage frequencies above 12Hz define wakefulness (table 2.1).

STAGE	EEG	EOG	EMG
1	Alpha, theta, Vertex waves	Slow rolling	↓
2	Theta waves, K-complexes, spindles	-	↓
3	20-50% delta waves	-	↓
4	>50% delta waves	-	↓
REM	Low voltage: alpha, theta, sawtooth waves	REMs	↓↓/phasic
WAKEFULNESS	Alpha, sigma, beta waves	blinks	↑↑

Table 2.1. Sleep/wakefulness characteristics based on the R&K classification.
EEG: ElectroEncephaloGraphy, EOG: ElectroOculoGraphy, EMG: ElectroMyoGraphy.

The 3-dimentional electrical activity changes are detected in two dimensions by electrodes placed on the scalp.

The electrodes are placed according to the ten twenty system [167] (figure 2.1) and the tracings are either unipolar, commonly C3, C4, O1, O2 referenced to the contra-lateral mastoids, or bipolar, e.g. F_zP_z. Frontal electrodes are associated with prominent appearance of SWS, occipital placement enhances the detection of alpha waves. Due to these topographical differences it is essential to state the placement of the electrodes.

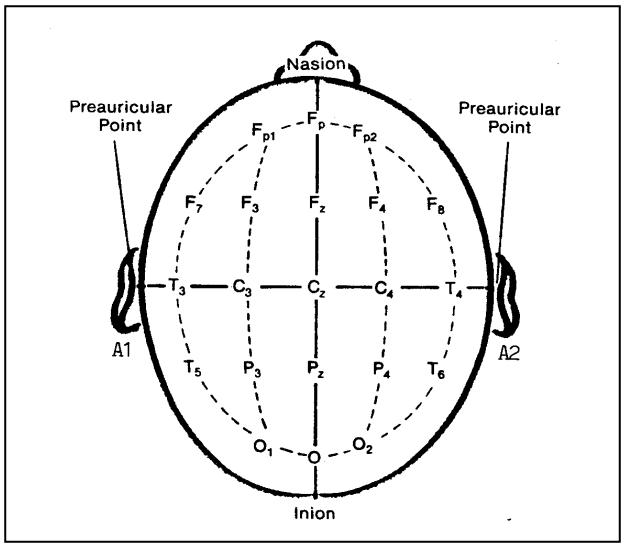


Figure 2.1. Diagram showing the placements of the 10-20 electrode system. Measurements are made at 10% and 20% of the distances from inion to nasion, from left to right preauricular points around the circumference of the head. Commonly used tracings are the unipolar C3A2, C4A1, O1A2, O2A1 with reference to the contra-lateral mastoids or the bipolar F_zP_z. Some centres use the unipolar ipsilateral tracings FP1-LEOG, FP2-REOG. (Re-drawn from Harner PF, Sannit T: A review of the international ten-twenty system of electrode placement. Quincy MA, Grass Instrument Company, 1974) [150].

The R&K rules have standardised the recording and scoring techniques and enabled comparisons between laboratories. The Committee suggested revisions of the manual in the light of any new information and encouraged the use of alternative concepts of sleep analysis in order to understand physiology and improve pattern recognition. However, the R&K rules became the only widely used method and consequently the gold standard of sleep analysis. This unintentionally constituted a restriction to the development of subsequent sleep research.

One of the limitations of the R&K rules, is the 30 second-based scoring: for example, a pattern existing for 16 of the 30sec-epoch determines the whole epoch, irrespective of the patterns in the remaining 14 seconds. Although this may represent a useful means of data reduction in healthy individuals with few stage changes and electrophysiological characteristics that fit the scoring criteria, the heterogenic content of epochs in patients with disturbed sleep and frequent stage changes may result in complete loss of short-term changes and bias the accuracy in the overall outcomes of visual analysis. The recommended tracings and scoring rules refer to healthy sleep

patterns with few interruptions. Arousals from sleep due to apnoeas or hypopnoeas and their detection were not considered in the recommendations, as the syndrome was not recognised at the time. This consequently implies that neither the recommended placement of electrodes, nor the 30 sec-based scoring may be appropriate for the detection of pathologies, recognised later than 1968.

A further limitation of the R&K scoring is that it is based on voltage patterns, but the objective physiological processes behind them are not completely known. Of importance for the detection of voltage changes is the placement of electrodes. The Committee recommended the central tracings for the quantification of sleep because at the time, no regional differences critical to sleep scoring were known. More recent studies have shown that slow wave activity may be of low amplitude in the central leads but more prominent and well represented frontally [197]. The addition of a frontal lead may also improve the detection of short awakenings, as recently shown by O'Malley et al [259]. Thus the time spent awake, in NREM stage 2 and in SWS may clearly depend on the tracings used for sleep scoring.

The need to revise the R&K recommendations and improve analysis is reflected in the lower inter-scorer agreement in individuals with impaired sleep quality, increased frequency of arousals and sleep stage transitions. In healthy individuals, Norman et al found that the mean epoch-based inter-scorer agreement was 76% (range 65-85%) which is significantly higher compared to the average agreement of 71% (range 65-78%) in the group of patients with sleep disordered breathing [256]. These findings support the generally held view that sleep fragmentation makes the application of the R&K rules less reliable.

In conclusion, with accumulating knowledge the R&K rule-based scoring can be seen as an insufficient description of sleep processes in terms of biology and morphology. It is therefore essential to continue research into sleep physiology and pathophysiology and explore alternative, more sensitive methods of sleep detection and analysis, its disruption and interaction with autonomic activity.

Alternative methods of sleep analysis will be discussed in the present thesis. A significant part of the research accomplished within this thesis aimed to contribute towards the further understanding of sleep pathophysiology in order to consequently improve the detection of voltage changes shorter than those detected by R&K, which represent brief arousals.

2.2. SLEEP PHYSIOLOGY

Knowledge of sleep physiology is necessary to understand the interactions between the state of vigilance, autonomic activity and the regulation of breathing.

The majority of NREM and REM sleep physiology studies have been carried out on animals. The most important aspects are explained below.

NREM SLEEP

The initiation and maintenance of sleep is a class of active states during which an equilibrium between inhibitory and excitatory neurons exists, modulated by the influences of the brainstem on the forebrain. The transition from wakefulness to sleep is modulated by serotoninergic raphe neurons via the hypothalamus to the thalamic reticular nucleus [174]. Lesions in this or other reticular formation regions may cause insomnia.

During NREM sleep, the activity of neural components is synchronised, modulated by a variety of aminergic neurotransmitters (serotonin, adenosine, GABA)[155]. The level of activity and degree of synchrony of neural networks is a dynamic process modulated by cellular mechanisms.

Serotonin contributes towards the initiation of sleep; damage to the serotoninergic raphe nuclei induces insomnia in cats [173]. Accumulation of adenosine contributes towards sleepiness and the maintenance of SWS; caffeine, an adenosine receptor inhibitor, counteracts its action and decreases the time spent in SWS [309].

The following forebrain structures are of importance:

Nucleus reticularis thalami: Performance of GABAergic inhibition on thalamocortical cells which results in the synchronised appearance of spindles on the cortex [329]. The thalamic reticular nucleus is the electrographic landmark in the transition from wakefulness to sleep. Its activity is associated with loss of perceptual awareness. Its inhibitory inputs also increase the interplay between slow corticothalamic oscillations (K-complexes < 1Hz) and thalamocortical delta oscillations (1-4Hz) enabling the synchronised appearance of δ-waves on the cortex. Maintenance of the synchronised slow-waved potential changes on the cortex is guarded by frontal neurons. Of importance for the physiology of sleep are the interactions between the thalamic reticular nucleus, the reticular formation and the cortex [328].

Ventrolateral preoptic neurons: There is increasing evidence to suggest that these act as the “master switch” that turns on the sleep process [318]. This group of neurons start firing at sleep onset and initiate the GABA-ergic inhibition of noradrenergic, serotoninergic and histaminergic

arousal mechanisms in the brainstem. As NREM sleep progresses and these neurons decrease their firing frequency, cholinergic neurons are no longer inhibited and REM sleep emerges.

Thalamocortical neurones: Hyperpolarisation and rhythmic membrane depolarisation in these cells, resulting from cortical excitatory low oscillations (K-complexes < 1Hz) and reticular thalamic inhibitory inputs, enable the generation and transmission of delta waves (1-4Hz). The hyperpolarisation is a progressive continuous process from drowsiness to SWS, when synchronised delta waves dominate the cortical EEG [328].

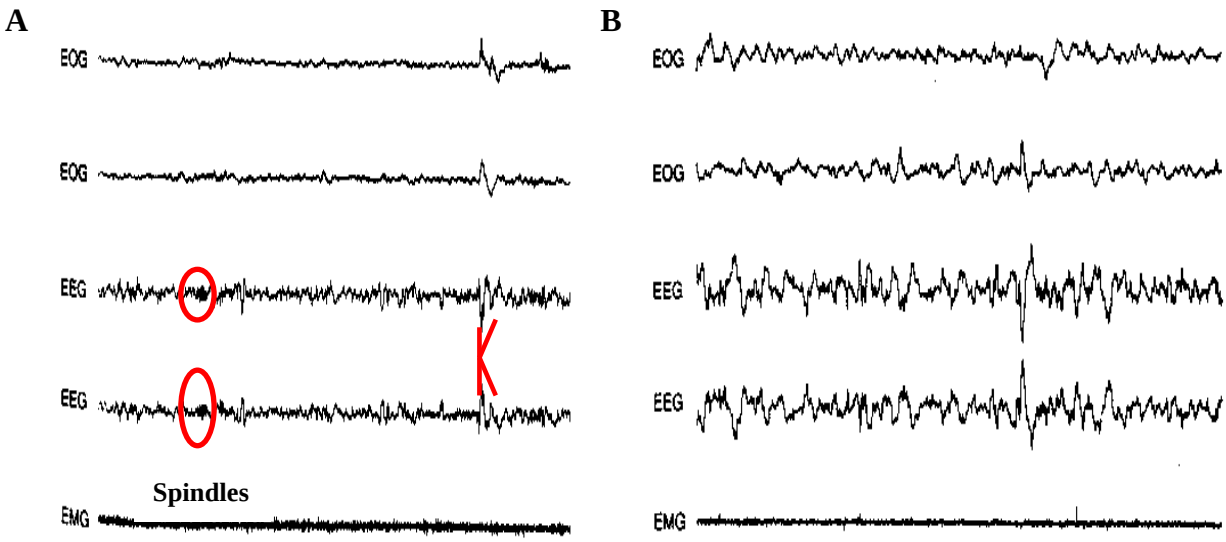


Figure 2.2. A: Sleep stage 2 with theta, alpha waves, a spindle and a K complex, i.e. a negative EEG deflection followed by a positive one, lasting ≥0.5s and with spindle activity riding over the complex. Ks are provoked by auditory stimuli. **B:** Sleep stage 4 consists of >50% delta waves.

REM SLEEP

Desynchronisation of cortical activity during REM sleep results from the abolition of GABAergic inhibition in the thalamocortical network. This transforms the high-amplitude voltage into fast changing rhythms of lower amplitude. These periods constitute the tonic REM sleep. Phasic REM consists of periodic, dream-related, bursts of eye movement and phasic muscle activity [156]. REM sleep is paradoxically similar to wakefulness and it would be indistinguishable if not for the atonic antigravity muscles. Electrodes placed in hippocampal, gyrus dentatus and pyramidal cells have detected synchronised theta wave activity generated by cholinergic neurons [258]. The hippocampal theta waves accompany phasic and tonic REM periods. Despite increased cortical activity, perceptual awareness during REM sleep is lost. Muscle atonia is induced through the hyperpolarisation of brainstem modulated motoneurons.

Pontine and medullar regions are hypothesised to mediate the suppression of muscle tone in REM sleep and in cataplexy, the sudden onset of atonia during wakefulness in narcolepsy patients [200].

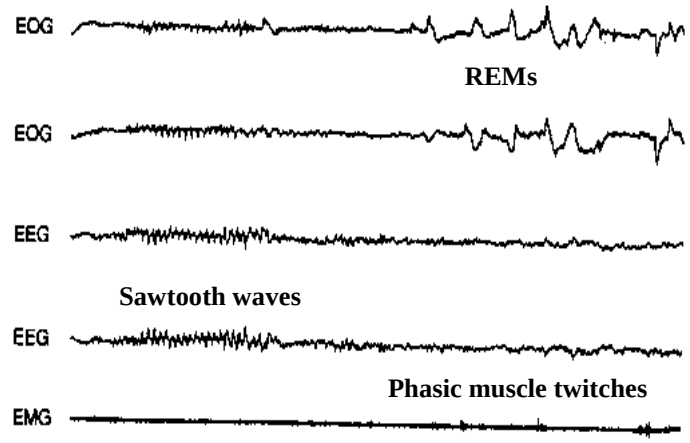


Figure 2.3. Epoch of REM sleep demonstrating sawtooth waves, phasic increase of the muscle tone parallel to rapid eye movements.

Brainstem lesions or diffuse brainstem degeneration are conditions associated with REM sleep behaviour disorder, the "acting out" of REM-related dreams. In infants, the detection of REM sleep is solely based on the eye movements and the phasic muscle activity, as muscle atonia and desynchronised cortical activity are absent.

SLEEP CYCLES AND EFFICIENCY

A healthy sleep pattern in an adult usually consists of 5 cycles, each cycle lasting approximately 90 minutes. Physiologically, the first sleep cycle begins with stage 1 followed by the transition to stage 2, SWS and results in REM sleep [57] (figure 2.4).

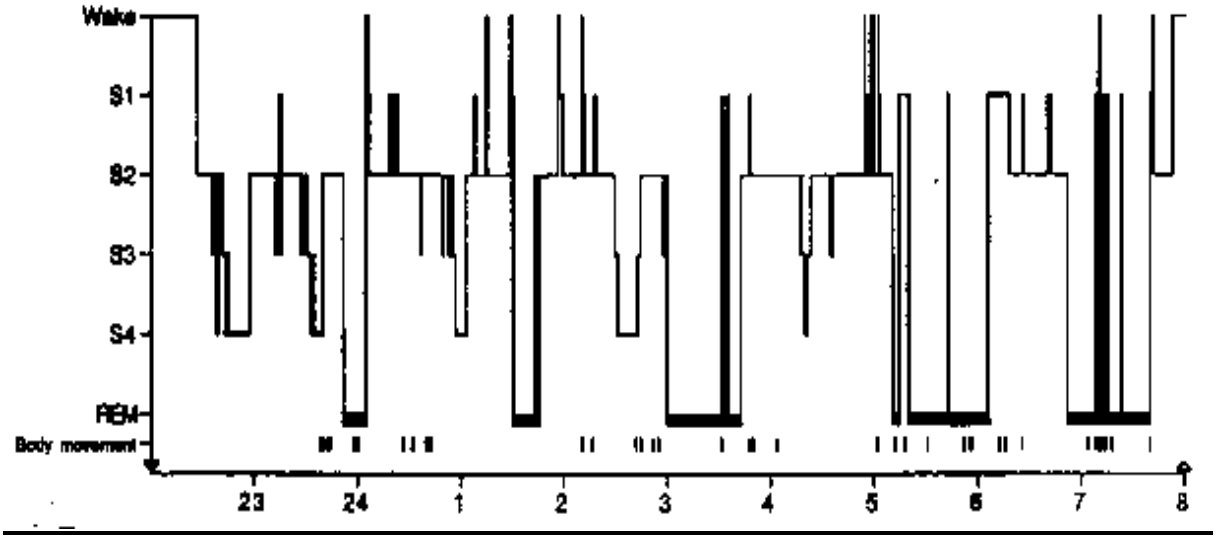


Figure 2.4. A physiological hypnogram of a healthy adult, consisting of 5 sleep cycles, each beginning with sleep stage 1 and progressing to SWS and REM sleep.

Sleep onset is defined as an EEG change to stage 1 accompanied by definite slow eye movements or, the first scored 30-second epoch of sleep stage 2 [57].

Total Sleep Time (TST) is calculated as the total time spent asleep.

Sleep Period Time (SPT) is calculated as the total time between sleep onset and the last epoch of sleep.

Sleep efficiency **[%]** is defined as the ratio between TST and SPT, multiplied by 100 and expressed in percent [%].

$$SE\ [\%] = [TST/SPT] \times 100$$

A healthy adult spends approximately 60% of TST in NREM 1&2, 20% in SWS and 20% in REM sleep.

Time spent in SWS decreases with age or the use of benzodiazepines, whereas tricyclic antidepressants and monoamine oxidase inhibitors tend to suppress REM sleep.

AROUSAL

Wakefulness is initiated spontaneously or through visceral, somatic and sensory afferent impulses. Arousal from sleep is an ordered process that starts in the basal forebrain and pontomesencephalic tegmentum and radiates to the cortex via rostral thalamo-cortical projections, with the posterior hypothalamus being a major contributor [172].

Cortical arousing system: Moruzzi and Magoun`s [244] description of the Ascending Reticular Activating System (ARAS) was the first step in the understanding of the arousal mechanisms. Recent studies have shown that the arousing mechanism consists of various ascending activating systems, located in the upper brainstem, posterior hypothalamus and basal forebrain. Their activation is conditional to the release of acetylcholine, noradrenaline, dopamine, histamine and glutamate [31] and influences the activity of Nc. reticularis thalami, of thalamocortical neurons and the cortex resulting in the suppression of low and promotion of high frequencies (12-80, mean 40Hz) [330]. There is some evidence to suggest sustained inhibition of low voltage waves during SWS subsequent to a stimulus [146]. In OSAHS patients this would mean a sleep stage dependent change of the cortical arousal-threshold following apnoeas or hypopnoeas.

The definitions and scoring criteria of EEG arousals are described in detail in chapter 4.2.

In conclusion, important morphological substrates for the state of vigilance are thalamic, hypothalamic, hippocampal regions which constitute the limbic system, their projections to the cortex in conjunction with visceral, somatic and sensory inputs. The synchronised excitation or inhibition of these substrates, modulated by different neurotransmitters, results in the different levels of cerebral vigilance: arousal, synchronised or desynchronised sleep.
Closely related to the state of vigilance is the autonomic cardiovascular and respiratory regulation due to functional interactions of the above mentioned morphological substrates.

2.3. SLEEP, AUTONOMIC ACTIVITY AND BREATHING

2.3.1 Autonomic Activity

Frontal cortex, basal forebrain and brainstem are morphological substrates involved in the regulation of autonomic activity, of sleep and arousal [320]. Supportive evidence is provided by trans-sectional studies which reversed blood pressure elevations in hypertensive animals [340], studies which increased arrhythmogenesis and sudden death through electrostimulation of the mentioned cerebral regions [366][219] and studies which blocked centrally mediated cardiovascular sympathetic activity through intracerebral injection of propranolol [381].
Homeostasis – continuous adjustment - of the autonomic functions (heart rate, stroke volume, vascular resistance, blood pressure) results from the feedback between internal and external stimuli and inputs from the following entities:
Frontal cortex: engaged in higher cognitive function, learning, memory, speech, higher-order motor control, behaviour, selective attention, processing of sensory inputs and psychosocial stress with intensified autonomic support for possible enhanced requirements upon somatic behaviour, associated with increased cardiovascular risk [320]. To maintain higher cognition, learning and memory, sleep is necessary [70].
Corpus amygdaloideus: With projections from frontal neurones and towards forebrain and brainstem nuclei, this part of the limbic system supports autonomic and lower cognitive functions.
Nucleus tractus solitarii: Primary receiver of visceral sensory afferents, e.g. from sinoatrial, atrioventricular nodes, baroreceptors, projections from and to other brainstem regions and from the frontal cortex.
Nucleus ambiguus: Centre of parasympathetic (vagal) neurons, part of the brainstem.

Vasomotor nuclei: Centre of sympathetic neurones, part of the brainstem; efferents reach organs via paravertebral ganglia.

Baroreflex: Heart rate and blood pressure interact in a reflex manner, the baroreflex. The baroreflex sensitivity (BRS) for heart rate is a marker of the capability to reflexly increase vagal activity and to decrease sympathetic activity in response to a sudden increase in blood pressure. The baroreflex results in a control of heart rate. Baroreflex can often be evoked through external massage of the neck. The arterial baroreflex buffers abrupt transitions of blood pressure. The reflex originates from stretch receptors in the wall of the carotid sinus, the aortic arch, the heart and pulmonary vessels (the 2 latter also being referred to as the cardiopulmonary receptors). Afferent fibres from carotid sinus baroreceptors join the glossopharyngeal nerve and project to the nucleus tractus solitarii in the dorsal medulla, which is under cortical command and in turn projects to efferent cardiovascular neurones in the medulla and spinal cord. The extra-carotid baroreceptors transmit their afferent information along the vagus nerves to the same brainstem nuclei. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibres to the heart and sympathetic fibres to the peripheral blood vessels. There is strong evidence from studies on dogs [153] and in humans [325] to support interaction between chemo- and baroreceptor reflexes at brainstem level.

Baroreceptor reflex sensitivity for heart rate is suppressed in individuals with arterial hypertension [269], heart failure [111], OSA [250] [265] and following myocardial infarction [199] resulting in attenuation of the buffer effect for heart rate with adverse cardiovascular and prognostic consequences [199][72][213][238].

2.3.2. Sleep and Autonomic Activity

Variations in autonomic activity occur during stress, during the transition from wakefulness to sleep and across the different sleep stages. Somers et al studied the haemodynamic changes and peripheral sympathetic activity, measured invasively from the peroneal nerve - the Muscle Sympathetic Nerve Activity (MSNA) - in healthy individuals and found a progressive reduction in sympathetic traffic, heart rate and blood pressure during the transition from NREM 1 to 4, in association with reduced cardiac output and peripheral vascular resistance [323]. During REM sleep MSNA, blood pressure and heart rate returned to levels similar to those during wakefulness. Phasic increases in blood pressure were detected during K complexes in NREM 2 and during phasic muscle twitches and bursts of eye movement during REM sleep, indicating an

attenuated phasic inhibition of the sympathetic efferents - a sign of poikilostatic autonomic regulation.

Cardiac electrophysiology is influenced by the sleep-related decrease of sympathetic traffic resulting in slowing down of the heart rate, slowing of the conduction in the AV node and prolongation of the atrial, AV and ventricular refractory periods [63]. Sinus bradycardia is commonly observed during SWS [299]. These effects suppress arrhythmogenesis and may explain the low incidence of sudden cardiac death found during sleep in contrast to the morning hours [246]. Arousal from sleep may be associated with electrical instability evoked by the increased sympathetic tone and may promote premature ventricular depolarisation and ectopy [211].

In conclusion, sleep is accompanied by a physiological suppression of sympathetic outflow. Disturbed sleep architecture, altered circadian autonomic balance and/or recurrent hypoxic episodes due to OSA [134] or severe Chronic Obstructive Pulmonary Disease (COPD) [316] may predispose to arrhythmogenesis. The overall severity and frequency of arrhythmias is concordant to the pre-existing myocardial dysfunction and the character and severity of the accompanying disorder.

2.3.3. Breathing

The respiratory muscles do not have a built-in pacemaker. Frequency and duration of the respiratory cycle, innervation of the effectors (diaphragm, intercostal muscles, upper airway) and synchronisation between inspiration and expiration are adjusted to mechanical, behavioural, autonomic and metabolic changes in a homeostatic approach, co-ordinated by a neural network which consists of the following entities:

Brain stem:

Dorsal respiratory group -Nc. tractus solitarii: This region influences the phrenic and intercostal motor neurone activity via the spinal cord in response to vagal, glossopharyngeal afferent impulses from the carotid bodies (PaO2 and PaCO2 chemoreceptors), pulmonaly and tracheobronchial stretch receptors [30].

Ventral respiratory group -Nc. ambiguus, Nc. retroambigualis, Botzinger cells: Efferent fibres innervate the upper airway along with trigeminal, facial, hypoglossal and vagal neurons and interact with afferent fibres from stretch receptors in the lung and upper airway.

Ventral medullary centre -Nc. paragigantocellularis: Provides tonic excitatory response to changes in the pH of the cerebrospinal fluid [53].

Pontine respiratory group: Exhibits inspiratory, expiratory and phase-spanning activity in conjunction with autonomic, somatic sensory afferents and forebrain structures related to emotional behaviour (crying, laughing, speaking) and change in vigilance [30].

Chemoreceptors: Adjustment of respiration in response to alterations in oxygen levels, carbon dioxide and hydrogen ions in the body fluids are mediated by central and peripheral chemoreceptors. The peripheral arterial chemoreceptors, located in the carotid and aortic bodies**,** are responsible for the immediate ventilatory and arterial pressure changes during acute hypoxia. They contain glomus (type I) cells, which release neurotransmitters in response to hypoxia, causing depolarisation of afferent nerve endings [285]. Peripheral chemoreceptors also play a minor role in the sensing of changes in arterial PaCO2 and pH. Sensory fibres from the carotid and aortic bodies course through the glossopharyngeal and vagus nerve respectively towards medullary centres, including the nucleus tractus solitarii. Central chemoreceptors located in the ventrolateral medulla respond to changes in the hydrogen ion concentration in the cerebral interstitial fluid and are responsible for ventilatory and circulatory adjustments during hypercapnia and disturbances of acid-base balance.

Lung stretch receptors: Respond to inflation, deflation, irritation and congestion of blood vessels. Afferent sensory fibres transmit their information centrally through the vagus nerve.

Muscle activity: The upper airway does not have rigid support to be held open. Its patency results from the balance between a number of forces acting in opposing directions. Forces which tend to close the airway, i.e. gravitational and surface adhesive forces, interact with tonic and phasic (synchronous with inspiration) dilatory forces. The genioglossal muscle is the most active phasic dilator contra-acting the dorsal movement of the tongue, the tensor veli palatini is the tonic dilative muscle. The latter is innervated by the mandibular branch of the trigeminal nerve, the genioglossal muscle by the hypoglossal nerve.

Haemodynamics: The respiratory cycle is associated with oscillations in heart rate (HR) and blood pressure (BP), resulting from physiological haemodynamic changes. During inspiration, the decline in intrathoracic pressure (negative pressure values) induces an increase in venous return, resulting in increase in HR, BP and cardiac output (CO). Expiration is associated with increase in intra-thoracic pressure towards zero and decrease in HR, BP and CO. The interplay between respiration and haemodynamics is regulated through the baroreflex loop.

2.3.4. Sleep and Breathing

Changes in respiration during sleep are concomitant with changes in cerebral activity, muscle tone and receptor sensitivity.

Neuromechanical changes: Sleep onset is associated with a generalised muscle relaxation, which includes the muscles in the oral cavity, the oro- and hypopharynx. The tone of the palatal muscles, including tensor veli palatine, and the tongue-controlling genioglossal muscle decrease during NREM sleep and decrease further during REM sleep resulting in reduced patency of the retropalatal and retroglossal regions [161]. The sites of inspiratory narrowing within the upper airway during sleep are therefore either at the level of the palate or the hypopharynx – quantitative differences in the degree of narrowing occur between individuals.

As the genioglossal negative pressure reflex diminishes during sleep, inspiratory resistance increases and minute ventilation decreases [86] despite increased ribcage and diaphragmatic muscle activity and prolonged ventilatory effort [341]. Studies of the palatoglossal and levator palatini muscle tone during NREM sleep have shown that although the tone decreases during sleep stage 2, during SWS the tone increases with the palatoglossal peak inspiratory activity returning to near waking levels [343].

Tabachnik et al studied the intercostal and diaphragmatic muscle activity during sleep in healthy adolescents and found that transition from wakefulness to NREM sleep is associated with increased activity of the intercostal and diaphragmatic muscles [341]. REM sleep is associated with a decrease in intercostal muscle activity and a diminished rib cage contribution, possibly related to supraspinal alpha motoneuron inhibition, but an increase in diaphragmatic tone. The decreasing negative pressure during inspiration along with increased inward forces within the upper airway during sleep contribute to the narrowing of the oropharynx. Collapse occurs if the negative airway pressure exceeds the dilatory muscle forces. The narrower the airway, the higher the airway resistance. Resistance-related arousals, a sign of ventilatory response to increased inspiratory resistance, are less frequent from SWS than from stage 2 or REM sleep in healthy individuals [131]. These findings have been confirmed in OSAHS patients by Berry et al, who found that the increase in inspiratory resistance correlated with the increase in delta power on the EEG, i.e. the airway patency threshold during SWS was higher than in NREM1&2 or REM sleep [32].

Chemical/ metabolic changes: The ventilatory response to hypoxia and hypercapnia is reduced during NREM sleep and reduces further during REM sleep compared to wakefulness

[34][87][88]. These findings may contribute to the observed longer apnoeas during REM sleep [60], which often are associated with more severe hypoxia. An increase in minute ventilation with or without associated brief arousal may represent a response to hypoxia. However, the studies by Berthon-Jones [34] and Douglas [87] have shown that isocapnic hypoxia is a poor stimulus to arousal, with many healthy individuals remaining asleep despite oxygen saturation values as low as 70%. No differences between REM and NREM sleep were noted during these studies in the relationship between oxygen saturation and arousal. A response to hypoxia may occur at oxygen levels so low that when acute, they pose a risk of tissue damage. Hypoxia increases the sensitivity to hypercapnia - a combination of both possibly reduces arousal threshold and may explain the REM-related arousals in COPD patients [108].

The tolerance to hypoxia and hypercapnia may in part explain the observed reduction in minute ventilation during sleep [86]. Ventilatory chemosensitivity worsens in sleep-deprived healthy individuals [368]. The impaired ventilatory response may account for the development of hypoventilation and hypoxaemia not only in sleep apnoeics but also in patients with COPD [83] or patients with restrictive defect due to cystic fibrosis [247] or kyphoscoliosis [230]. Also in these conditions, hypoxia is most marked during REM sleep.

The cerebral blood flow during sleep in healthy individuals has been studied by Townsend et al [352]. The group observed an increase in cerebral blood flow during REM sleep and a reduction during SWS, when compared to wakefulness. These changes can not be entirely attributed to the sleep-induced changes in oxygen or carbon dioxide concentration. Sleep-related changes in blood flow are most likely related to changes in brain metabolism and/or neurogenic control.

Minute ventilation: Respiration is profoundly affected by sleep, with state-related decreases in minute ventilation and changes in breathing pattern. Minute ventilation during the transition from wakefullness to sleep and during the different sleep stages in healthy individuals, has been studied by Douglas et al [86]. Minute ventilation falls during sleep, with progressive reduction from stage 2 to SWS and further reduction during REM sleep. These changes are associated with increase in frequency and reduction in flow, resulting in rapid and shallow breathing – more so during REM sleep. These observations are in agreement with the previously described findings by Bülow et al [54]. During the same study, Douglas et al [86] measured the end-tidal partial oxygen (PO_2) and carbon dioxide (PCO_2) tensions of the expired flow and concluded that as a result of the diminished minute ventilation there is a steady and significant decline in the PO_2 and a rise in the PCO_2.

In animals, observational studies showed different pattern to humans. In cats breathing becomes deeper and slower during NREM sleep and more rapid and shallow during REM sleep with an overall reduction in minute ventilation [261].

Diminished chemo- and mechano-receptor sensitivity during drowsiness results in fluctuations of the breathing amplitude, which occurs in approximately 60% of healthy individuals and may persist until the onset of stable sleep. This pattern is known as periodic breathing and may be manifested as Cheyne-Stokes breathing or Biot's breathing.

Cheyne-Stokes breathing: A progressive decrease in breathing amplitude before an apnoea or hypopnoea followed by a progressive increase.

Biot's breathing: A progressive decrease in breathing amplitude before an apnoea/hypopnoea followed by an abrupt increase thereafter [54].

Apnoeas are defined as a complete cessation of airflow lasting 10 seconds or longer [141].

Hypopnoeas are defined as a clear amplitude reduction of more than 50%, of a valid respiratory measure – thoracoabdominal movement or airflow - compared to the amplitude during the previous 2 minutes, lasting 10sec or longer [126]. In 1999, the Task Force of the American Academy of Sleep Medicine issued a report on scoring criteria and definitions [5]. According to this report, if the >50% amplitude reduction criterion on a valid measure of breathing is not fulfilled, the reduction should be associated with either oxygen desaturation of >3% or an arousal.

The requirement of 10 seconds as part of the apnoea and hypopnoea definition is somewhat arbitrary. In children, due to the differences in functional residual capacity and chest compliance, oxygen desaturations occur more rapidly. Consequently, paediatric sleep centres define apnoeas or hypopnoeas if these occur with 2 or more consecutive breaths even if they last less than 10 seconds.

The definitions used in this thesis refer to the adult population.

Based on their pattern, there are 3 types of apnoeas/hypopnoeas:

Obstructive: There is a cessation/reduction of the airflow whilst the respiratory effort is preserved or increased. This is the most commonly found abnormal breathing during sleep and will be extensively discussed in this thesis.

Central: There is a cessation/reduction of both, the airflow and the respiratory effort – the latter is measured through oesophageal manometry. In healthy individuals, central apnoeas/hypopnoeas may occur during the transition to sleep. They are common in heart failure patients, often in form of Cheyne-Stokes respiration. Central apnoeas/hypopnoeas will not be discussed in this thesis.

Mixed: There is a cessation/reduction of the respiratory effort which normalises midway through the event followed by a cessation/reduction of the airflow.

Haemodynamic changes: During sleep, the breathing-related oscillations in heart rate and blood pressure decrease progressively from NREM 1 to 4. During REM sleep they return to levels similar to those during wakefulness [323].

In conclusion, there is morphological and functional overlap between sleep, ventilation and cardiovascular autonomic activity. Sleep is associated with decrease in baro-, chemo- and mechanoreceptor sensitivity (figure 2.5). These are of major importance for the genesis, the pathophysiology and the long-term consequences of the OSAHS.

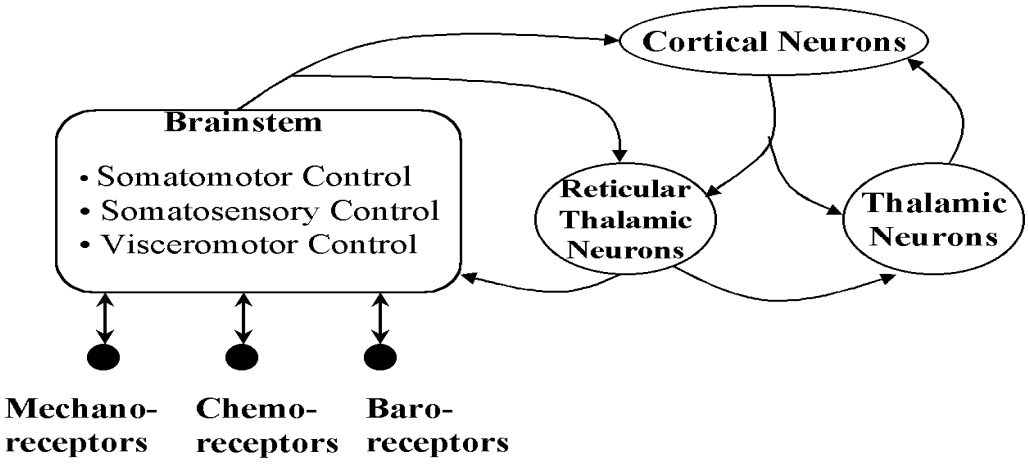


Figure 2.5. Key elements of the functional and morphological interactions between sleep, autonomic activity and breathing.

CHAPTER 3

OBSTRUCTIVE SLEEP APNOEA HYPONOEA SYNDROME

3.1. HISTORICAL REVIEW

One of the first descriptions of the syndrome in the medical literature, dates back to 1877 in an article by WH Broadbent, a London physician, published in the Lancet [48]. In this article, the author gave a detailed description of the clinical presentation and sequence of events.

In the year 1919, Sir William Osler used the term “Pickwick Syndrome” to describe the obese and hypersomnolent patient, who resembled C. Dickens’s “Joe the fat boy” from The Pickwick Papers (1837) (figure 3.1).

In the years 1965/66 Gastaut et al [119] and independently Jung et al [175] published the first studies on breathing during sleep in Pickwickian patients. Both authors described repetitive apnoeic episodes associated with oxygen desaturations, frequently followed by EEG arousals which disrupted sleep and caused excessive daytime sleepiness.

During a landmark symposium organised by Sadoul and Lugaresi in 1972 [306], sleep apnoeas were recognised as the cause of the disease which was graded into different levels of severity. It was recognised that only the more severe forms are associated with the Pickwickian characteristics, i.e. obesity, hypoventilation, polycythaemia or right heart failure.

Figure 3.1. Samuel Pickwick is a retired businessman, founder and chairman of the Pickwick Club. Along with his friends, he travels around England in search of adventure. One of the characters described in The Pickwick Papers is Joe the fat boy, who is a servant and has an amazing ability to fall asleep anytime, unless he's eating. Joe is red-faced (plethoric), has dropsy (peripheral oedema), snores heavily “as if roaring of cannon were his ordinary lullaby” and his perception is slow (cognitive impairment).

3.2. EPIDEMIOLOGY

Recording of the OSAHS prevalence is important for the understanding of the disease burden, the allocation of appropriate health care resources, the recognition of associations and etiological factors and the identification of high risk groups aiming at targeted diagnosis and treatment.

Outcomes of prevalence studies may vary significantly due to methodological issues including selection bias of the populations studied or the use of different diagnostic criteria.

The use of more restrictive apnoea/hypopnoea definitions, higher AHI cut-points or an additional requirement for symptoms of sleepiness lower prevalence estimates and affect values expressing associations.

Following careful consideration of methodological differences in the selection process and the diagnostic criteria, a meta-analysis of 12 epidemiological studies conducted by Davies et al concluded that the OSAHS affects 1-5% of men, 1-2% of women worldwide and increases in proportion to the degree of obesity [76].

Some of the important studies carried out in large community cohorts using universally accepted definitions are discussed below.

One of the most comprehensive surveys of the OSAHS prevalence is based on data from the Wisconsin Sleep Cohort Study, initiated in 1988 to address the public impact of sleep related breathing disorder [378]. This prospective population-based study used polysomnography to investigate the epidemiology and severity of OSA among middle-aged men and women and to assess age, sex and obesity as risk factors. A random sample of 602 state employees, 352 male, aged 30-60 years, was included in the study. An AHI $\geq$5/hr slept on polysomnography and daytime sleepiness were the diagnostic criteria. The overall prevalence in the middle-aged work force was 3% (n=19); 4% among men (n=14) and 2% among women (n=5) with a male:female ratio of approximately 3:1. The prevalence of OSA (AHI$\geq$5/hr) regardless of the presence or absence of hypersomnolence was prevalent in 21% (n=128) of the population (16% in men, n=97; 12% in women, n=31). Using a diagnostic cut-off of AHI$\geq$15/hr and regardless of the presence or absence of sleepiness, the prevalence was 9% (n=54; men: 12%, n=42; women: 5%, n=12). Multiple logistic regression analysis confirmed that male sex and obesity in people aged 30 to 60 years, are important risk factors for the OSAHS.

Bixler et al assessed the effect of gender and age on the prevalence of the OSAHS (AHI≥10/hr and clinical symptoms) in 741 men and 1000 women randomly selected, aged 20 to 100 years [38]. The prevalence among men was 3.9%, among women 1.2%, yielding a men:women ratio of 3.3:1. These findings are similar to the epidemiological observations by Young et al in the Wisconsin Cohort [378]. Bixler et al [38] found that premenopausal women and women on Hormone Replacement Therapy (HRT) had similar prevalence (0.6% vs. 0.5%), whereas the prevalence in postmenopausal women not on HRT was 2.7%, i.e. comparable to that among men. A further study by the same group examined the effects of age on the prevalence of OSAHS (AHI>10/hr plus symptoms) among men, aged 20 to 100 years [36]. A peak prevalence of 4.7% was found in men between 45 and 64 years.

The Sleep Heart Health Study cohort, consisting of 5615 men and women aged between 40 and 98 years, was used to assess cross-sectional associations of selected characteristics with OSA - defined as an AHI≥15 [380]. Multiple logistic regression modelling confirmed that male gender, age (40-79 years), BMI (> 30kg/m^2), waist-to-hip ratio (>1), neck circumference (>42cm), snoring and reported frequent breathing pauses are significant independent correlates of OSA.

These large cohort studies confirm that OSA is strongly associated with obesity, male sex and age, with a peak prevalence in middle-aged men. The effects of progesterone (and estrogen) on the genioglossal activity [283], ventilatory stimulation, chemosensitivity [383] and body fat distribution [351] are the likely causes of gender and menopause-related differences.

In summary, OSA is a common disease which remains underdiagnosed and undertreated and is therefore a public health issue. To date no epidemiological data are available on the proportion of undiagnosed patients who would benefit from treatment.

3.3. SYMPTOMS AND ASSESSMENT

DAYTIME SYMPTOMS

Daytime sleepiness is the cardinal symptom, reported in around 87% of OSAHS patients surveyed by Whyte et al [370]. Excessive daytime sleepiness among OSAHS patients is associated with impaired concentration, impaired performance and difficulties learning new tasks at work [353].

Sleepiness is becoming acknowledged as the most common cause of accidents on major highways [365]. Road traffic accidents (RTAs) are becoming the third leadining cause of death worldwide [248].

Studies have shown that OSAHS patients are at higher risk of having a RTA than age and gender matched controls [21][345][121]. In a prospective case control trial, Barbe et al compared the number of RTAs in 60 OSAHS patients (AHI>20/hr) with age and gender matched controls during a 3-year period prior to the diagnosis of the syndrome [21]. Data were obtained from both, the participants and their insurance companies. OSAHS patients were more likely to have had more than 1 accident during the 3-yr period than their controls (OR:5.2; 95% CI:1.07-25.29, $p<0.05$). The increased risk was independent of the number of kilometres driven, the patients' age or average alcohol consumption. Disease severity – based on the AHI, ESS, mean SaO2 [%], anxiety and depression scores, driving simulator performance - did not correlate with the risk of RTAs.

In a further case-control study, Teran-Santos et al assessed the prevalence of OSA (AHI$\geq$10/hr) among 102 drivers presented to the Accident and Emergency departments of 2 Spanish hospitals following a RTA and compared it to the prevalence in 152 age and gender matched controls [345]. After adjusting for the annual number of kilometres driven, alcohol consumption, age and years of driving, OSA was found to be associated with increased risk of RTAs (OR:7.2; 95% CI:2.4-21.8).

Many professional drivers are men between the age of 40-60 years, i.e. the age and gender where the OSAHS is most prevalent [380]. Among 159 long-haul truck drivers studied by Stoohs et al, 77% (n=123) had an ODI$\geq$5hr, 46% (n=73) had an ODI$\geq$10/hr and 10% (n=15) had severe OSA (ODI$\geq$30/hr). Based on a questionnaire used to assess the drivers' perception of symptoms, only 3% considered themselves as being hypersomnolent [331]. A study by the same group confirmed that commercial truck drivers diagnosed with the OSAHS have a 2-fold higher accident rate per mile than non-OSAHS drivers [333]. The findings demonstrate that the OSAHS may be more prevalent among professional drivers due to the characteristics of this subgroup, i.e. age, gender and body habitus [333]. Sleepy drivers may underestimate and underreport symptoms of sleepiness. Detailed history taking and questions regarding sleepiness whilst driving as well as the rapid and cost-effective diagnosis and treatment in this group of patients is therefore paramount.

The most commonly used tools to assess subjective and objective daytime sleepiness, are the Epworth Sleepiness Scale, the Multiple Sleep Latency Test and the Maintenance of Wakefulness Test.

The Epworth Sleepiness Scale (ESS) is a widely used self-assessing questionnaire which evaluates the degree of the patients' subjective perception of sleepiness in passive and active situations. In the original validation study control subjects (n=30) without sleep disorders all had an ESS $\leq$ 10 and among OSAHS patients the severity of the disorder correlated with the ESS [170]. In the study by Johns et al, an ESS score of 10 has been reported to be 2 SDs above the mean in healthy individuals and therefore a score of greater than 10 is commonly used to define significant sleepiness. However, a larger study found that healthy controls had ESS scores in the range of 0 -14 (n=188) [268].

Sleepiness is not always described as "excessive" or "abnormal" by the patient either because it is not perceived as such or due to the potential consequences that the admittance of sleepiness may have on the patient's work or driving ability [91]. Careful interview of the patient, the bed-partner [223] or other family members usually provides a detailed description of the symptoms, sleep hygiene and may exclude other disorders such as PLM, narcolepsy, post-traumatic or idiopathic hypersomnia or hypothyroidism.

Objective daytime sleepiness is measured using the Multiple Sleep Latency Test (MSLT) or the Maintenance of Wakefulness Test (MWT). The MSLT evaluates the sleep onset time during 4 or 5 20-minute periods at 2 hour intervals, while individuals are asked to avoid resisting sleep [58]. Abnormal daytime sleepiness is confirmed through a mean daily score of <5min. Non sleepy adults score 10-20 minutes; scores between 5-10 minutes are the diagnostic grey area. The MWT evaluates the ability to resist sleep during 4 or 5 40-minute periods at 2 hour intervals. Sleep latencies $\geq$20min are considered normal [81].

Impaired cognition, concentration, memory, manual dexterity and changes in mood and personality are frequent symptoms in OSAHS patients, detected through psychometric and cognitive function tests [60]. Morning headaches, irritability, depression and sexual dysfunction are also common. These symptoms have long-term effects on the patients' quality of life and may predispose to impaired work performance [353] with financial and social implications.

Questionnaires commonly used to assess well-being are the generic Sort Form 36, the Hospital Anxiety and Depression Scale and the non-generic Functional Outcomes of Sleep Questionnaire.

The Short Form 36 (SF-36) is a generic, disease non-specific health status questionnaire, which measures the self-assessed quality of life. It consists of 36 questions and addresses 8 important

aspects: physical and social function, general and mental health, extent of limitations linked to physical and mental impairment, vitality and pain [362]. Bennett et al conducted a correlation analysis between AHI, arousal index and autonomic changes (detected through the Pulse Transit Time), markers of objective and subjective sleepiness as well as markers of energy and vitality – using the SF-36 questionnaire- before and after CPAP treatment in sleep apnoea patients [28]. Although the correlation was not close, it was significant and the markers improved with treatment.

The Functional Outcomes of Sleep Questionnaire (FOSQ) is a non-generic disease specific questionnaire, which measures how sleepiness affects daily functioning and quality of life [364]. The FOSQ consists of 30 questions and addresses 5 areas: general, social and sexual activity, productivity and vigilance. The mentioned questionnaires are the most commonly used to identify patients requiring treatment, to monitor improvement and treatment compliance as well as for across-disease comparisons. Scores should be interpreted with caution, as some patients may under- and others overestimate their symptoms.

In this thesis we used the non-generic ESS to recruit symptomatic sleepy patients [Appendix].

NOCTURNAL SYMPTOMS

Sleep-related symptoms may be snoring, choking, gasping, restlessness with increased turning and tossing, PLM, sweating, nocturia, excessive salivation, gastro-oesophageal reflux and frequent arousals, occasionally perceived and described by the patient as insomnia.

3.4. CLINICAL EXAMINATION

The clinical examination begins with the recognition of the body habitus and inspection of the upper airway.

OSAHS patients are usually obese with a thick, short neck. Obesity is defined as a BMI greater than 30 kg/m^2 (BMI= weight/$[height]^2$). Central obesity - abdominal and chest wall fat assessed by the waist circumference and the waist-hip ratio - may impair respiratory muscle activity and reduce lung volume [129]. Fatty infiltration of the neck and throat – soft-palate, uvula, lateral to

the pharynx between pterygoid muscles and carotid arteries – and pharyngeal oedema with enlarged soft palate and uvula induce changes in upper airway structure and function. The patients' neck circumference is often greater than 42 cm [380].

Macroglossia associated with acromegaly, hypothyroidism or amyloidosis, hypertrophied tonsils and craniofacial dysmorphism such as retro- or micrognathy (Pierre-Robin Syndrome) may contribute to the narrowing of the oro- and hypopharyngeal region. Obesity [337] and disproportionate craniofacial anatomy [217] are to a large extent genetically determined factors predisposing to the familial aggregation of the OSAHS. Further genetic disorders associated with the OSAHS are Marfan's, Trisomy 21 and Prader-Willi Syndrome.

3.5. DIAGNOSTIC CRITERIA

The diagnosis of the OSAHS is based on the combination of clinical symptoms and the outcomes of overnight monitoring [370][5].

Based on the recommendations of the American Academy of Sleep Medicine published in 1999, the clinical symptoms which constitute the syndrome are [5]:

1. excessive daytime sleepiness not explained by other factors OR
2. at least two of the following: chocking or gasping during sleep
   recurrent awakenings from sleep
   unrefreshing sleep
   daytime fatigue
   impaired concentration

The diagnostic criteria for nocturnal abnormalities have been modified over the years. In 1976, the diagnostic requirement was $\geq$30 apnoeas over a 7-hour sleep period [141]. In 1983 the diagnostic threshold increased to 10 apnoeas per hour slept [203]. In 1988, Gould et al added the scoring of hypopnoeas to the diagnostic criteria [126]. An Apnoea-Hypopnoea-Index (AHI), i.e. the sum of apnoeas and hypopnoeas divided by the hours slept, of 15/hr slept was the proposed diagnostic threshold.

In 1999 the Task Force of the American Academy of Sleep Medicine proposed a reduction of the diagnostic threshold to 5/hr slept [5].

Based on these recommendations, there are 3 severity groups:

Mild OSA: 15 > AHI > 5

Moderate OSA: 15 ≥ AHI ≥ 30

Severe OSA: 30 > AHI

Currently, the diagnostic AHI threshold varies between 5 and 15/hr slept across sleep centres.

At the mildest end of the range is simple snoring. Guilleminault et al found decreased daytime alertness in snorers [140] suggesting that snoring is not just a social nuisance but has pathophysiological implications and clinical consequences. In a further study, the Stanford group used oesophageal manometry to demonstrate periods of increased intrathoracic pressure linked to flow limitation and increased upper airway resistance, which failed to meet the apnoea or hypopnoea criteria [139]. These episodes are associated with transient EEG arousals resulting in daytime sleepiness and were therefore named the Respiratory Effort Related Arousal (RERA). According to the recommendations of the American Academy of Sleep Medicine [5], their scoring along with apnoeas/hypopnoeas may contribute towards the diagnosis of the OSAHS. Alternatively, scoring of >5 RERA/hr slept in the absence of apnoeas/hypopnoeas plus clinical symptoms, constitute the Upper Airway Resistance Syndrome (UARS). The UARS as a distinct entity is not widely recognised [82].

In conclusion, the OSAHS is a complex of signs and symptoms resulting from the occlusion/narrowing of the upper airways. Controversy still exists over the diagnostic threshold of polysomnographic findings when conventional analysis – visual scoring- is used. Part of this thesis will explore the potential of non-conventional signal analysis in the assessment of OSAHS patients.

3.6. RECORDINGS

POLYSOMNOGRAPHY

Polysomnography (PSG) is the classical nocturnal recording (figure 3.2), carried out in sleep laboratories. The limited number of sleep centres and beds often results in long waiting lists for diagnosis and treatment. PSG consists of the following parameters (table 3.1):

Function	Biosignal
Sleep/Awakenings	EEG, EOG, EMG of chin muscle
Cardiovascular system / Autonomic activity	ECG (three leads): Heart rate & regularity
	Blood pressure (not standard)
	PTT (not standard)
Movement	EMG of calf, Body position
Breathing	Airflow
	Respiratory movement of thorax & abdomen
	Snoring detection
	Blood oxygen saturation level (SaO_2)
	Oesophageal manometry (not standard)

Table 3.1. EEG: Electroencephalography, EOG: Electrooculography, EMG: Electromyography, ECG: Electrocardiography. PTT: PulseTransitTime.

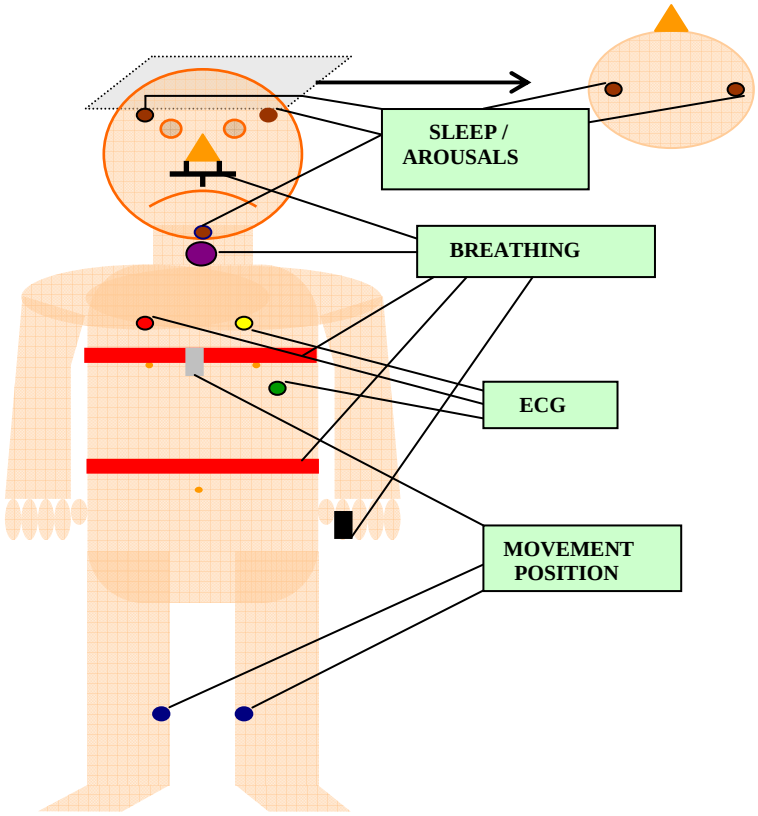


Figure 3.2. Application of the tools required for the polysomnography. These consist of: EEG electrodes, a chin EMG electrode, digital microphone, ECG electrodes, thoracic and abdominal wall movement detectors, body position detector, calf EMG electrodes and a pulseoximeter.

PORTABLE DIAGNOSTIC DEVICES

The OSA diagnosis can also be home-based. There are many commercially available portable devices, which differ in their diagnostic validity, configuration and price. Existing devices require expertise in scoring and evaluation of the outcomes, so most are in the hands of

specialised staff. The devices can be divided into 3 categories, based on their monitoring features, diagnostic validity and price (table 3.2). Cost-effective, user-friendly portable system can simplify diagnosis, reduce waiting lists and related costs.

Characteristics	**Category 1**	**Category 2**	**Category 3**
Features	Breathing monitoring	PSG	PSG
Number of tracings	1-6	7-8	$\geq$ 12
Biosignals	Airflow	Airflow	EEG,EOG,EMG
	Respiratory sound	Respiratory sound	Airflow
	SaO2	SaO2	Respiratory sounds
	Heart rate	ECG	SaO2
	Respiratory movements	Respiratory movements	ECG
	Body position	Body position	Respiratory movements
			Body position
Diagnostic validity	Low to moderate	Moderate to good;	Very good.

Table 3.2. Categorisation of the portable diagnostic devices.

Home-based sleep studies have potential advantages in terms of costs [369], patients' convenience as well as sleep quality compared to hospital based studies. A study by Kingshott et al compared the sleep quality of in-hospital to home-based studies and found that patients studied at home had better sleep efficiency with less arousals and had spent more time in REM and SWS [187].

The expenses related to the purchase and operation of portable devices increase with the number of biosignals. Portable devices with many biosignals require specialised staff for their application and data analysis and are therefore usually applied in specialised centres.

Less sophisticated portable devices provide different levels of diagnostic validity depending on the number of biosignals and the disease severity.

Pulseoximetry, a single channel screening tool, uses the oxygen desaturation index (ODI) as diagnostic criterion. In 1990, the British Thoracic Society (BTS) defined the diagnostic threshold for OSA as at least fifteen 4% desaturations per hour in bed (ODI $\geq$ 15/hr bed) [47]. In a prospective study, Ryan et al. assessed the diagnostic accuracy of the ODI criteria set by the BTS [304]. An AHI > 15/hr slept on polysomnography was used as the objective measure of disease.

The authors found that the BTS criteria are highly specific when positive (100% specificity) but may miss sleep apnoeics with no significant desaturations (31% sensitivity).

Pulseoximetry has good diagnostic validity in severely ill patients with significant oxygen desaturations, but not in mild or moderately ill patients.

It is important to validate every new diagnostic device against polysomnography, which is the diagnostic "golden" standard. Part of this thesis will examine the diagnostic accuracy of a portable diagnostic device, the Embletta (Flaga, Iceland).

The recording of airflow and thoracoabdominal movement may vary across the different recoding systems. The most frequently used and most important tools for the purposes of this thesis are explained below:

CHEST AND ABDOMINAL MOVEMENT RECORDINGS

Mercury strain gauges are placed around the chest and/or abdomen. The sensor, mercury in silastic tubing, covers only a fraction of the gauges' surface. Calibration is difficult, consequently the tracing is more qualitative than quantitative.

Piezoelectric belts are placed around the chest and/or abdomen. The sensor covers only a fraction of the belts' surface. The belts cannot be calibrated, the tracing is more qualitative.

Electrical impedance pneumography uses 2-3 electrodes attached to the patient's chest wall, similar to the 3-lead ECG. The signal is a qualitative indication of chest-wall movement.

Inductance plethysmography is accomplished by placing elastic belts around the chest and/or abdomen. The detectors are thin parallel wires along the surface of the belts. Calibration usually provides a reliable quantitative signal except in morbidly obese people.

AIRFLOW RECORDINGS

Thermistor and thermocouples detect oronasal airflow sequence based on temperature differences between inhaled and exhaled air. The devices cannot be calibrated, the tracing is therefore qualitative.

Nasal cannulae connected to a pressure transducer system and corrected for their nonlinear pressure-flow relationship provide a quantitative measure of nasal but not oral flow [236].

Pneumotachometer connected to a snug-fitted face mask measures tidal volume.

SCORING

Sleep staging is based on the R&K classification [288], as described in chapter 2.

Arousal scoring is based in most sleep centres on the guidelines proposed by the American Sleep Disorders Association (ASDA) [9]. The guidelines enable the scoring of shorter awakenings and therefore improve the R&K ruled arousal scoring, which is based on awakenings longer than 15 seconds. The ASDA arousal definition includes all episodes of abrupt shift in cortical activity of 3 seconds or longer, which may include theta, alpha and/or frequencies greater than 12 Hz but no spindles. Arousals during NREM sleep are scored irrespective of a concomitant increase in EMG activity. During REM sleep a transient rise in EMG activity, no matter how brief, is part of the arousal definition. The ASDA definition succeeded in identifying shorter, physiologically important arousals.

Mean arousal frequency in healthy individuals is 21 per hour slept, when brief arousals (i.e. longer than 3 seconds) are included. Arousal frequency increases with age [218].

Apnoeas are defined as a complete cessation of airflow lasting 10 seconds or longer [141].

Hypopnoeas are defined as a clear amplitude reduction of more than 50%, of a valid respiratory measure – thoracoabdominal movement or airflow- compared to the amplitude during the previous 2 minutes, lasting 10sec or longer [126]. Gould et al found that the reduction in thoracoabdominal amplitude – measured by inductance plethysmography -correlated better with the number of arousals and $\geq$4% oxygen desaturations, than did the reduction in airflow – measured by thermocouples [126].

Because different sleep laboratories have been using different hypopnoea definitions as well as different tools to monitor thoracoabdominal movement and airflow, in 1999, the Task Force of the American Academy of Sleep Medicine issued a report on scoring criteria and definitions [5]. According to this report, if the >50% amplitude reduction criterion on a valid measure of breathing (pneumotachometer or 2 independent techniques to allow for sensor failure) is not fulfilled, the reduction should be associated with either an oxygen desaturation of >3% or an arousal.

Obstructive events are the commonest sleep-related breathing disorder and the main focus of this thesis.

CHAPTER 4
PATHOPHYSIOLOGY OF THE OBSTRUCTIVE SLEEP APNOEA HYPONOEA SYNDROME

The major pathophysiological aspects of the OSAHS are:

- upper airway mechanical effects
- central nervous system implications
- autonomic, haemodynamic and neurohumoral effects

4.1. MECHANICAL EFFECTS

In the section on respiratory physiology in chapter 2, I described the balanced interplay between the different muscle groups that allows the upper airway to remain patent during negative inspiratory pressure. During inspiration, especially in the supine position, the increased negative pressure along with increased adhesive forces contribute to the narrowing of the oropharynx on the basis of the Bernoulli phenomenon (for a given pressure difference between the oropharynx and the lungs, the rate of flow and inward forces increase as a tube narrows). During sleep, as muscle tone and dilatory forces decrease, dorsal movement of the tongue is induced and the Bernoulli phenomenon becomes more marked [161][341]. Collapse of the upper airway occurs if the negative upper airway pressure generated by the inspiratory pump muscles exceeds the dilatory force of the muscles. The genioglossal force counteracts the decrease in pharyngeal pressure and oropharyngeal collapse. This is supported by the observations of Remmers et al, who studied the genioglossal EMG activity during sleep in 10 sleep apnoeics and found a periodic increase at the end of the apnoeic cycle, inducing occlusion release [292]. During sleep, gradual decline in genioglossal activity was associated with progressive increase in pharyngeal resistance to a point of pharyngeal occlusion. Carrera et al studied the genioglossal structure and function in sleep apnoeics before and after initiation of treatment and compared them to healthy individuals [56]. The authors found differences in the histology of the muscle, which was associated with greater fatigability amongst sleep apnoeics compared to healthy individuals; initiation of CPAP treatment corrected these changes. Bradley et al compared the upper airway

of healthy controls to that of OSAHS patients using acoustic reflection techniques and found anatomical and physiological differences: the pharyngeal cross-sectional area was smaller and more compliant in the patients group [44]. The increase in upper airway compliance implies that changes in flow and lung volume have a significant impact on the pharyngeal lumen; a reduction in lung volume as it occurs in the end-expiratory or early inspiratory phase is associated with a decline in pharyngeal lumen [44]. These mechanisms may explain why the airway collapses during early inspiration. The persisting occlusion during attempted inspiration results from the combination of decreased dilatory muscle tone and increased surface tension within the upper airway. Mouth breathing and supine position influence inversely the genioglossal activity and increase the propensity to upper airway collapse during sleep [84][107]. This is related to the change in mandibular position which affects the retroglossal airway diameter but also to the change in the length-tension relationship of the dilator muscles which affects their contractile force; the combination of the 2 factors results in increase in upper airway resistance during mouth breathing [229]. Increased resistance may induce snoring, airway narrowing and/or occlusion. These findings suggest that apnoeas/hypopnoeas are caused by sleep-related decrements in pharyngeal muscle activity in individuals with underlying structural narrowing of the upper airway. In support of this conclusion are the observational study outcomes by Mortimore et al, who compared the pharyngeal fat deposition in non-obese and obese sleep apnoeics to that of healthy individuals using T1-weighted MRI [243]. The imaging confirmed that even non-obese sleep apnoea patients have excess fat deposition, in particular anterolateral to the upper airway, compared to healthy individuals with the same level of body mass index and neck circumference. Cephalometric studies comparing sleep apnoeics to healthy individuals confirmed that craniofacial differences contribute further to the structural narrowing of the upper airway. Using a morphometric model based on the mandibular and maxillary size, their overlap, the palatal height but also the neck circumference and body mass index, Kushida et al applied the model on 300 symptomatic individuals referred to the sleep clinic, in order to test its accuracy as disease discriminator [198]. The sensitivity of the model was 97.6%, which is higher than that of BMI (93.8%) or neck circumference (89.8%) alone; its specificity was 100%. The findings indicate that disproportionate craniofacial anatomy or craniofacial dysmorphism predispose to the obstructive sleep apnoea syndrome, as do obesity and increased collar size [180]. As craniofacial anatomy is to a large extend inherited, the findings imply that the sleep apnoea syndrome has a strong familial predisposition. The latter is confirmed by the findings of Mathur et al who conducted morphometric studies on 1st degree relatives of 51 non-obese sleep apnoeics

and found that relatives had narrower upper airways with retroposed maxillae and mandibles and longer soft palates with wider uvulae compared to healthy controls [217]. The prevalence of the syndrome among relatives was also higher compared to the controls. In conclusion, in non-obese sleep apnoeics the craniofacial anatomy may be of importance for the development of the disease, whereas in obese patients fat deposition or a combination of the 2 factors may be the predisposing factors [99].

4.2. AROUSALS

4.2.1. CORTICAL AROUSALS

4.2.1.1. Detection, Definition and Analysis

Sleep fragmentation is the term used to describe the interruption of sleep by brief periods of increased EEG activity, which occur spontaneously or in response to apnoeas and hypopnoeas. These periods are known as arousals.

The initial arousal scoring definition was proposed by Rechtschaffen and Kales [288] and it refers to Movement Arousals, which are short periods of changes in any EMG channel accompanied by a change in pattern on the EEG and/or the EOG channel. The changes in the EMG channel may consist of either an increase in activity or an amplified blocking artefact. The accompanied changes in the EEG pattern may consist of a decrease in amplitude, increase in alpha activity, a paroxysmal burst of high voltage activity or amplifier blocking artefacts. EOG changes may consist of blink artefacts, amplifier blocking artefacts or increased muscle activity. Movement arousals are used as a signal for sleep stage change. If these periods extend to 15 seconds or longer, they define an epoch scored as Movement Time. Movement time and arousals should precede or follow sleep.

The detection of apnoeas and hypopnoeas during sleep and the recognition of their importance in the pathogenesis of sleep fragmentation and daytime sleepiness [135][280], created the need to re-define arousals. Several investigators contributed towards the present, modified, generally accepted but not universally agreed criteria on arousal scoring. Roth et al included the increase in muscle tone into the scoring criteria [301], Zwillich et al set the duration criterion at 5 seconds [384], Gould et al reduced it to 1.5 seconds [126]. In 1984, Stepanski et al introduced a four-

level arousal scoring system: the lower degree of arousal included an increase in EEG frequency and EMG activity, whereas the intrusion of alpha rhythm was considered as an intermediate level of arousal [327]. In 1992, Cheshire et al included alpha and theta frequencies into the arousal scoring criteria [60]. During the same year, the American Sleep Disorders Association (ASDA) published new rules on arousal scoring, which consider the different arousal stimuli (apnoeas, hypopnoeas, leg movements) and incorporate the mentioned, suggested modifications [9]. According to the ASDA recommendations, arousals can be scored from a central or an occipital EEG tracing. A minimum of 10 continuous seconds of sleep is necessary, before an arousal can be scored. An arousal is defined as an abrupt shift in EEG frequency 3 seconds or greater in duration, which may include alpha, theta and/or frequencies greater than 16 Hz but not spindles (figure 4.1). Arousals in NREM sleep may occur without concurrent increases in the chin EMG amplitude, in REM sleep only when accompanied by increase in muscle tone (figure 4.2). Delta waves, K complexes or artefacts are included to meet duration criteria only when they occur within the EEG frequency increase but not when they precede the period of frequency shift.

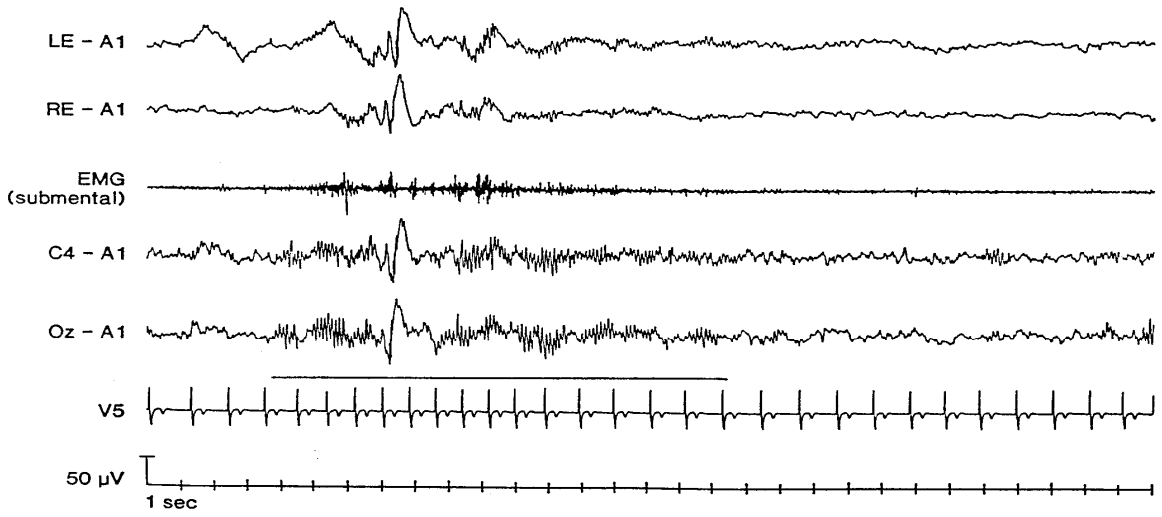


Figure 4.1. Example of an arousal during NREM sleep.

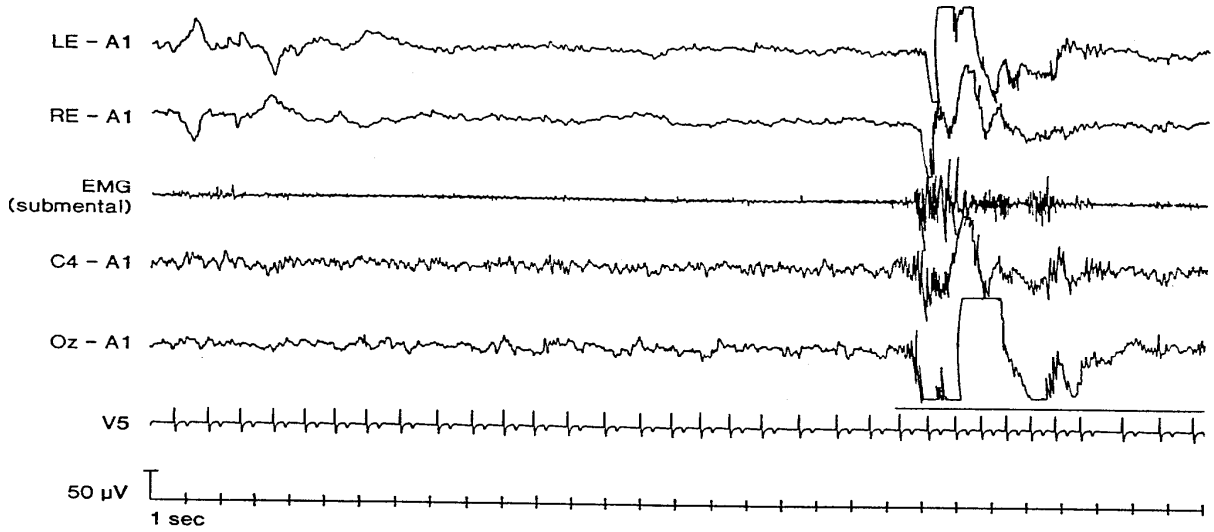


Figure 4.2. Example of an arousal during REM sleep.

Fragmented sleep is less restorative than consolidated sleep and, like sleep deprivation, leads to sleepiness and impaired daytime performance [214][297]. Martin et al demonstrated that experimental sleep fragmentation not only interrupts sleep but also alters its quality by reducing the time spent in SWS and REM sleep [214]. The Total Sleep Time (TST) was carefully controlled during the study, to ensure that the effects of sleep fragmentation were not produced by partial sleep deprivation. One night of sleep fragmentation increased objective sleepiness and impaired daytime performance in healthy volunteers. Typical laboratory tasks sensitive to sleep loss and fragmentation are vigilance, mental flexibility, sustained attention and reaction time. Psychomotor tasks most affected are those that are long, monotonous, externally paced without feedback.

One example of an applied task containing many of these elements is driving. George and co-workers [120] demonstrated that OSAHS patients perform worse in simulated driving situations than controls, as a result of sleep fragmentation and sleep deprivation. Sleepiness and cognitive impairment are the likely causes of decrements in driving ability and the substantially increased risk of road traffic accidents (RTAs) found in drivers with OSAHS [21][345]. However, although patients with severe disease are at higher risk of RTAs [120], no significant correlation has been found between reported driving performance and disease severity [21][121].

A number of studies have been conducted to explore and quantify the causes and consequences of sleep fragmentation on daytime performance. As their outcomes have been controversial, the optimal approach to the quantification of sleep fragmentation continues to be debated. Roehrs

and co-workers [298] performed multiple regression analyses and found that arousal frequency and Total Sleep Time (TST) are the best predictors of objective daytime sleepiness, measured by the Multiple Sleep Latency Test (MSLT). Cheshire and co-workers found that sleep fragmentation and hypoxia correlated with cognitive impairment but not with objective measures of daytime sleepiness [60]. Colt et al treated sleep apnoea patients with CPAP to reduce sleep fragmentation and daytime sleepiness [67]. While on CPAP, patients were exposed to periods of hypoxia. The episodes of oxygen desaturation alone, did not induce the recurrence of sleepiness. Kingshott et al found no significant correlation between sleep fragmentation and objective daytime sleepiness but did confirm a weak correlation with cognitive impairment [188]. Since sleep fragmentation is not the only factor affecting daytime sleepiness, correlation analyses between measures of sleep fragmentation and sleepiness have significant limitations. For example, a subject with severely fragmented sleep will show increased sleepiness during the day. However, the overall correlation may be reduced because lack of fragmented sleep does not guarantee that the level of sleepiness will be low. Multivariate statistical modelling may account for sources of variance simultaneously in the prediction of daytime sleepiness and may help identify the optimal definition of sleep fragmentation.

The conflicting outcomes of correlation analyses between measures of sleepiness and traditional measures of EEG arousals as well as lacking evidence to support that cortical arousals are necessary to terminate apnoeas and hypopnoeas have pushed investigators to explore alternative ways of detecting, analysing and defining sleep and arousals [77][259][266][291][335][339][348].

Moreover, a number of investigators have suggested that the definition proposed by the ASDA allows a proportion of cortical arousals to remain undetected, indicating that alternative, more sensitive methods for arousal detection are necessary [259][291][335].

AROUSAL DEFINITION

An important factor in the detection of arousals is the scoring definition used. The ASDA recommendations intended to serve as a basis for comparison between scorers and laboratories. The 3-second criterion is a methodological decision which is not based on sleep physiology or its significance on symptoms or treatment benefits. This duration was recommended, as it was thought that identification and agreement on events of shorter duration would have been difficult to achieve. During a multicentre study, Drinnan et al [89] examined the reproducibility of the ASDA definition and found an overall moderate inter-observer agreement (k=0.47), best during

Slow Wave Sleep (k=0.60), moderate during REM sleep (k=0.52) and poor during light sleep (k=0.28). The differences in arousal reproducibility across the sleep stages may be attributed to the fact that during NREM sleep, increase in EMG activity is not part of the ASDA arousal scoring criteria. During an analysis of respiratory events associated with recognisable EEG arousals, Rees et al concluded that of the 72% of all respiratory events found to be associated with an arousal, 30% were associated with clear EEG changes lasting between 1 and 3 sec [291]. Had the 3-second ASDA criterion been applied, these arousals would have been missed. These findings suggest that shorter events, which are of physiological importance, are not included in the ASDA arousal scoring criteria. Loredo et al demonstrated that interscorer reproducibility for arousals between 1.5 and 3 sec was low and increased for arousals longer than 3 seconds [209]. Reproducibility was highest for arousals associated with respiratory events or increased EMG activity. These findings suggest that although the ASDA arousal scoring criteria may succeed higher rates of reproducibility, the definition underestimates the number of physiologically important events. In support of this observation are the findings by Berry et al [32]. Using oesophageal manometry, Berry et al monitored the respiratory effort during sleep in 9 patients with severe OSAHS, in addition to the spectral analysis of their EEG recordings. The authors observed a cyclical increase in cortical arousal-threshold across the night, parallel to an increase in delta power. These findings confirm previous observations by Roehrs et al [297], supportive of differences in the arousal threshold across the different sleep stages. This may contribute to the lack of arousal detection at the end of all apnoeas and hypopnoeas. The current thesis will explore factors which may be contributing towards the induction of arousals at the end of apnoeas and hypopnoeas and differences between events which predispose to more marked arousal reactions. These might include event duration and severity of oxygen desaturation. Furthermore, there may be differences between sleep stages in the arousal threshold or in the arousal detection.

AROUSAL DETECTION

The ASDA recommends a central or an occipital lead for arousal detection and scoring. A study by O'Malley and co-workers examined whether the addition of a frontal lead improves arousal detection [259]. The authors analysed the recordings of 15 sleep apnoea patients. Arousals were scored according to the ASDA criteria. The number of arousals scored on the conventional central lead was compared to the number scored on the non-conventional frontal lead (Fz). The frontal lead increased detection of arousals by 19%, increasing the number of arousal-associated

respiratory events to 92%. The autonomic changes, i.e. increase in heart rate, were similar during the central and frontal arousals. The authors concluded that the addition of a single frontal lead (Fz) yields additional physiologically relevant information in terms of respiratory-related arousals.

Further multi-central studies with larger numbers of patients and healthy controls are needed to assess the significance of topographic differences in the arousal detection and physiology of sleep fragmentation. The reproducibility and clinical relevance of frontal arousals needs further exploring.

4.2.1.2. Alternative Analysis Methods

Following the findings by Rees et al [291], a number of investigators have explored alternative methods of EEG analysis in order to improve arousal detection and correlation with respiratory events and clinical symptoms. Svanborg et al analysed the EEG power spectrum of 5 sleep apnoea patients [339]. Albeit based on a small number of patients and apnoeas/hypopnoeas (53 during NREM and 28 during REM sleep) studied, the observations demonstrated a consistent increase in delta band power (0.5-3.5 Hz) towards the end of apnoeas and hypopnoeas during NREM sleep. Berry et al [32] analysed the EEG power spectrum parallel to the monitoring of upper airway resistance using oesophageal manometry in 9 sleep apnoea patients and found that the increase in EEG delta power was associated with increases in upper airway resistance.

These findings support the hypothesis that visual arousal detection is less sensitive compared to non-conventional proposed methods, such as Power Spectrum analysis. Furthermore, arousal threshold may vary across the different sleep stages, parallel to the increase in delta power. However, the diagnostic validity of the novel analysis methods needs further exploring.

A significant part of the work within this thesis will explore apnoea-/hypopnoea–related changes in the power spectrum of the EEG and the significance of those changes.

Frequency analysis will be described in detail in the following section.

4.2.1.2.1. Frequency Analysis

The process of separating a signal into its frequency components is known as frequency or power spectral analysis. Frequency analysis detects the frequency content over time.

The DC (Direct Current) -free EEG signal is usually low-pass filtered at 25 Hz; frequencies above this cut-off are “noise”. Of importance for the analysis are the power values within following frequency bands:

- delta frequency band: 1-4Hz
- theta frequency band: 4-8Hz
- alpha frequency band: 8-12Hz
- sigma frequency band: 12-16Hz

The original signal, i.e. amplitude as a function of time, is converted to an amplitude spectrum – a graph of amplitude as a function of frequency (figure 4.3).

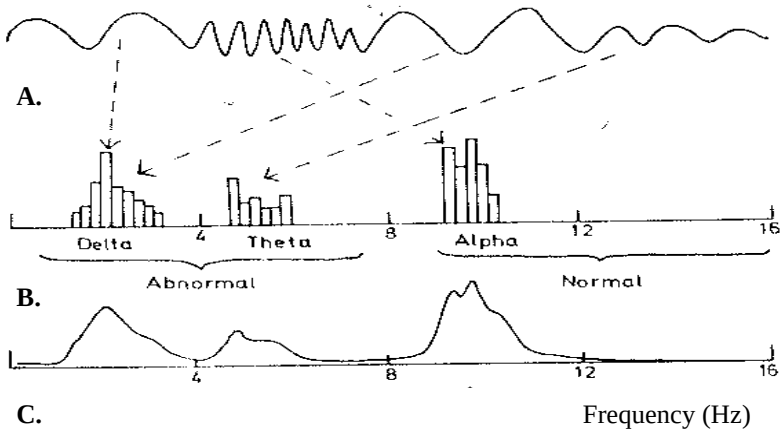


Figure 4.3. The top trace shows a simulated EEG recording, which contains fast and slow frequencies (A). The tracing is analysed into the delta, theta and alpha frequency bands (B) and the spectral power for the tracing is calculated (C).

The graphical presentation of the power spectrum can be in form of 2-dimensional plots with time on the horizontal axis, frequency on the vertical axis and amplitude reproduced by the colour scale (figure 4.4.A). Alternatively, it may be as a discrete line spectrum in which only specific frequencies are present and their amplitudes are represented by vertical lines on the frequency axis (figure 4.4.B).

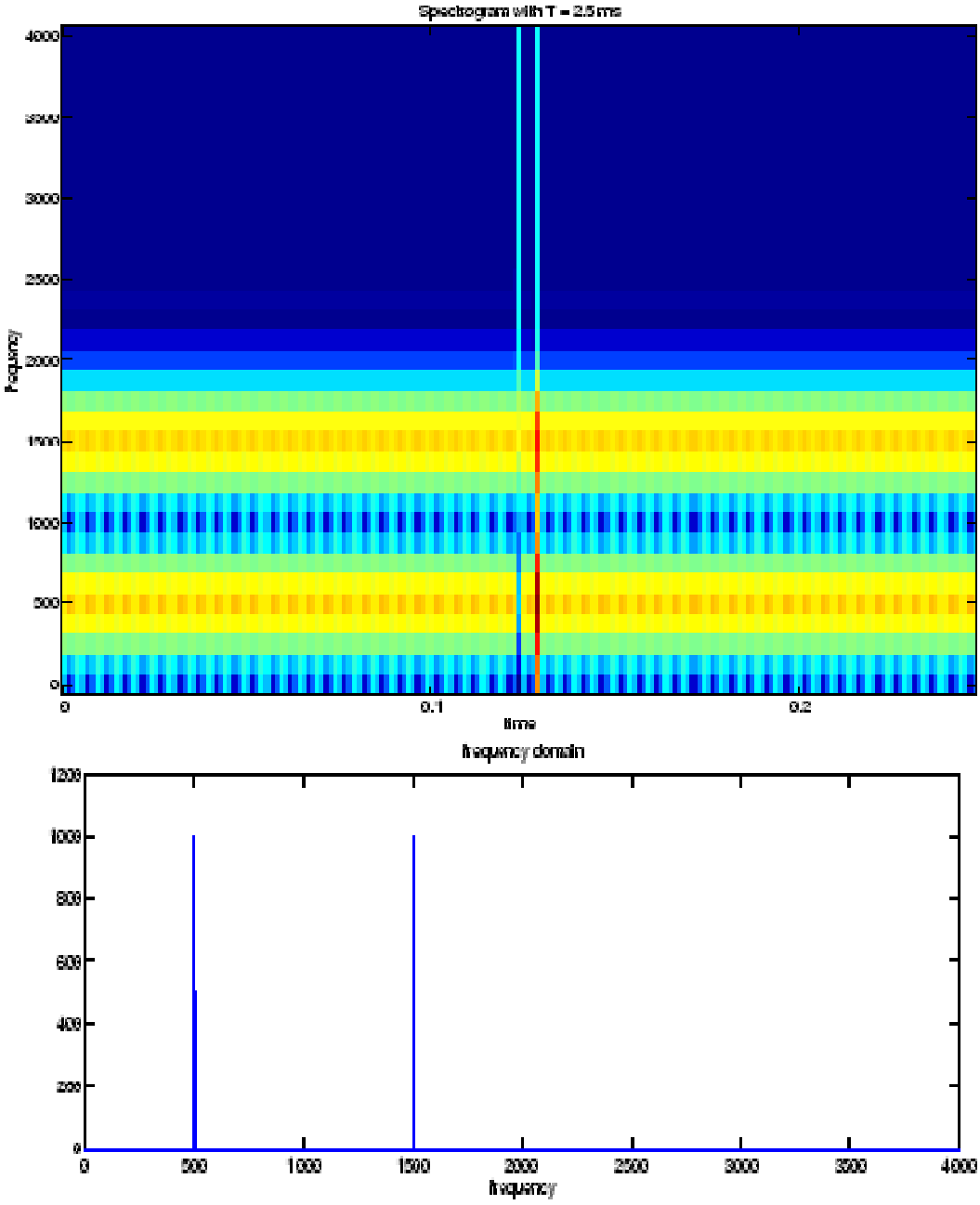
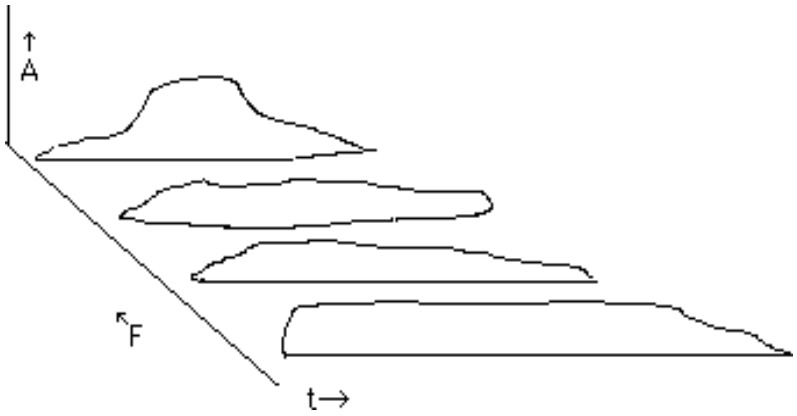


Figure 4.4. Graphical presentation of the power spectrum as a: **A.** 2-dimentional plot of 3 measures: time on the horizontal axis, frequency on the vertical axis and amplitude reproduced by the colour scale. **B.** line spectrum in which specific frequencies are present and their amplitudes are represented by vertical lines on the frequency axis.

When the values in a line spectrum are joined by a curve, they give the appearance of a continuous spectrum. The amplitude changes as a function of frequency over time, can be presented 3-dimensionally, the amplitude being plotted on the third axis (figure 4.5).



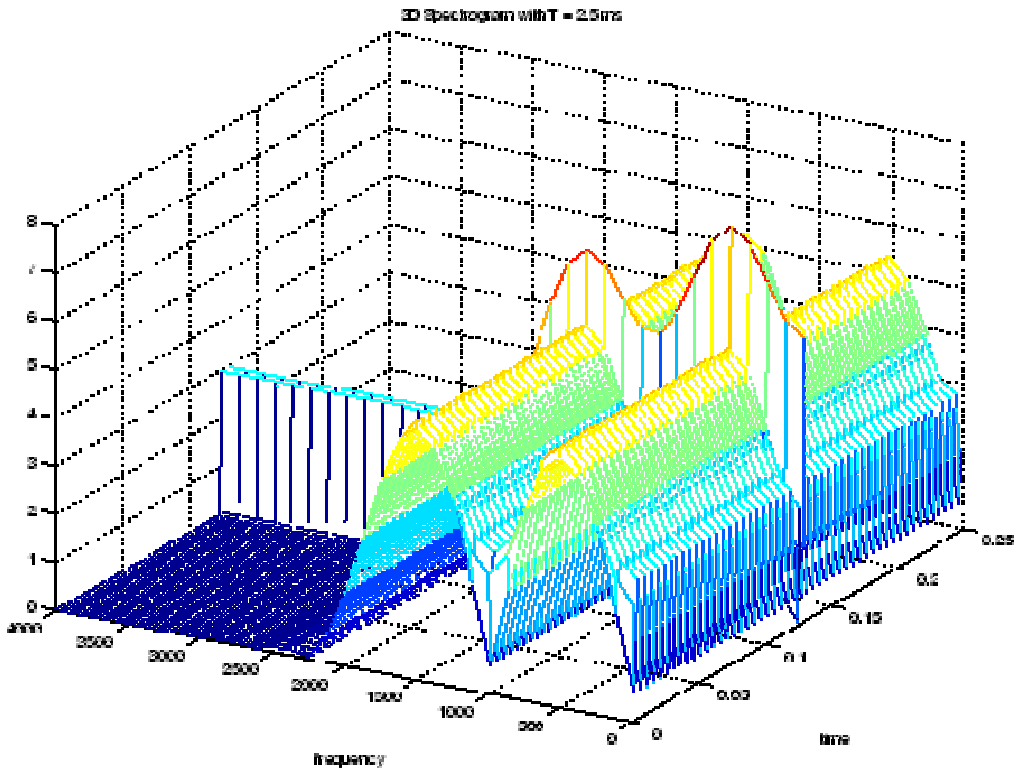


Figure 4.5. Three-dimensional presentation of frequency over time, with amplitude plotted on the third axis.

Power spectral density (PSD) analysis provides the basic information of how power (variance) distributes as a function of frequency. Density is calculated as the amount of power per unit of frequency [power/Hz].

Different mathematical models have been applied for the analysis of the EEG frequency components - the primary methods in recent years are fast Fourier transform (FFT) analysis and autoregressive modelling (AR). A basic assumption in both methods is that the statistical characteristics of the EEG signal (the average frequency and the average amplitude) do not change with time, i.e. that the EEG is stationary. It is self-evident that this assumption is not always justified, and there are many examples of non-stationarities in the EEG such as the transition from wakefulness to sleep, paroxysmal slow waves or apnoea-related arousals. For the separation and characterisation of the different patterns in a nonstationary EEG, two approaches have been used.

In the first, the EEG is subdivided into fixed short equal-length segments and their power spectrum is calculated. The features for all the segments are then grouped (clustered) among themselves or matched against a standard reference group. Adjacent segments may be joined to form longer, stationary segments, the length of which would be a multiple of the basic segment length. This approach is termed fixed-interval or non-adaptive segmentation.

In the second approach, the segment boundaries are determined for the specific EEG signal and the resultant variable-length segments are then clustered according to their features, which are extracted as part of the segmentation process. This approach is termed variable-interval or adaptive segmentation.

In the FFT method, the spectrum is determined from the signal directly, without any assumption as to its nature other than stationarity and without reference to its particular characteristics. In AR modelling the particular characteristics of the signal are specifically taken into account; a mathematical model is fitted to the signal and an output with same statistical parameters but not identical with the EEG signal is generated, from which the spectrum is extracted. Autoregressive analysis is therefore known as a parametric method because during the analysis the parameters for a mathematical model of the signal are determined. In contrast, Fourier transformation is termed a nonparametric method.

Below, the two methods are depicted in further detail.

4.2.1.2.1.1. Fast Fourier Transform (FFT)

In 1807, Joseph Fourier presented a mathematical analysis of problems associated with heat conduction. The “Fourier Transform” developed into an important area of signal analysis, usually implemented in the form of a fast Fourier Transform algorithm. Classically, the Fourier Transform is a harmonic analysis, which consists of multiplication of the signal by sine and cosine waves (i.e. at 90° phase with respect to each other), summing the resulted cross-products for each frequency (power) and repeating the procedure for the series of frequencies (power spectrum). The power spectrum - or periodogram - is obtained by squaring the sum of sine and cosine components for a series of closely spaced frequencies and so the area under the spectrum is equal to the mean square value of the original signal. For a random (stochastic) signal such as the EEG, the resulting raw periodogram is quite irregular and requires smoothing over adjacent frequencies or averaging over successive epochs. The whole process of obtaining and smoothing the periodogram yields the *Discrete Fourier Transform* (DFT).

In 1965, Cooley and Tukey designed the fast Fourier Transform algorithm (FFT), which revolutionised the calculation of power spectra in that it eliminates the redundant computation of the original DFT by sequentially combining progressively larger sums of products to obtain the sine and cosine coefficients [69]. The Fast Fourier Transform is a highly efficient short-cut method for carrying out the calculations of the frequency components.

There are different implementations of the FFT algorithm, most of which are usually computed for powers-of-two multiples of a basic length (number of samples).

The advantages of the FFT analysis are the simplicity of the algorithm and the high processing speed. The method can analyse any waveform as a combination of waves of different amplitude, frequency and phase.

For a non-stationary signal, such as the EEG, the standard Fourier Transform analysis is not useful. Time localization can be achieved by windowing the signal, i.e. by cutting off a well-localized slice and then taking its Fourier Transform. This gives rise to the *Short Time Fourier Transform* (STFT) or *Windowed Fourier Transform*. The portions of signal for analysis can be selected visually – if they appear to the eye to be unchanging. Alternatively, statistical methods (e.g. the two-sided Kolmogorow-Smirnov test) can be employed to detect the absence of significant differences in the average frequency and amplitude and select the appropriate length of EEG for analysis [227].

The *Welch* method splits a signal into smaller sets of data and calculates the periodogram of each set [367]. The frequency domain coefficients arising from calculating the periodograms are then averaged over the frequency components of each data set. This results in a power spectrum which is a smoothed version of the original, with less noise. In this way, the Welch method is a form of low pass filtering data. The Welch method was used for data analysis during the present thesis. Signal windowing may result in “leakage” in the spectral domain, i.e. the energy in the main lobe of the spectral response “leaks” into the sidelobes, obscuring and distorting other spectral responses that are present. Weak signal spectral responses can therefore be masked by higher sidelobes from stronger spectral responses.

One solution to the problem of FFT leakage is windowing. The most common windowing functions are the Triangular, Hamming and Hanning. These functions smoothly "tail off" the signal at both ends, reducing FFT leakage. Each point in the signal is multiplied by a window scaling factor before the FFT is calculated. The main differences between the windowing functions are their shape and whether or not they intersect the x-axis – Hamming and Hanning functions are both bell-shaped windows; the latter intersects the x-axis, the former doesn’t. Skilful selection of data windows can reduce the sidelobe leakage, sometimes at the expense of reduced resolution.

Data windowing and signal resolution are the most important performance limitations of the FFT approach, particularly troublesome when analysing short data records or strong varying time series that may be considered constant only for short record lengths.

Recognising these limitations, the Welch method and windowed FFT have been applied to analyse the EEG signal during the present thesis.

4.2.1.2.1.2. Autoregressive Modelling

Autoregressive (AR) modelling was originally developed for geophysical data processing, where it was termed the maximum entropy method. In biomedicine, AR modelling has been applied as an alternative spectral estimation procedure in an attempt to alleviate the inherent limitations of the FFT approach.

The term autoregressive analysis arises as follows: in ordinary statistics the relationship between two variables can be found by “regressing”, i.e. projecting the one onto the other, from which a

regression line can be drawn with the correlation coefficient (r) being a measure of the amount of scatter of the points on either side of the regression line. An estimation (prediction) of the value of one variable, knowing the particular value of the other variable, can be given from the regression line and an estimate of the error of the prediction can be derived from the correlation coefficient.

The same procedure can be carried out for a time series, such as the EEG (sequence of sample points) by “regressing” it linearly upon itself, hence the term “autoregression”. Correspondingly, a value – sample point of the signal – can be extrapolated based on the autoregressive coefficients and the preceding values of the time series. Hence, AR modelling is the mathematical modelling of a time series based on the assumption that each value of the series depends only on a weighted sum of the previous values plus "noise" – an unpredictable, uncorrelated random component. The coefficients are time dependant parameters, updated with every segment according to the error between that sample and the corresponding prediction. If the time series is totally predictable (e.g. a sine wave), the prediction error – the difference between the predicted value of the current point on the time series and its actual value) is zero. A set of autoregressive coefficients – collectively termed the state vector – constitute the “mathematical model” for the signal; the coefficients formulate the “autoregressive filter” which, for a totally random noise input, will have an output that is statistically indistinguishable from the original signal. A change in the output of the filter can thus be used as an index of non-stationarity of the signal [23].

An important parameter in autoregressive modelling is the selection of the order of the model or filter, i.e. the number of coefficients (p) to be used. The order of the model is a measure of its complexity. In practice, the value of p varies between 3 and 15 – for EEG spectral estimation the coefficients are usually 9 or 10.

The length of recording to be analysed can be flexible and does not need to be a multiple of a minimal interval, as is usually the case with FFT algorithms. Kalman modified the classic linear prediction theory which could also be used for nonstationary signals. In the model, known as Kalman filter, the autoregressive coefficients are themselves allowed to become time-varying, and are continuously adapted or updated to fit the signal during the analysis. The analysis can be viewed as a multidimensional tracking process - the number of dimensions being equal to the order of the model.

In comparison with FFT, autoregressive techniques produce smoother spectra with fewer peaks, easy identification of the central frequencies and accurate estimation of the PSD even on a small

number of samples. The degree of smoothness and the number of peaks depend on the choice of model and its complexity (model order) used in the autoregressive algorithm. However, this may not reflect the underlying reality of the number of frequencies characterising the signal, and may bias the data according to a population-wide model of frequency characteristics. Therefore, need of verification of the chosen model constitutes the main disadvantage of AR modelling.
In the present thesis, FFT was chosen because of its easier implementation without hypotheses about the choice of order.

4.2.1.2.2. Cyclic Alternating Pattern (CAP)

The identification of cyclic variations of the EEG dates back to 1955, when Fischgold and co-workers observed the pattern in a comatose patient [105]. Terzano et al studied the periodic changes further on a recovered post-coma patient and suggested that the pattern may represent a mechanism of arousal not only in coma patients [106] but generally during sleep [346].
The Cyclic Alternating Pattern (CAP) is characterised by sequences of transient electrocortical events distinct from background activity. Its analysis is based on the standard bipolar or monopolar EEG tracings. This periodic activity signifies synchronised sleep instability, recurring at intervals between 2 and 60 seconds, which may or may not be associated with sleep disturbance. CAP sequences are mainly detected during NREM sleep and commonly precede the transition from NREM to REM sleep. As REM sleep is characterised by lack of EEG synchronisation, CAP does not occur during REM sleep under physiological conditions. The pattern is an equilibrium between a greater arousal level (Phase A) and a diminished arousal level (Phase B); both phases constitute a cycle C (figure 4.6).

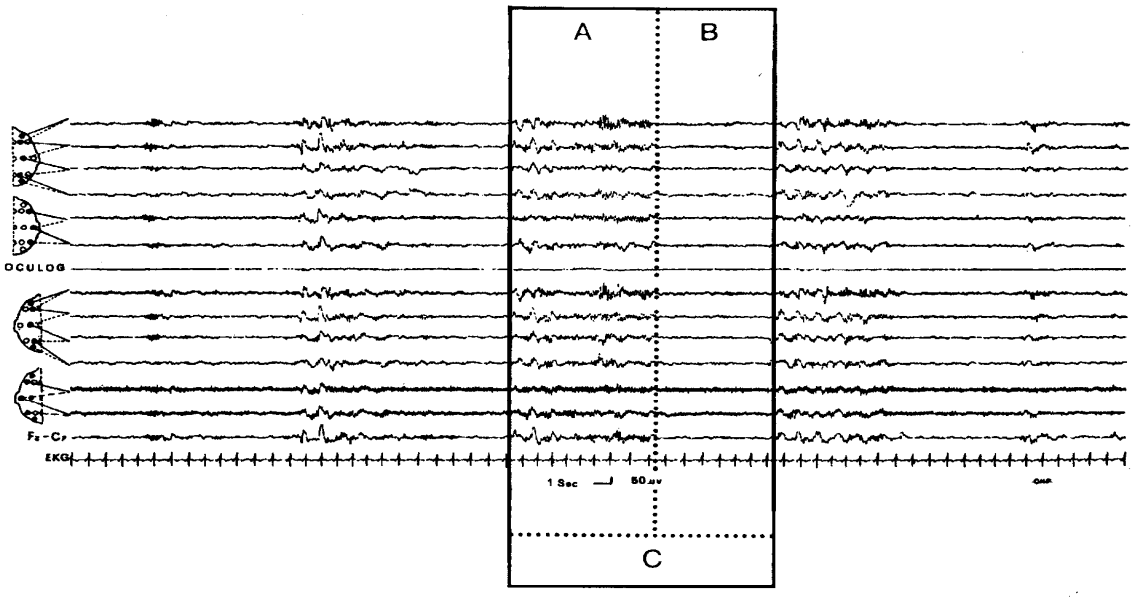


Figure 4.6. Illustration of the Cyclic Alternating Pattern (CAP) during sleep stage 2. The box outlines a Cycle (C) consisting of Phase A and Phase B. Reprinted from the Consensus Report by Terzano et al [348].

Phase A is characterised by increase in cardiorespiratory and attenuation in myotonic activity. Phase B separates 2 successive A phases, consists of theta-delta activities and is characterised by powerful attenuation of the myotonic and cardiorespiratory activity. Phase A may include delta bursts, Vertex sharp waves, K-complexes, intermittent alpha waves and/or EEG arousals and is therefore subdivided into 3 subtypes. Subtype A1 is characterised by synchronised slow waves – mainly K-complexes, vertex waves and delta bursts- A2 by mixed high and low voltage activities and A3 by fast desynchronised activities – subtypes A2 and A3 are consistent with the ASDA arousal definition. All CAP sequences begin with a phase A and end with a phase B. The period of sleep between two successive A phases separated by an interval longer than 60 sec is scored as non-CAP (figure 4.7).

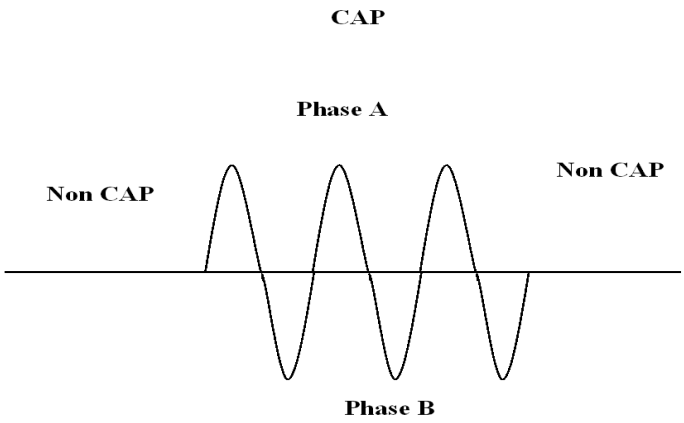


Figure 4.7. Graphical presentation of a Cyclic Alternating Pattern sequence consisting of Phase A and Phase B.

The degree of electrocortical desynchrony, cardiorespiratory and myotonic activity are less prominent during subtype A1 and increase towards subtype A3. Increased CAP rates reflect a state of disturbed sleep. The absence of CAP, the non-CAP, coincides with sustained sleep stability. CAP marks the brain's attempt to preserve sleep; if sleep becomes too unstable, then a frank arousal will appear on the cortex. The pattern can appear spontaneously or in conjunction with conditions, which disrupt sleep such as apnoeas or Periodic Limb Movement Disorder. These conditions may induce CAP sequences during REM sleep. Phase A features in REM sleep consist mainly of fast, desynchronised low amplitude patterns, separated by a mean interval of 3-4min. Apnoeas and hypopnoeas usually occur during phase B, while they are extremely rare during non-CAP.

Analysis of the CAP is not meant to replace sleep stage or arousal scoring; it aims to extend the analysis of sleep, include markers of prearousal activation and supplement the ASDA criteria for sleep fragmentation. CAP may provide additional information on the sleep continuum, physiology and autonomic activity. The pre-arousal states consist mainly of K-complexes and delta bursts. Although there is some evidence in support of the significance of synchronised electrocortical frequencies in terms of neurovegetative activation [158][138], the definitive clinical and pathophysiological importance of the CAP subtypes requires further exploring. In a recent review, Halasz has described a hierarchical arousal model: K-complexes are considered elementary forms of arousal, which may appear without periods of awakening or may precede the ASDA-type microarousals [147]. The latter are higher levels of arousal and are associated with EEG desynchronisation and increased neurovegetative and muscular activity. In contrast to these phasic events, the CAP phenomenon offers a global framework for measuring arousal instability. Thus, microstructural evaluation of the cyclic and phasic components of vigilance provides a picture of both the preprogrammed and the reactive changes. The analysis may contribute towards the understanding of sleep regulation and its interaction with neurovegetative activity. Terzano et al compared the agreement between CAP subtypes and ASDA arousals [347]. The study found that 95% of the A3 subtypes met the ASDA arousal criteria. Halasz et al observed that during NREM sleep 90% of ASDA-like arousals were preceded by K-complexes or delta bursts [148]. These findings support the hypothesis that the ASDA arousal definition covers only one side of the multi-faceted activation complexes, whereas the phase A subtypes of CAP provide a graded picture of the activating features. The differences between visible ASDA arousals and non-visible arousals, i.e. when ASDA criteria are not fulfilled and autonomic activation occurs unrelated to conventional EEG arousals, may therefore represent an artificial

dichotomy as a result of the scoring rules used and not due to differences in the regulatory physiological process. During the same study, Terzano et al [347] observed an increase in the number of A1 periods parallel to a drop in the number of A2 and A3 periods during SWS. During light sleep the number of A2 and A3 periods increased significantly. These findings demonstrate differences in the physiology of sleep across the different states of vigilance, which reinforce the hierarchical arousal model [147]. A significant part of the work in this thesis will analyse the pathophysiological differences across the sleep stages.

The analysis of the cyclic and phasic components of sleep microstructure, as currently performed, is not always complementary but sometimes contradictory. Figure 4.8 demonstrates 2 episodes of electrocortical disturbance, which do not fulfill the ASDA arousal criteria but do fulfill the CAP criteria for subtypes A1 and A3, respectively. In order to better understand the pathophysiology and clinical implications of sleep disruption, future research may contribute towards the elimination of methodological differences of visual sleep analysis.

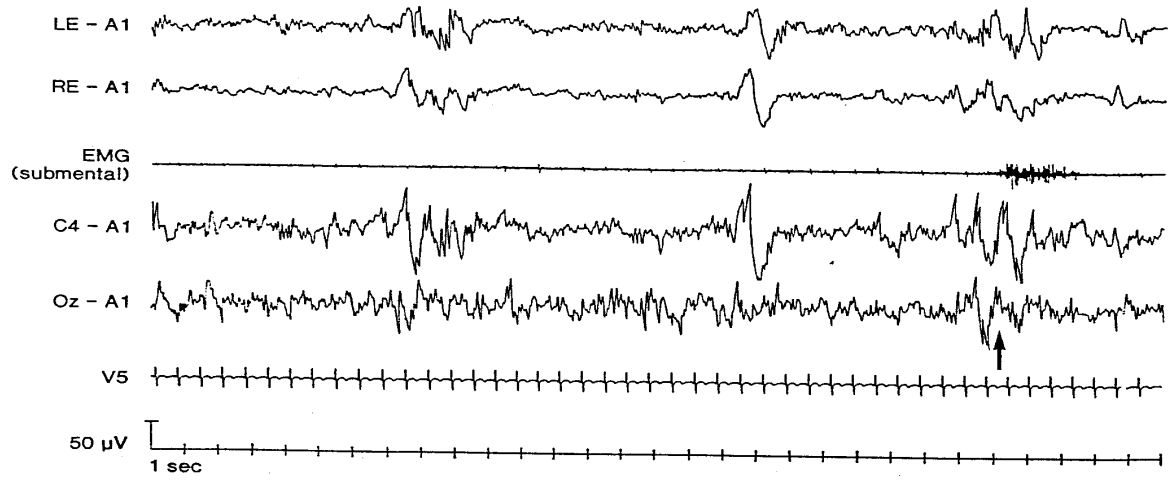


Figure 2.8.A. Delta bursts in NREM, no ASDA arousal but CAP.

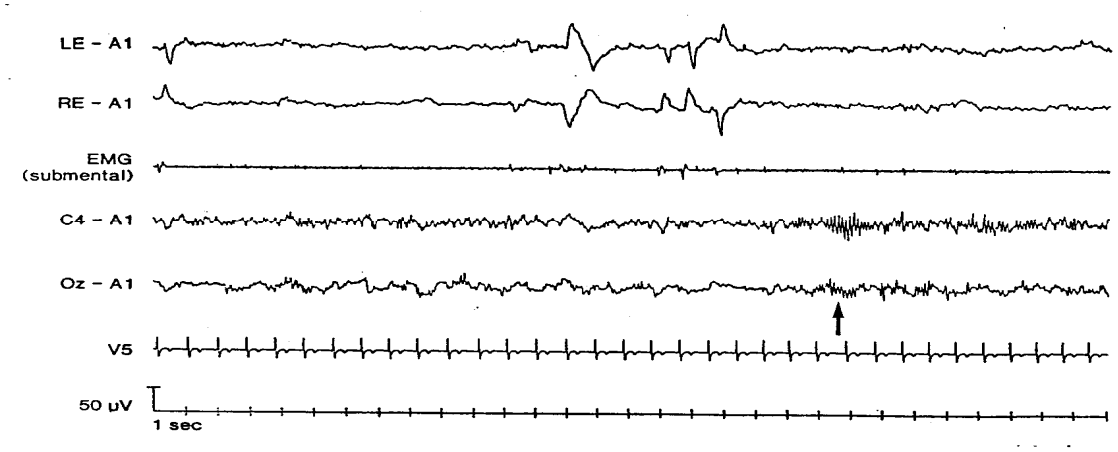


Figure 4.8.B. REM, alpha, no muscle activity, no ASDA arousal, but CAP.

One way to minimise those methodological differences is to use non-conventional, non-visual analysis. Many research groups have therefore analysed the power of the EEG frequency spectrum. Two research groups analysed the spectral power of periods around ASDA arousals in patients suffering from the sleep apnoea syndrome, periodic limb movement disorder, insomnia and partial epilepsy [77][339]. Both studies described periods of increased low delta power (1-2Hz) prior to ASDA arousals during NREM sleep. These findings are in keeping with the Phase A1 pre-arousal periods of delta bursts and K-complexes. Berry and co-workers [32] identified a cyclical delta power increase in sleep apnoeics parallel to increased respiratory effort, which may represent the cyclic pattern of CAP/ non-CAP [102] or it may be consistent with cardiorespiratory fluctuations [51]. Spectral analysis may therefore contribute towards the understanding of sleep microstructure – arousal and prearousal states - and its association with changes in neurovegetative activity. A significant part of the work within this thesis will analyse the changes in the EEG frequency power around apnoeas and hypopnoeas, as a contribution towards the understanding of sleep pathophysiology.

4.2.1.2.3. Artificial Neural Networks (ANN)

ANNs are computational models inspired by the biological neural networks. The aspiration for the design of ANN dates back to the 1930s, when Raschevski proposed the use of differential equations and physical concepts to describe how the behavior of neural networks may resemble psychological processes, such as Pavlovian conditioning [287]. McCulloch and Pitts are popularly remembered for having contributed to the idea that artificial networks can carry out

logical inferences [226]. In 1949, Hebb contributed towards the ANN's learning process by proposing a novel version of connectionism, the cell assembly theory of cognition [151]. Further researchers contributed towards the modelling of the widely used feed-forward multilayer perceptron (MLP).

An ANN consists of a set of processing units which simulate neurons and are interconnected through weights (analogous to synaptic connections in the nervous system) in a way which allows signals to travel through the network in parallel as well as serially. The units are very simple computing elements and are based on the observation that a neuron behaves like a switch: when sufficient neurotransmitter has accumulated in the cell body an action potential is generated. This has been modelled mathematically as a weighted sum of all incoming signals to a unit, which is compared with a threshold. If the threshold is exceeded the unit fires, otherwise it remains quiescent. The connections between the units, known as synapses or weights, may be uni- or bi-directional. Usually the units of an ANN are identical, the computation of the network is therefore determined by its weights. Computational power in a network derives not from the complexity of each processing unit (as in a conventional computer) but from the density and complexity of the interconnections. The computational knowledge in the ANN is distributed throughout the weights and is modified through experience, in contrast with conventional computers which operate a fixed programme of instructions through one or more very complex central processing units. The topology or connectivity of the network describes its architecture, i.e. the layers and the information flow of the network. A multilayered network consists of units, structured into input, hidden and output layers (figure 4.9). The information flow is usually fed forward but it may also recur in preceding layers. The best architecture for a particular task is usually developed by experimentation and observation. The computational knowledge of the ANN is acquired through training algorithms. Learning can be supervised or unsupervised. The commonest supervised learning mechanism, is the backpropagation algorithm [303]. This attempts to minimise the mean-square output error over the entire training set. The output of the network, when fed with the input data, is compared with the desired output and the error is propagated backwards through the network, to adjust the weights, reduce the mean-square error and make the network's response more nearly correct. Once the ANN has been trained, it is tested on a new data set. In the testing period only the input data are given to the network and the network's prediction is compared with the known outcome. If the result of the testing is favourable then the trained network can be assessed as a possible decision-support tool.

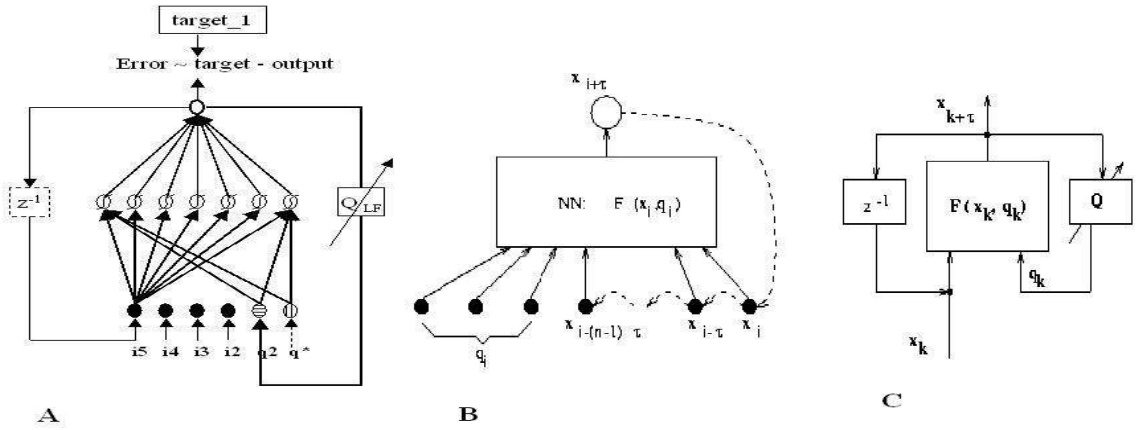


Figure 4.9.A: A multilayer, feed-forward artificial neural network with inputs, hidden layer and output. The architecture and function of the network is as followed:

Step 1: The inputs are i2,i3,i4,i5, the control variables are q2 (the modules own control unit) and q* (propagated by other modules, if present in the network). Step 2: The inputs are propagated to the hidden units through the weights. Step 3: The hidden units calculate their activations according to their weighted signals. Step 4: The activation is propagated from the hidden layer to the output unit. Step 5: The output unit calculates and compares the actual with the desired output. Step 6: The error is back-propagated and a mathematical equation is used to re-arrange the weights. Step 7: Estimation of the optimal control sequence for the given weights. Step 8: Shift of the input vectors to the right, i2 is dropped; the next inputs are i3,i4,i5,i6.

B and C : depict the ANN structure in mathematical formulas.

When the network is supervised during training, a class label is provided every time a new data set is presented. Because it knows the desired response, the training algorithm can generate an error signal which is backpropagated to adjust the weights. Unsupervised algorithms are closely related to the clustering techniques of pattern recognition and statistics. The most prominent is the “self-organising feature map” which can group into clusters automatically discovered by the network itself. Because unsupervised learning tends to be rapid and the task (number of inputs) will be simplified through selection of few key clusters, unsupervised networks are frequently used as pre-processors for the development of supervised systems. At the second, supervised stage networks tend to be slow to learn, especially if there are many inputs.

Once a network trained on a fixed data set has been trained, its learning mechanism is suppressed. The device becomes static, with potential implications for live applications. To enable an ANN to continue the learning process, the introduction of a feedback mechanism to protect previously learned knowledge from being overwritten by new information or noise, is

required. Networks based on the Adaptive Resonance Theory (ART) have this property. An ART network compares stored information with the current input. If the pattern fed back is a sufficiently good match to the current input, it becomes a member of the class. If the match is poor the search continues until a better one is found. If there is no improvement, a new class is established; the state of knowledge about the class is thus updated.

The ability of the ANN to evolve has enabled their application in discrimination tasks and pattern recognition. When approached from the perspective of discriminating analysis, ANN provide a framework for automatically selecting the appropriate form of boundary and locating it. Unlike statistical techniques, ANN can be used to select items that are important in performing discrimination, without a priori assumptions about the "best" discriminating variables, their underlying distribution or their interactions. ANN are therefore used to model higher order relationships of transient, non-stationary states and may prove useful diagnostic support tools [71].

Sleep is a highly transient, non-autonomous dynamical system, which includes non-measurable interactions between the subsystems involved. The dynamics of such a high dimensional, non-linear system with non-stationary behaviour can be modeled using ANN.

In 1996, Pardey et al proposed a model of automated sleep quantification through the application of ANN [266]. The model was based on the parameterisation of consecutive 1 sec EEG segments in terms of autoregressive (AR) modelling and the calculation of the tenth-order AR coefficients, using the Burg algorithm [181]. These coefficients were used as inputs to a self-organising feature map for the maximum separation of the different types of signal activity in a high-dimensional output-space. This clustering technique required no a priori assumptions or rules and was performed in an unsupervised manner. The insights gained using the self-organising feature map led to the development of a MLP that utilized the pattern recognition capabilities and provided a second-by-second quantification of sleep/wakefulness continuum with much higher resolution than the rule-based R&K scoring, enabling the detection of micro-arousals.

Schaltenbrand et al designed a multilayer ART network for automated sleep analysis, aiming to reproduce visual sleep stage scoring [310]. The spectral power values of the EEG, EOG and EMG, calculated during 2-second intervals through the application of the fast Fourier Transform (FFT) algorithm, were used as inputs of the MLP. The network's outcomes were summarised to cumulative values over 30-second intervals, similar to visual scoring. The automatic classifier achieved an average agreement of 82% with visual sleep stage scoring. However, considering the

limitations of the R&K scoring rules, novel methods of automated sleep analysis should aim to improve, not reproduce the currently used ruled-based scoring.

To improve second-by-second analysis of the sleep/wakefulness continuum and enable the detection of microarousals, our research group implemented a new architectural network model [13]. The Mutual-Control-Neural-Network (MCNN) consisted of 2 interactive MLPs, for the parallel analysis of the 2 most important, interacting "subsystems" of the system sleep: the activity of the central and the autonomic nervous system. The novelty of the second-by-second quantification of the sleep/wakefulness continuum through the MCNN, is the inclusion of autonomic activity into the arousal-scoring algorithm. The inputs of each module were the spectral power values of the EEG during 5-second intervals and of the ECG RR-interval time series during 15-second intervals, respectively. The power spectrum was calculated using the non-parametric FFT algorithm. The overall MCNN outcomes were 1sec-based sleep or wake states. Training was performed on 1-hour recordings and the model was validated on further 1-hour intervals. Preliminary data on 5 OSAHS patients showed good agreement with visual scoring with an increase in the number of arousals associated with apnoeas and hypopnoeas. The study will not be discussed in further detail, as it does not constitute part of the present thesis.
In conclusion, ANN may have a place in the automatisation and the improved resolution of the second-by-second quantification of the sleep/wakefulness continuum. Although frequently used in research, the outcomes of these studies suggest that the different ANN models require further validation in order to exclude over-sensitivity resulting in spurious arousals due to signal noise. Furthermore, estimation of a cut-off value for the differentiation between physiological and abnormal patterns would be necessary. A further aim is to establish the clinical significance of the ANN outcomes, their association with symptoms and their reproducibility before they can be applied routinely for diagnostic purposes.

Arousals are important components of the pathophysiology of the OSAHS. They disrupt the physiological sleep architecture, impair sleep quality and are major contributors towards the clinical symptoms. Different studies have found poor correlation between the severity of clinical symptoms and polygraphic findings such as AHI and cortical arousal index [28] [60] [188]. The latter may be due to limitations of visual scoring to detect all EEG arousals. It has been suggested that differences in arousability are associated with the depth of sleep [32].

Despite the different definitions used to score EEG arousals, aiming to improve their visual detection and correlation with apnoeas/hypopnoeas and clinical symptoms, around 28% of apnoeas/hypopnoeas were not terminated by visually detectable EEG changes [291]. This may be linked to differences between events which predispose to more marked arousal reactions, including event duration and severity of oxygen desaturation. Furthermore, there may be differences between sleep stages in either the visibility of arousals or the arousal-threshold. These issues have been investigated in this thesis.

The application of alternative techniques, such as the EEG spectral analysis in order to improve detection of changes at apnoea/hypopnoea termination, constitutes a further substantial part of the present thesis.

4.2.2. AUTONOMIC AROUSALS

In 1960 Sokolov et al described the Orienting Reflex (OR) as a nonspecific reflex, independent of the modality of the stimulus, which involves autonomic, somatic and EEG components [321]. During the process of arousal, autonomic control centres are involved that produce the characteristic “orienting” reflex. The regions of the brain that organise this response include the hypothalamus and brainstem [172] and their activation is associated with a rise in blood pressure and heart rate and an inhibition of the baroreceptor reflex. Sokolov et al studied the reflex during wakefulness and concluded that it is subject to extinction or habituation on repeated presentation. Johnson et al studied the OR in response to tone stimuli during wakefulness and sleep in healthy individuals [171]. The observations were based on different parameters but for the purposes of this thesis, I shall concentrate on the measurements of heart rate, finger plethysmography and EEG. The authors concluded that during wakefulness the tone stimulus resulted in an orienting response which habituated with repetition for all variables.

During sleep there was habituation of the EEG cortical response to auditory stimuli but very little attenuation of the rise in heart rate to auditory stimuli was found. After 7 hours of sleep, no significant decrease in heart rate or vasoconstriction responsiveness were observed - the OR was not suppressed, therefore habituation was absent. As a result of the rapid habituation during wakefulness, the mean heart rate response to the tone stimuli was high during stages 1, 2 and REM but lower during stages 3 and 4. The vasoconstriction response, monitored through finger plethysmography, was higher during sleep stages 1, 2 and SWS but lower during REM sleep,

compared to wakefulness. The response magnitude of these autonomic variables was significantly higher when an evoked K complex was associated with the stimulus. This observation indicated that brainstem and diencephalic “arousal” mechanisms involved in the response to alerting stimuli are always active but the arousal may or may not propagate to the cortex, depending on other factor such as possibly, the sleep stages.

In recent years, modelling studies aimed to establish the pathophysiological consequences of nocturnal fluctuations in autonomic activity and their impact on daytime sleepiness. Davies et al used tone stimuli on healthy volunteers during nocturnal sleep to induce rise in blood pressure and heart rate with or without associated cortical arousal response on the EEG [74]. The authors observed that tone stimuli induced an increase in blood pressure and heart rate, which in many but not all cases was associated with a visible increase in cortical activity on the EEG. Therefore, Davies et al concluded that the rise in the markers of autonomic activity was a direct consequence of the stimulus and not a confounding factor of the cortical arousals. A further modelling study on healthy volunteers conducted by Martin et al used tone stimuli during sleep to induce a rise in heart rate and/or blood pressure without any visible cortical arousal response on the EEG [215]. Following a mean of 6.9 hours of nocturnal sleep, the volunteers who were exposed to the stimuli had significantly shorter mean sleep-onset latencies in the Multiple Sleep Latency Test (MSLT) the following day and were therefore objectively sleepier. The study showed that fluctuations in autonomic activity impair sleep quality with less time spent in SWS and induce daytime sleepiness.

Observations in patients with disturbed sleep pattern due to apnoeas and hypopnoeas confirmed that fluctuations in blood pressure following the abnormal breathing pattern represent non-EEG markers of arousals, which potentially could be useful as a diagnostic screening tool for this disorder [75]. Pitson et al confirmed these findings using a different method for the detection of autonomic activity, the Pulse Transit Time (PTT) – the time taken for the arterial pulse pressure wave to travel from the aortic valve to the periphery [281].

Despite the different methods used to detect autonomic activity, the above studies drew similar conclusions: the activation of sympathetic drive represents a form of “arousal”, the “autonomic arousal”. Autonomic arousals are associated with brainstem activation and are important for the understanding of the pathophysiology, symptoms and clinical implications of the OSAHS.

Rees et al studied the changes in the EEG and blood pressure associated with apnoeas and hypopnoeas during the polysomnographic recordings of 15 OSAHS patients [291]. The EEG criterion used to score arousals during the study was 1 second or longer - shorter than the ASDA

definition of 3 seconds. Of all apnoeas/hypopnoeas, 72% were associated with a ≥1 sec cortical arousal detected on the EEG. In 28% of the apnoeas/hypopnoeas no change in the EEG frequency was detected. However unlike the inconsistencies in the EEG frequency shift, a rise in blood pressure was consistently present at apnoea/hypopnoea termination.

These findings support the concept of the hierarchical model of arousal, i.e. that the arousal response consists of a spectrum of pathophysiological changes and is therefore a continuous, variable rather than a discrete response. Intermittent increases in sympathetic activity in response to changes in intrathoracic pressure during apnoeas/hypopnoeas and associated haemodynamic changes constitute a useful marker of disrupted sleep physiology and represent "subcortical", brainstem arousals. This is the fundamental hypothesis behind most of the research carried out and presented in this dissertation.

In the chapter on sleep physiology, the morphological overlap and interactions between autonomic activity, breathing and state of vigilance under physiological conditions have been described. The purpose of this detailed exploration was to depict the implications of abnormal physiology in one or more of these components on the physiology of the others. In particular, how abnormal fluctuations in sympathetic activity may interfere with sleep, disrupt its quality and have consequences similar to those induces by cortical arousals. Therefore, the use of the term "autonomic arousal" adequately describes the pathophysiology and consequences of abnormal autonomic activity during sleep.

Autonomic brainstem arousals and cortical arousals are induced by afferents from baroreceptors, intrathoracic stretch receptors, upper airway mechanoreceptors and chemoreceptors among others. To what extent each receptor group contributes towards the imbalance and dysregulation of sleep is difficult to ascertain. Upper airway narrowing/occlusion in combination with reduction in blood oxygen, influence tidal volume and minute ventilation, which in turn cause changes in intrathoracic pressure, venous return, heart rate and blood pressure. These changes are transmitted through afferent fibres towards the brainstem. If the reflex loop is regulated at brainstem level, the induced changes are termed "autonomic arousals". If the reflex loop includes the cortex, the reaction is termed "cortical arousal". Which parameters influence the arousal type is not clear. A number of studies contributed towards the recognition of factors, which may determine the generation of one or the other type of arousal. Some of these study outcomes are discussed below.

Apnoeas/hypopnoeas may cause different levels of blood oxygen reduction between and within individuals. A number of studies assessed the importance of hypoxia on arousal induction.

During an animal modelling study, Bao et al found that hypoxia is a potent stimulus to acute blood pressure elevation [19]. During a modelling study in severe sleep apnoea patients, Narkiewitz et al monitored the sympathetic activity in the peroneal nerve (MSNA) and its response to supplemental oxygen therapy during 15 minutes of wakefulness and compared it to the response in healthy subjects [251]. The authors found that chemoreceptor deactivation through oxygen supplements reversed the acute excitatory sympathetic response to hypoxia in sleep apnoea patients. The outcomes of these modelling studies were not confirmed by Ali et al [3], who monitored the apnoea-induced changes in blood pressure during sleep and following supplemental oxygen therapy in a group of normotensive, severe sleep apnoea patients. Correction of apnea-related oxygen desaturations did not reverse the associated rise in blood pressure. These findings suggest that activation of chemoreceptor afferents does not contribute significantly to increased phasic efferent sympathetic response to apnoeas/ hypopnoeas. The study did not differentiate between autonomic and cortical arousals – the postapnoeic rise in blood pressure may have been associated with cortical arousals; if so, correction of oxygen desaturation prevented neither the sympathetic nor the cortical arousals. The authors also found that the rise in blood pressure was proportional to the apnoea duration. Kimoff et al studied the influence of chemo- and mechanoreceptor stimulation on the generation of arousal at apnoea termination [186]. The group measured apnoea duration and inspiratory effort while breathing room air, during supplemental oxygen and carbon dioxide administration. Supplemental oxygen, associated with attenuated chemoreceptor stimulation resulted in apnoea prolongation; hypercapnia, associated with increased chemoreceptor stimulation resulted in apnoea shortening. The authors monitored oesophageal and gastric pressures to calculate the transdiaphragmatic pressure; the latter, divided by time (diaphragmatic tension time index) was an estimate of the inspiratory effort. There was a consistent increase in inspiratory effort prior to apnoea termination; the rate of increase was not attenuated during oxygen delivery and was not accelerated during hypercapnia. These findings suggest that mechanoreceptor afferents play a predominant role in apnoea termination, whereas chemoreceptor afferents exert an indirect effect through stimulation of ventilatory muscles at brainstem level. The influence of inspiratory effort on autonomic activity was studied by Argot et al [10], who monitored intrathoracic pressures using oesophageal manometry while measuring Pulse Transit Time (PTT), which is inversely related to arterial blood pressure. During apnoeas there was a progressive increase in inspiratory effort, evidenced by a decline in the intrathoracic pressure, paralleled by an increase in PTT. Apnoea termination was associated with a reduction in PTT.

Garpestad et al examined the influence of sleep state on the post-apneic rise in blood pressure and found the haemodynamic response to be more pronounced during REM compared to NREM sleep. The differences in the level of post-apnoeic blood pressure rise between REM and NREM sleep were independent of the degree of oxygen desaturations [117].

Cortical arousals, detected on the EEG, are associated with increases in sympathetic activity. This has been confirmed in a number of studies using different markers of autonomic activity. Bonnet et al assessed the heart rate variability in healthy individuals using spectral analysis of the RR-interval time series and detected increases in sympathetic activity parallel to EEG arousals [42]. Pitson et al detected the arousal-related increases in sympathetic activity in healthy controls, based on changes in heart rate and Pulse Transit Time [281]. Davies et al monitored the arousal-related fluctuations in blood pressure in a large group consisting of healthy individuals and OSAHS patients [75]. Morgan et al used a wide range of auditory stimuli in a group of healthy individuals to produce EEG and/or autonomic arousals, detected through fluctuations in blood pressure but also through intraneural recordings of postganglionic muscle sympathetic activity in the peroneal nerve (MSNA) [240]. The stimuli produced a graded response ranging from a rise in blood pressure to EEG arousals. Arousals were associated with a larger increase in blood pressure accompanied by a phasic rise in MSNA.

The above observations in combination with advances in technology, led to the recognition that EEG recordings may not be essential in the detection of apnoea-/hypopnoea-induced disruption of sleep physiology [335]. Detection of autonomic activity changes may supplement and even replace EEG arousals in the recognition of the extent of sleep fragmentation and deprivation of REM and SWS and improve analysis of sleep disruption.

In conclusion, upper airway and chest wall mechanoreceptors in combination with chemo-, baro- and lung stretch-receptor stimulation generate a graded response to apnoeas/hypopneas ranging from a tonic increase in sympathetic activity regulated at brainstem level to cortical arousals (figure 4.10). This thesis aims to contribute towards the recognition of factors which may induce such arousals.

The importance of cortical and sub-cortical arousals for daytime function is not yet fully understood. The detection of autonomic activity changes may contribute to the understanding of all mechanisms through which sleep fragmentation impacts on daytime function and will improve our knowledge and understanding of the syndrome, its clinical implications and long-term consequences. Moreover, monitoring of the autonomic activity changes may simplify the diagnosis of OSAHS.

In the next chapter, the pathophysiological importance of nocturnal sympathetic activation will be discussed and methods of its detection will be explored.

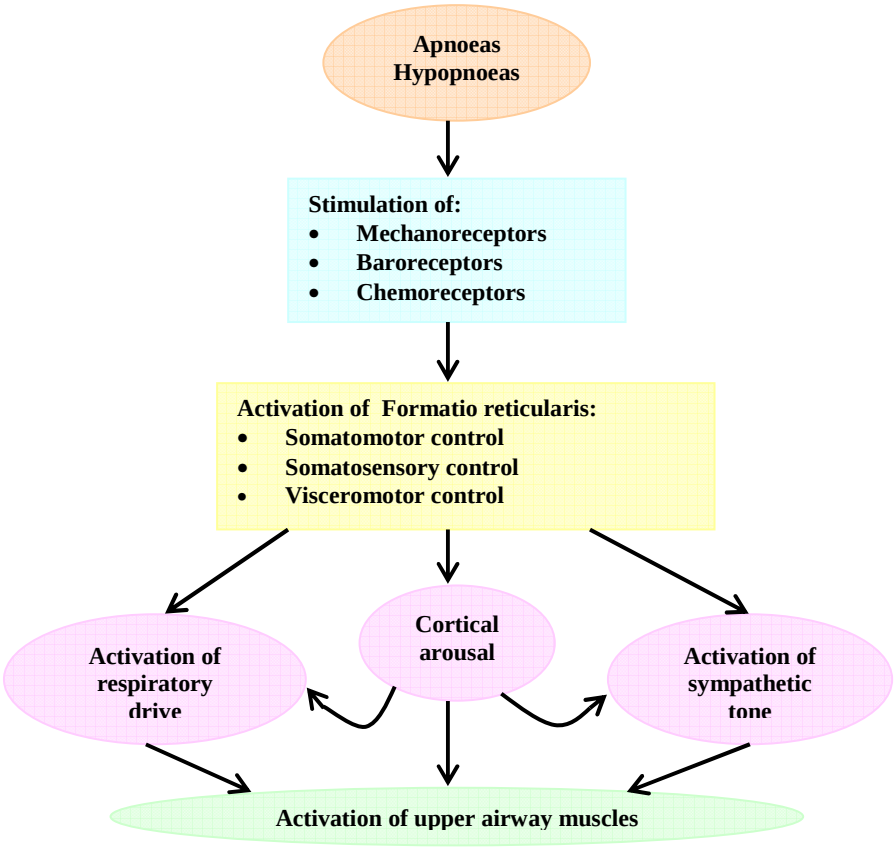


Figure 4.10. Sequence of events induced by apnoeas/ hypopnoeas consisting of afferent impulses to the brainstem and Cortex and efferent impulses resulting in restoration of pharyngeal patency.

4.3. AUTONOMIC ACTIVITY

The tonic and phasic sympathovagal balance is modulated by the interaction of central and peripheral reflex mechanisms. Apnoeas/hypopnoeas prevent the physiological attenuation of sympathetic outflow during sleep through the stimulation of chemoreceptors [242][251], baroreceptors [179][322] and upper airway stretch receptors [131]. Receptor stimulation increases peripheral and central cardiac sympathetic traffic to the sinus node and the myocardium resulting in surges of heart rate (HR) and systemic blood pressure (BP) at apnoea/hypopnoea termination. Baroreceptors co-influence the regulation of HR and peripheral sympathetic outflow. Receptor stimulation activates the afferent limb of the baroreflex loop,

which mediates negative feedback mechanisms by exciting parasympathetic and inhibiting sympathetic outflow (figure 4.11).

Peripheral sympathetic activity is measured invasively from a nerve fascicle to muscle blood vessels in the peroneal nerve, posterior to the fibular head (MSNA). In the early apnoeic period MSNA is increased, HR and BP are reduced. Towards the end of apnoeas and at apnoea/hypopnoea termination, MSNA drops as HR and BP surge [134][152]. Despite the decreased cardiac output during the respiratory events, BP increases towards the end of apnoeas/hypopnoeas. This suggests that peripheral sympathetic but not central cardiac efferents are implicated in the regulation of apnoea-/hypopnoea-related BP changes, resulting in vasoconstriction and increased total peripheral vascular resistance. A modelling study in awake healthy individuals demonstrated the contribution of peripheral sympathetic activity in the regulation of BP, as pharmacological ganglionic blockade abolished MSNA during, and BP elevation after sustained eucapnic Mueller manoeuvre (inspiratory attempts against occluded upper airways during apnoea at maximal exhalation) while HR rise remained preserved [179]. In the absence of ganglionic blockade, hypoxia was not a prerequisite for the apnoea-related elevation of BP and HR. The mechanisms and interactions involved in sympathetic activation and the baroreflex loop are multifactorial and not entirely understood. Although there is strong evidence in favour of chemoreceptor involvement in sympathetic activation and baroreflex regulation [242][251], recent evidence suggests that oscillations in ventilation can amplify changes in BP and HR in the absence of hypoxia or arousals from sleep [210]. A further modelling study in healthy awake individuals confirmed the influence of respiratory pattern on the centrally mediated sympathetic activity by demonstrating a dissociation between peripheral neurocirculatory (MSNA) and central (HR) sympathetic activation during apnoeas and hyperventilation [355]. During normal breathing, the reflex arising from pulmonary stretch receptors suppresses central sympathetic discharge, resulting in expiration induced bradycardia. During apnea this reflex ceases, disinhibiting central sympathetic outflow.

Baroreflex control of heart rate is assessed non-invasively from co-variations of systolic pressure and RR interval, which can be detected using different techniques described in the next section on measures of autonomic activity. The baroreflex-related suppression of sympathetic activity in response to blood pressure reduction is diminished in awake normotensive OSAHS patients compared with healthy controls, indicating reduced baroreflex sensitivity (BRS) associated with diminished ability to activate the parasympathetic reflexes [250]. The HR responses to blood pressure changes are preserved. Unlike in healthy individuals, BRS fails to increase during sleep

in OSAHS patients [265]. Potential causes of the increased baroreceptor threshold are the high sympathetic drive and the nocturnal BP fluctuations [322]. Increased sympathetic outflow would be expected to impair the baroreflex response to increases in BP [101]. Repetitive nocturnal BP increases may reduce BRS similar to the reduced BRS found in hypertensive relative to normotensive individuals and the negative correlation between BRS and BP status with stepwise reductions in the transition from borderline to established hypertension [342]. The suppressed BRS is one of the underlying pathophysiological mechanisms which predispose OSAHS patients to the increased risk of arterial hypertension [254][276]. Reversal of apnoeas/hypopnoeas with Continuous Positive Airway Pressure (CPAP) ventilation attenuates sympathetic increase [322], increases BRS in normotensive OSAHS patients during sleep and wakefulness [43] and reduces diurnal and nocturnal BP in normotensive and hypertensive OSAHS patients [27].

The described interactions are graphically presented in figure 4.11.

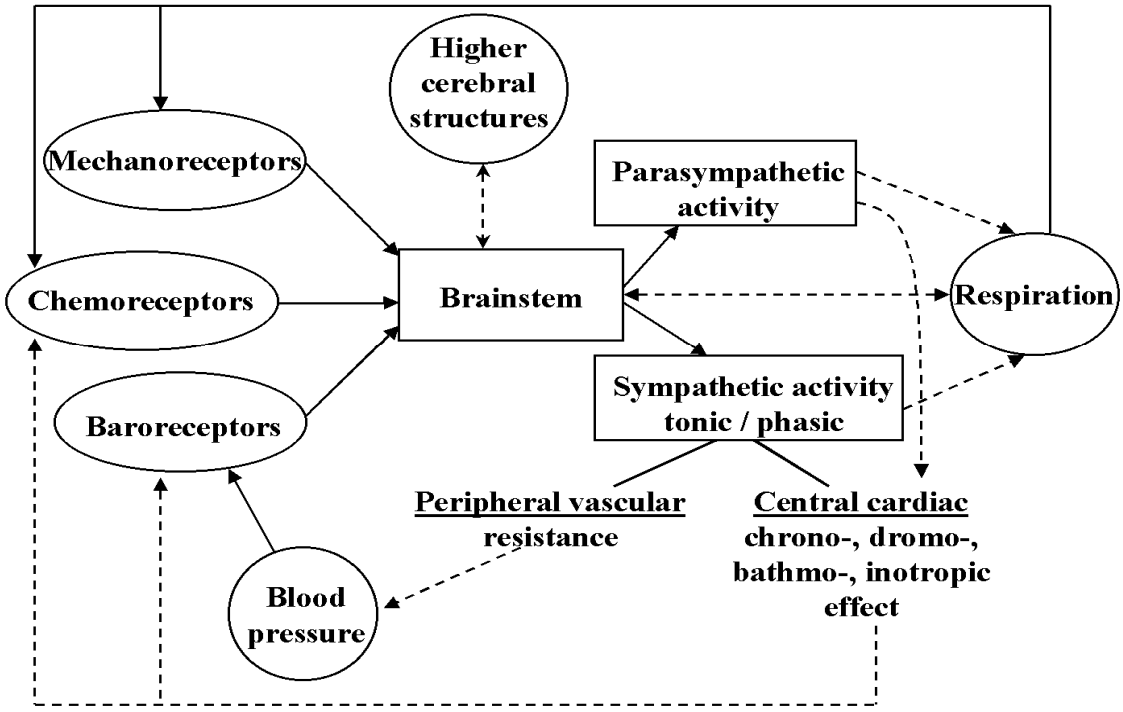


Figure 4.11. Main contributors towards cardiac and peripheral sympathetic activity and their interaction with baroreceptors in the regulation of blood pressure and heart rate. The loop is influenced by sleep, wakefulness, respiration and higher cerebral structures.

Sympathetic activity is a major contributor towards the pathophysiology and long-term effects of the OSAHS. Increased sympathetic activity in normotensive OSAHS patients is not confined to sleep but remains high during daytime wakefulness compared with healthy individuals

[249][322]. As described earlier, the mechanisms involved in the sympathetic up-regulation include baroreflex dysfunction [250][265], mechanoreceptor [10][131][210][355] and tonic chemoreceptor [250] activation. The maintenance of high sympathetic activity is associated with increased release of humoral vasoconstrictors, higher BP and endothelial dysfunction. Frequently used measures of sympathetic activity are the invasive monitoring of MSNA and the non-invasive HR analysis. The latter is described in the next section on measures of autonomic activity.

A significant part of the present work explores the analysis of Heart Rate Variability (HRV) and its importance in the detection of apnoea-/hypopnoea-related autonomic activity changes during sleep in OSAHS patients.

4.3.1. MEASURES OF AUTONOMIC ACTIVITY

4.3.1.1. Heart Rate Variability (HRV)

The sinus rhythm varies with time. The variability depends on the inputs of sympathetic and parasympathetic activity, influenced by the state of cerebral vigilance (sleep, wakefulness), breathing and vasomotion. Changes in one or more inputs of the control systems produce oscillations in the controlled variables. Analysis of HR fluctuations over time and of the frequency of fluctuation, is a non-invasive quantitative measure of the cardiac sympathovagal modulations. The clinical relevance of HRV was first recognised in 1965, when Hon and Lee noted that foetal distress during labour was accompanied by diminution in the inter-beat intervals before any appreciable changes occurred in HR itself [157]. Since then, HRV analysis has been used as a non-invasive measure of autonomic cardiac control in various conditions including the detection of autonomic neuropathy in diabetics and the assessment of mortality risk in post myocardial infarct patients. The latter established the clinical importance of HRV analysis as strong and independent predictor of post-infarct mortality [191]. The variations in HR can be evaluated by a number of methods. The most frequently used and the most important for the purposes of this thesis are explained below:

TIME DOMAIN ANALYSIS

The simplest to perform are the time domain measures of HRV which can be described as heart rate or cycle length (RR-interval duration) of normal-to-normal (NN) intervals, i.e. QRS

complexes resulting from sinus node depolarisation. Further measures are the standard deviation (SDNN) which is an estimate of the overall HRV, the average standard deviation of intervals (SDANN), usually 5 minutes, which is an estimate of long-term components of HRV and the square root of the mean squared differences of successive NN intervals (RMSSD), an estimate of short-term changes in HRV. In this thesis, mean RR duration is the calculated time domain measure.

FREQUENCY DOMAIN ANALYSIS

Frequency domain analysis of HRV refers to the analysis of the HR signal in its constituent frequencies. The HR signal is derived from the ECG with the HR samples (RR intervals) evenly spaced in time [cycles per second]. For the extraction of the HR signal, different algorithms have been proposed. HRV has become a conventionally accepted term to describe the analysis of the RR-interval tachogram, a signal of cycle length on a consecutive beat count axis [cycles per beat], not evenly spaced in time. While the number of cycles per beat can be converted to an average number of cycles per second by multiplying the former by the average heart rate, the frequency spectrum of this signal is different from the "true" HR signal spectrum. Within this thesis, the term frequency domain analysis of HRV refers to the fluctuation analysis of the "true" HR signal. For its extraction from the ECG signal, we used the algorithm implemented by Berger and co-workers [29].

The frequency spectrum of the centrally mediated cardiac autonomic activity is divided into following frequency bands, based on the recommendations by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [97]:

Ultra Low Frequency: 0.0001-0.003 Hz

Very Low Frequency: 0.003-0.04 Hz

Low Frequency (LF): 0.04-0.15 Hz

High Frequency (HF): 0.15-0.4 Hz.

Information on the power (variance) distribution of a time series across its frequency spectrum is provided by the Power Spectral Density (PSD), calculated as the amount of power per unit (density) of frequency [power/Hz]. Different algorithms are used for the estimation of PSD. The most popular in the field of cardiovascular signal analysis are the nonparametric fast Fourier Transform (FFT) and the parametric autoregressive modelling of a time series. The advantages of the FFT method are the simplicity of the algorithm and the high processing speed. Autoregressive techniques produce smoother spectra with fewer peaks and easy identification of

the central frequencies. The degree of smoothness and the number of peaks depend on the choice of model and its complexity (model order) used in the autoregressive algorithm. The need of verification of the chosen model constitutes the main disadvantage of this method. In the present thesis, PSD calculation was based on the FFT algorithm. To perform an accurate analysis, both methods require signal stationarity, i.e. the mean value and standard deviation of the signal must remain the same during the periods analysed. To fulfil this requirement, non-stationary signals are split into short stationary intervals (windows), which are analysed separately. The stationarity requirement constitutes an inherent limitation of the frequency domain analysis.

PSD is usually calculated for frequencies between 0.04 and 0.4 Hz, i.e. the LF and HF components. The power values (i.e. areas under the curve) of each component are extracted from the PSD curves. The frequency power of the HR signal is expressed in absolute units [$msec^2$] and in normalised units (n.u.), the latter representing the relative value of each power component in proportion to their sum (LFn, HFn) [212]. Normalised power emphasises the controlled and balanced behaviour of the 2 branches of the autonomic nervous system, increases sensitivity and correlation with attendant changes in MSNA [262]. The relation of efferent cardiac sympathetic and parasympathetic nerve traffic to LF and HF power has been demonstrated in clinical and experimental conditions. Efferent vagal activity is the major contributor to the HF power confirmed during vagal stimulation, muscarinic receptor blockade and vagotomy in animals and during clinical manoeuvres in humans [2][212]. The interpretation of LF power is more controversial; although some investigators consider it as a variable that includes both sympathetic and vagal influences particularly when expressed in absolute units [2], there is increasing evidence to support that LF power is a marker of central sympathetic inputs conditional to brainstem activation [142][212]. Reduced HRV, i.e. reduction in total HR frequency variance (power), may result from autonomic withdrawal or from high levels of sympathetic input [97]. When expressed in absolute units, LF may therefore appear unchanged on reduced; normalisation and/or calculation of the LF/HF ratio minimises the effect of the changes in total power on the values of LF and HF components and enhances the detection of the fractional energy distribution. In resting awake healthy individuals, LF power is greater than HF power (LF/HF>1) [262]. The transition from wakefulness to non-REM sleep is associated with a trend toward a decrease in LFn power and a marked increase in HFn power, which describes the traditional parasympathetic dominance expected in quiet sleep. Transition to REM sleep causes a decrease in vagal contribution to HR control compared with wakefulness, resulting in a relative prevalence of LF variability, indicating sympathetic dominance in the autonomic balance during

REM sleep [354]. Controlled breathing during wakefulness with frequencies within the physiological range enhances the vagal contribution to HR and increases HF power [50][212]. Apnoeas and hyperventilation induce a dissociation between peripheral (MSNA) and central (HR) sympathetic activity [134][152][355]. These findings confirm the common central control mechanisms of respiratory and cardiac autonomic modulations and the influence of respiration on cardiac autonomic activity (figure 4.11).

Hypertension [319] and myocardial infarction [35][207] are associated with diminished HRV, i.e. a reduction in total power. Singh et al [319] found that HRV diminution was associated with reduction in the absolute LF and HF power values in hypertensives whereas Lombardi et al [207] observed an associated increase in the normalised LFn power values in post myocardial infarction patients. Both clinical conditions have been associated with the prevalence of OSAHS [163][276]. HRV during daytime wakefulness is altered in normotensive, untreated OSAHS patients compared with healthy controls; the changes are characterised by increase in the LFn and decrease in the HFn component of HR power [249]. These findings confirm the increase in daytime sympathetic activity in untreated normotensive OSAHS patients [249][322] which may be implicated in the development of cardiovascular disorders in these patients.

A significant part of this thesis explores the sympathovagal balance during sleep and around apnoeas and hypopnoeas in OSAHS patients based on the time and frequency domain analysis of HRV.

TIME-FREQUENCY ANALYSIS

Wavelet transform of the HR signal or the RR-interval tachogram enables the three dimensional analysis of spectral power as a function of real time. The method is not restricted to stationary signals. Prior to the analysis, wavelets are developed and adapted to the specific signal.

4.3.1.2. Baroreflex Sensitivity (BRS)

Heart rate and blood pressure interact in a reflex manner, the baroreflex. The baroreflex sensitivity (BRS) for heart rate is a marker of the capability to reflexly increase vagal activity and to decrease sympathetic activity in response to a sudden increase in blood pressure. The baroreflex loop is important in buffering the interplay between heart rate and peripheral vascular resistance in order to maintain blood pressure requirements.

The following techniques are frequently used for the quantification of the BRS:

SEQUENCE TECHNIQUE

Estimation of BRS is based on the calculation of the slope of the regression line between spontaneous co-varying increases or decreases in systolic BP and the corresponding lengthening or shortening in the RR interval (ΔRR / ΔSBP [ms/mmHg]), occurring over spontaneous sequences of three or more consecutive beats. Only the intervals with concurrent increase or decrease in both values are measures of BRS and are included in the graph.

SPECTRAL TECHNIQUE

The power spectra of the RR-interval (RRI) tachogram and the corresponding systolic BP signal are calculated using the FFT algorithm. BRS equals the squared route of RRI power / SBP power [ms/mmHg], with the power values integrated over the LF and/or HF bands or other bandwidths associated with the baroreflex function.

Commercially available devices monitor the beat-to-beat finger BP based on digital photoplethysmography, from where the time series used for the BRS calculation are extracted.

4.3.1.3. Pulse Transit Time (PTT)

Pulse Transit Time, an indirect indicator of blood pressure fluctuations, is the time delay between aortic valve opening during systole and the arrival of the pulse pressure wave to a peripheral artery, usually the finger. This is typically around 250 msec (0.25sec), i.e. much faster than the time required for the actual blood volume, ejected from the left ventricle to reach the periphery – usually 2.5 sec. PTT is the time delay between each systolic R-wave (ECG) and the corresponding peak on the finger pulse wave (digital photoplethysmography). PTT is inversely related to BP; increase in BP is associated with an increase in the speed and reduction in the time of pulse wave transition and vice versa. The initial phase of apnoeas/hypopnoeas is associated with a lengthening in PTT parallel to the prolonged increase in negative intrathoracic pressure and the decrease in cardiac output and in BP. Towards the end and at apnoea/hypopnoea termination, PTT drops parallel to the increase in sympathetic activity, HR, BP and cardiac output [282]. In conclusion, different measures of autonomic activity confirm that sympathovagal balance is altered in OSAHS patients. A significant part of this thesis focused on the analysis of autonomic activity around apnoeas/hypopnoeas during sleep in OSAHS patients based on time and frequency domain analysis of HRV.

4.4. HAEMODYNAMIC EFFECTS

In the late 19th and early 20th century, Otto Frank and Ernest Starling studied the mechanics of the heart on animals and found that the strength of myocardial contraction is proportional to the initial length of the muscle fibres. Increase in venous return results in an increase in left ventricular filling pressure. This is associated with an increase in the precontractile stretch of the myocardial fibres, increases contractility and results in increase in stroke volume. This myocardial response is independent of neural and humoral mechanisms. The ability of the heart to change its force of contraction and adapt its stroke volume in response to changes in venous return and end-diastolic volume is called the Frank-Starling mechanism (or Starling's Law of the heart) and is summarised in the Frank-Starling curve (figure 4.12.A).

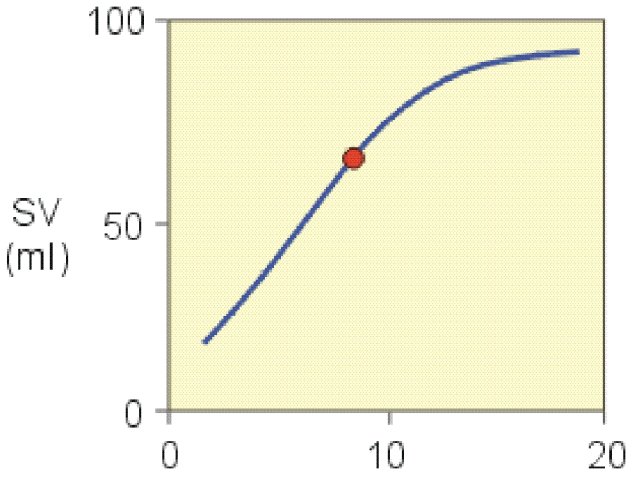


Figure 4.12.A.
The Frank-Starling curve states that myocardial contraction is proportional to the initial length of the muscle fibre.
Increasing venous return to the left ventricle increases left ventricular end-diastolic pressure (LVEDP) and volume, thereby increasing ventricular preload which under physiological conditions in healthy individuals results in increase in stroke volume (SV). Physiological starting points are a LVEDP of approximately 8 mmHg and a SV of 70mls/ beat.

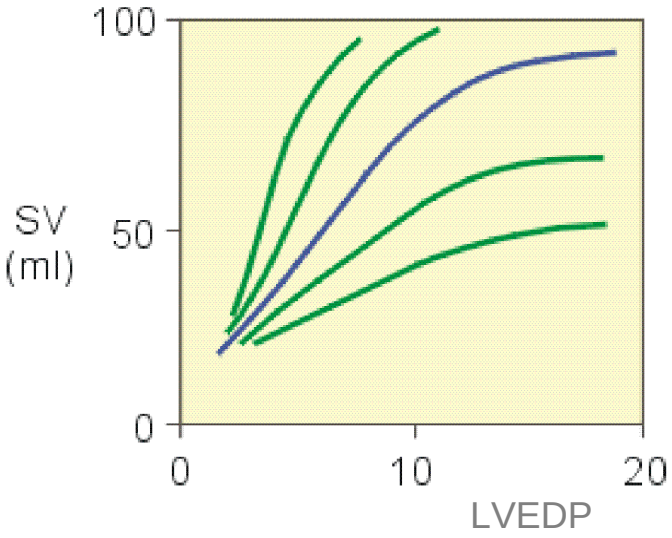


Figure 4.12.B.
The point of the Frank-Starling at which the heart operates under different physiological and pathological conditions is defined by the pre-load, the afterload and the inotropic state of the heart. For example, in heart failure or cardiogenic shock the curve shifts down and to the right. During exercise, the curve shifts up and to the left.

In contrast to the Frank-Starling mechanism, which defines the myocardial contractility as a length-dependant function not influenced by neural or humoral factors, the inotropy is a length-independent contractile function defined by humoral and neural inputs.

Changes in venous return cause the ventricle to move along a single Frank-Starling curve that is defined by the existing conditions of afterload and inotropy, shifting the curve on which the ventricle operates to the left or the right (figure 4.12.B). For example, increasing afterload or decreasing inotropy in conditions such as heart failure or cardiogenic shock shifts the curve down and to the right; decreasing afterload and increasing inotropy during exercise shifts the curve up and to the left.

While the Frank-Starling curves show how changes in ventricular preload lead to changes in stroke volume, they don't show how changes in venous return affect the end-diastolic and end-systolic volume. Increase in venous return equals increase in preload and results in increase in end-diastolic volume. The ability of the ventricle to increase its contractility – under constant afterload – to maintain the same end-systolic volume through the increase of stroke volume is illustrated in the Pressure-Volume loop (figure 4.13). Under physiological conditions, the healthy ventricle increases its stroke volume to match physiological increases in venous return and maintain the same end-systolic volume. This is achieved through increase in inotropy. The increased stroke volume is manifested by an increase in the width of the pressure-volume loop. This is not the case in ventricles that are in failure or during pathophysiological conditions such as apnoeas/hypopnoeas.

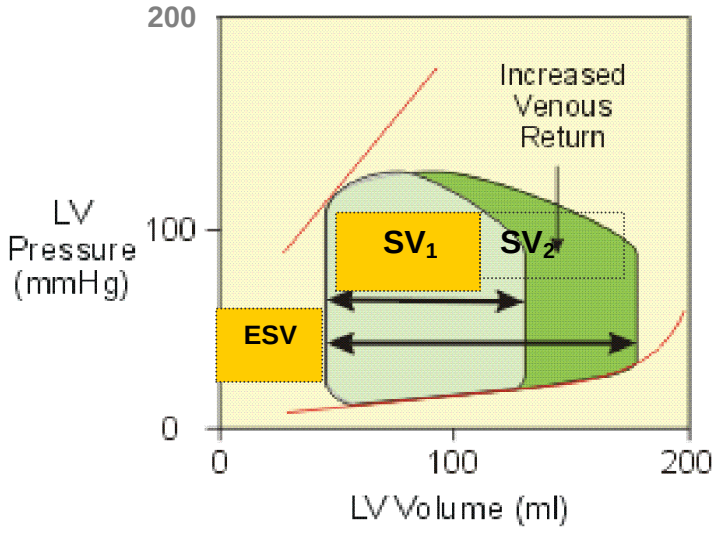


Figure 4.13. Changes in venous return result in changes in afterload under physiological conditions. The degree of shift is influenced by afterload and inotropy.

Haemodynamic changes result from the interplay between altered physiological variables such as changes in venous return and afterload and mechanisms aiming to maintain an adequate Cardiac Output (CO) - the product of Heart rate (HR) and Stroke Volume (SV). In a healthy heart under physiological conditions, the outputs of both ventricles are identical and equal to the venous return. Cardiac output changes to match changing metabolic demands of the body. For example during exercise there is a reduction in afterload due to peripheral vasodilatation and increase in preload due to increased venous return resulting in increased stroke volume and cardiac output. In addition, myocardial contractility and heart rate increase through central sympathetic efferents to contribute to the physiological adjustments of increased metabolism and perfusion demand.

The normalised cardiac output per square metre body surface is termed the Cardiac Index (CI) with values between 2.5- 4.0 l/min/m^2.

Stroke volume is determined by 3 factors: preload, afterload and contractility.

Preload is the ventricular volume at the end of diastole. Under physiological conditions and unchanged afterload, an increased preload leads to an increased stroke volume. Preload is mainly dependent on the venous return, which in turn is influenced by changes in position, intra-thoracic pressure, blood volume and tone in the venous system.

Afterload is the systemic vascular resistance to ventricular ejection. Vascular resistance is inversely proportional to the vascular diameter; it is controlled by the peripheral, postganglionic, noradrenaline releasing sympathetic efferents through alpha-noradrenergic receptors in the arterial muscle wall, resulting in changes in muscle tone and vascular diameter. Increase in postganglionic sympathetic activity results in increasing vascular resistance, which equals an increase in afterload. Under constant inotropic conditions with unchanged preload, an increase in afterload will result in stroke volume reduction with a downward shift of the Starling curve. Conditions associated with changes in stroke volume, such as shock or heart failure will result in an increase in afterload in order to maintain an adequate intravascular pressure and preserve organ perfusion. To achieve this, excitatory peripheral and central sympathetic impulses are necessary. Increase in central sympathetic activity results in increased contractility. The left ventricular transmural pressure (difference between left ventricular and intra-thoracic pressures) is an index of afterload – an increase in afterload is associated with an increase in transmural pressure.

Contractility or inotropy is the ability of the myocardium to contract in the absence of any changes in preload or afterload. It is directly influenced by central, noradrenaline releasing

sympathetic efferent nerve endings through beta-adrenergic receptors in the myocardium. Common negative inotropic factors are acidosis, myocardial ischaemia and beta-blockers.

Changes in preload, afterload, contractility and cardiac output are important pathophysiological consequences of the OSAHS. Guilleminault et al studied 17 OSAHS patients during sleep and monitored the fluctuations in their cardiac index [136]. Changes in cardiac index during and after apnoeas were studied using the method of thermodilution during Swan Ganz catheterisation. The authors found a decrease in CI during apnoeas, i.e. parallel to the decrease in negative intrathoracic pressure and a significant increase following apnoea termination.

Apnoeas/hypopnoeas during sleep are associated with an increase in inspiratory effort resulting from the inspiratory attempts against the occluded/narrowed airway. The Mueller manoeuvre simulates the effects of obstructive apnoea. Used in modelling studies in awake patients with normal blood oxygen levels, the manoeuvre is useful in identifying the haemodynamic effects of apnoeas due to changes in preload and contractility as well as the associated changes in postganglionic peripheral sympathetic activity under normoxic conditions. Using the echocardiographic transoesophageal doppler technique, Bradley et al studied the changes in stroke volume and cardiac output during and directly after the Mueller manoeuvre in healthy individuals and compared them to those of patients with heart failure [45]. The authors also monitored the intrathoracic pressure using oesophageal manometry, heart rate and blood pressure, using a finger plethysmographic devise. For the purposes of this thesis, of importance are the findings in healthy individuals: a steady decline in heart rate, stroke volume and blood pressure was observed during the manoeuvre, resulting in the reduction of CI as well as an increase in left-ventricular end-diastolic volume, associated with a fall in ejection fraction. The latter, in combination with the reduction in negative intrathoracic pressure during the manoeuvre, result in an increase in transmural pressure and afterload. The observed SV reduction is the result of reduced myocardial contractility, leftward shift of the interventricular septum, diminished left ventricular filling and increased peripheral vascular resistance.

Termination of the Muller manoeuvre was associated with an increase in intrathoracic pressure, increase in contractility, stroke volume, heart rate and cardiac index evidenced by the improved left ventricular systolic function parallel to a decrease in transmyocardial pressure and afterload resulting in blood pressure increase.

The changes observed in these haemodynamic parameters, both length-related and non-length related, are associated with a shift of the Frank-Starling curve down and to the right during apnoeas and its repositioning upwards and to the left following apnoea/hypopnoea termination.

The Pressure-Volume loop widens during apnoeas due to the increase in end-diastolic volume. As there is no associated increase in stroke volume, the result is an increase in end-systolic volume and a shift of the loop to the right. At apnoea termination, there is a shift of the loop to the left, to the pre-apnoeic end-systolic volume. Increased inspiratory effort during apnoea/hypopnoea induces a significant reduction in negative intra-thoracic pressure towards the end of apnoeas/hypopnoeas with pressures as low as -30 mmHg or lower [324]. The haemodynamic consequences are increased venous return to the right ventricle with a shift of the inter-ventricular septum to the left, which impedes left-ventricular filling. Increased peripheral sympathetic outflow is associated with an increase in afterload. Consequently, despite the increase in preload, stroke volume and heart rate decrease during the apnoeic episodes resulting in a reduction in cardiac output and blood pressure. These changes are associated with increased left ventricular transmural pressure and reflect compromised left ventricular systolic function. In the chapter on autonomic activity, emphasis was given to the differences in activity between centrally mediated sympathetic efferents to the myocardium and the sinus node which regulate contractility and HR, respectively, and the influences of the peripheral sympathetic efferents on peripheral vascular resistance, afterload and blood pressure [179][355]. Somers et al studied the changes in blood pressure and peripheral sympathetic nerve activity during the Mueller manoeuvre in healthy individuals. Initiation of the manoeuvre was associated with a significant decrease in both measures [324]. Resumption of ventilation induced a marked increase in blood pressure, while sympathetic nerve activity dropped. These observations demonstrate that apnoea onset is characterised by the suppression of centrally mediated sympathetic discharge resulting in a reduction in HR and cardiac output. Peripheral, postganglionic sympathetic discharge is also suppressed at apnoea onset resulting in vasodilatation and blood pressure drop. Resumption of breathing and re-inflation of the lungs is associated with reduction in transmyocardial pressure, increased central sympathetic discharge to the sinus node and the myocardium, resulting in an increase in myocardial contractility and in HR - positive ino- and chronotropy [45]. A consequence of the postapnoeic increase in cardiac index is the rise in blood pressure despite the associated reduction in afterload and vasodilatation, both being the result of reduced peripheral, postganglionic sympathetic discharge to the arterial muscle wall [179] (figure 4.14).

These events are repeated to every apnoea and hypopnoea during the night. Parker et al investigated the acute and chronic haemodynamic consequences of recurrent airway occlusion during sleep in dogs [267]. Obstructed inspiratory effort was found to be associated with increases in peak systolic transmural pressure, end-systolic volume and left ventricular afterload.

Chronic sleep-related airway occlusions led to a sustained decrease in left ventricular systolic performance with decrease in ejection fraction and an increase in end-systolic volume in previously healthy animals. The recurrent increase in systolic transmural pressure, afterload and end-systolic volume intensify myocardial stress and increase the myocardial metabolic demand parallel to reduced cardiac output, coronary perfusion and oxygen supply. These changes may result in nocturnal myocardial ischaemia [112], arrhythmias [176], contractile dysfunction [45], cardiac remodelling [65] and increased peripheral vascular resistance inducing persistent rises in blood pressure [322]. Reversal of apnoeas/hypopnoeas can reverse these pathophysiological processes, normalise sympathetic activity [322], restore BRS for heart rate [349], improve left ventricular function [177], and reduce blood pressure [27].

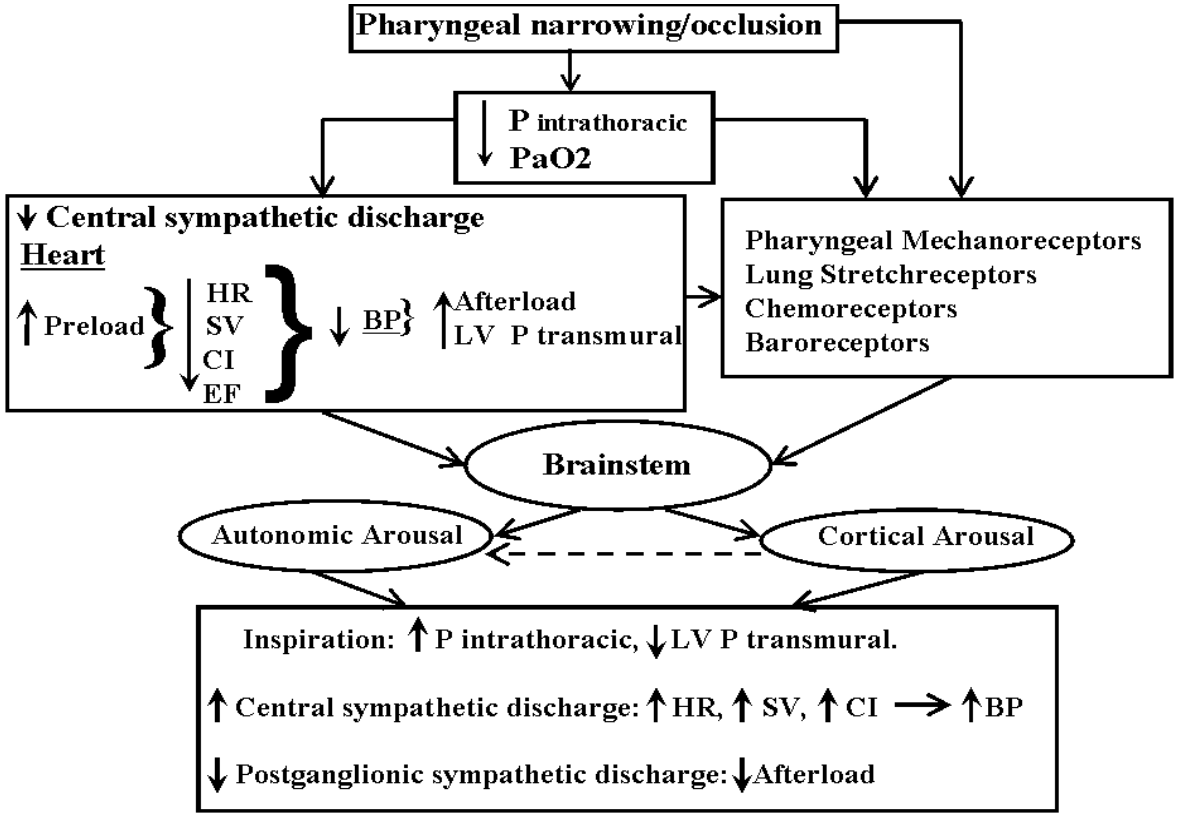


Figure 4.14: At apnoea/hypopnea onset, central and peripheral sympathetic activity is suppressed, whereas towards the end there is an increase in postganglionic sympathetic activity resulting in an increase in afterload. Resumption of breathing is associated with an increase in central and decrease in peripheral sympathetic discharge [179][355].

4.5. OXIDATIVE, INFLAMMATORY, NEUROHUMORAL AND VASCULAR ENDOTHELIAL EFFECTS

Recurrent episodes of apnoeas are associated with changes in sympathetic activity, cardiac output, tissue perfusion and oxygen delivery. Fluctuations in tissue perfusion and oxygen delivery are associated with oxidative stress resulting in increased intracellular free radical production [225]. Oxidative stress is a fundamental component in the pathogenesis of endothelial dysfunction, atherogenesis, cardiovascular and cerebrovascular disease.

4.5.1. Reactive Oxygen Species (ROS)

Free radicals, or reactive oxygen species (ROS), are molecules possessing an electron in the outer orbit and are therefore prone to react chemically – their main intracellular sources are the mitochondria, the peroxisomes and endoplasmic reticulum where cytochrome P450 is localized. A reaction between a radical and non-radical molecule yields a new free radical, propagating free radical chain reactions.

Free radicals are: superoxide anion ($O_2^{\cdot -}$) and hydroxyl free radical ($OH^{\cdot}$) – its precursor is hydrogen peroxide (H_2O_2).

$$O_2 \longrightarrow O_2^{\cdot -} \longrightarrow H_2O_2 \longrightarrow H_2O + OH^{\cdot -}$$

During inflammation, intracellular free radical production is increased; the radicals cause lipid peroxidation in the cytoplasm and the cellular membranes, which is associated with increased membrane permeability leading to influx of calcium and other ions with subsequent swelling of the cells, intracellular damage and apoptosis.

In recent years, nitrogen monoxide [nitric oxide ($NO^{\cdot}$)] – previously known as Endothelium Derived Relaxing Factor (EDRF) - a free radical diatomic gas, has been recognised as the signalling molecule with a key role in central and peripheral neurotransmission, in the regulation of bronchial and vascular tone, in the endothelial vascular homeostasis as well as in inflammation, host defence mechanisms and bacterial and tumour cytotoxicity, hence its application in acute lung injury [234]. In the 1980s, $NO^{\cdot}$ has been recognised as the most potent vasodilating agent. As a potent smooth muscle relaxant, nitric oxide is of major importance in the regulation of vascular tone - hence its clinical application in pulmonary hypertension. For the

purposes of this thesis, the author will explore the importance of NO in the inflammatory cascade and the association with vascular endothelial dysfunction.

The natural source is the guanidine group of the amino acid L-arginine:

$$\text{L-arginine} + \text{NADPH} \longrightarrow \text{L-citrulline} + NO^{\cdot} + NADP^{+}$$

The monooxygenation reaction is catalysed by one of the 3 isoforms of NO-synthetase: neuronal (NOS-I), macrophage or inducible (NOS-II) or endothelial (NOS-III); all isoforms catalyse the oxygenation of L-arginine and a reduction of NADPH in a cytochrome P-450 based reaction. NOS isoenzymes are strongly dependant on calcium and calmodulin. Conditions associated with an increase in intracellular calcium such as shear stress to the vascular endothelium, hypoxia, acidosis or receptor dependant cell stimuli such as bradykinin, an alkali sensitive modulator, are associated with increased NO synthesis [55].

In the vascular endothelial cells, NO is produced through the increased action of endothelial NO synthetase (eNOS) together with L-arginine, the precursor of $NO^{\cdot}$**,** nicotinamide adenine dinucleotide phosphate (NADPH) and other cofactors. NO is a lipophilic, inorganic gas able to diffuse from producer to target cells. It is in a class of its own because its action is not limited by a receptor distribution but by the rate of inactivation following reaction with other free radicals and/or its degradation through stepwise oxidation to the metabolic end products nitrite (NO_2^-) and nitrate (NO_3^-).

Intracellular NO induces univalent reduction of molecular oxygen to superoxide anion; the reaction between 2 superoxide anions with 2 hydrogen cations generates hydrogen peroxide. The reaction of superoxide with hydrogen peroxide generates hydroxyl radical; the latter can cause DNA strand breaks, oxidize proteins and initiate lipid peroxidation. In the presence of superoxide, NO precipitates the formation of peroxynitrite (ONO_2^-); the latter modifies the iron sulfur clusters in proteins resulting in the attenuated action of intracellular enzymes such as ribonucleotide reductase, which is associated with altered gene expression.

During ischaemia and decreased oxygen supply, oxidative phosphorylation is diminished resulting in reduced levels of intracellular ATP. In order to maintain cellular functions, anaerobic glycolysis is activated resulting in increased lactate production and intracellular acidosis. Reperfusion and reoxygenation are associated with increased intracellular free radical production, including peroxynitrite, which trigger the inflammatory cascade leading to leucocyte activation and further tissue injury. Antioxidants such as glutathione – a transporter of NO and

cofactor of NOS – and L-arginine increase the NO levels and protect against reperfusion injury and inflammation [55].

Therefore, in addition to its effect on the regulation of vasomotor tone and hence systemic blood pressure in healthy subjects, nitric oxide may play an important role in the vascular endothelial homeostasis through its ability to inhibit pro-inflammatory events such as platelet activation and aggregation [125], leukocyte adhesion [154] or the macrophage-mediated oxidation of low-density lipoproteins into its high uptake form [169], all of which may contribute towards atherosclerosis [300]. Fluctuations in tissue perfusion and oxygen delivery are associated with impaired expression of eNOS, reduced bioavailability of NO resulting in impaired endothelially mediated vasodilation and predispose to atherosclerosis and arterial hypertension [55]. NO circulates in the bloodstream and is excreted through the alveoli, whereas nitrates and nitrites are found in the bloodstream, urine, faeces and saliva. Asthma is associated with increased levels of exhaled NO, as a result of the inflammatory process within the airways [185].

As the half life of NO is short at around 1 second, the quantification of NO metabolites in biological samples provides information on NO production, bioavailability and metabolism. In plasma or other physiological fluids, NO is oxidized almost completely to nitrite. NO and nitrite are rapidly oxidized to nitrate in the bloodstream. The half life of NO_2^- in human blood is approximately 2 minutes, whereas that of NO_3^- is 5-8 hours. One of the mechanisms by which NO and NO_2^- are converted to NO_3^- is their oxidation through oxyhaemoglobin or oxymyoglobin. Quantification of NO_2^- and NO_3^- provides an index of NO bioavailability [182]. More recent studies confirm that nitrite is useful not only as a marker of NO metabolism but it is a circulating NO donor with properties as a signalling molecule [52].

Ip et al quantified the NO production in OSAHS patients through measurements of its stable metabolic end-products, nitrates (NO_3^-) and nitrites (NO_2^-) in serum and found lower nitrite/nitrate concentrations among OSAHS patients compared to healthy individuals [166]. Following CPAP treatment there was a significant increase in serum nitrite/nitrate levels. These findings demonstrate suppressed bioavailability of nitric oxide which is promptly reversible following CPAP treatment. Given the role of nitric oxide in the acute haemodynamic regulation and in particular its vasodilating, anti-inflammatory properties but also its ability to protect cells from ischaemia-reperfusion injury, these findings suggest that if untreated, OSAHS is associated with endothelial dysfunction.

The latter is supported by the findings of Imadojemu et al who studied reactive vasodilatation and vascular conductance following arterial forearm occlusion in OSAHS and healthy

individuals [165]. Both measures of vascular activity were attenuated in untreated OSAHS patients compared to healthy controls. Following CPAP treatment both measures increased in association with a reduction in sympathetic activity, which argues against irreversible structural vascular changes and in support of reversible neuro-circulatory physiological dysfunction as a cause of the impaired vasodilator capacity.

4.5.2. Inflammatory, Neurohumoral and Vascular Endothelial Effects

Free radicals result in altered gene expression [225]. In monocytes these changes are associated with increased production of adhesion molecules CD11c and CD15 [90]. In cell cultures, adherence of monocytes from OSA patients to vascular endothelial cells was found to be more pronounced compared to healthy controls. Following CPAP treatment the expression of adhesion molecules is down-regulated resulting in decreased leukocyte adherence to endothelial cells in cultures. Increased leucocyte adherence promotes vascular endothelial inflammation and injury, initiates vascular remodelling contributing towards altered endothelial reactivity and atherogenesis [286]. Plasma concentrations of the vascular endothelial growth factor (VEGF), an angiogenic substance which also contributes to the atherogenic process, have been found to be increased in OSAHS patients in proportion to the frequency of apnoeas and the degree of nocturnal hypoxia [201]. VEGF concentrations fall following CPAP treatment. Although these findings do not represent direct evidence of enhanced VEGF-induced atherogenesis in OSA, increased VEGF levels may influence the response of the vascular bed to tissue hypoxia. Endothelin-1 levels, a potent vasoconstrictor produced in endothelial cells, which raises blood pressure and contributes to vascular remodelling, have also been found increased in OSA patients compared to healthy controls [279]. The levels normalised following CPAP treatment. Plasma endothelin-1 levels correlated with changes in mean arterial pressure.

Atherogenesis is a process involving endothelial damage with proliferation of dysfunctional endothelial cells which form atheromatous plaques, attracting growth factors, cytokines, clotting factors and macrophages with activation of the thrombotic chain and proliferation of endothelial and smooth muscle cells. In the big vessels, the process is accompanied by media thickening, disruption of elastic fibres, increase in collagen and calcium deposit. These changes may also affect the morphology and function of the carotid and aortic baroreceptors and alter the baroreflex activity [250][265]. This sequence of events decreases vessel compliance and lumen resulting in a vicious circle: atheroma may contribute towards the development or deterioration

of arterial hypertension which in turn deteriorates atheroma as a result of increased pressure and turbulence [286]. Atherogenesis evolves through a combination of predisposing genetic and environmental factors such as nutrition, nicotine, stressors and may affect any part of the vascular endothelial surface: coronary, cerebral, retinal, renal arteries [286]. Plaque deposition/rupture may induce ischaemia, cerebral/myocardial infarction, arrhythmogenesis with deterioration of the left ventricular function and further worsening of ischaemia. Glomerular filtration and renal function may be also affected. Vascular remodelling is a multifaceted irreversible process. Although as of yet there is no direct evidence that OSA causes atherosclerosis, a number of studies on endothelial dysfunction in this group of patients suggest that facilitated atherogenic processes may play a role in the pathophysiology of cardiovascular morbidity in this syndrome. The increased production of superoxide from neutrophils [312], the elevated plasma levels of different adhesion molecules in OSAHS patients, such as the intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1), L-, E- and P-selectins [41][257] – adhesion glycoproteins originating from leucocytes, endothelial cells and platelets – the inflammatory cytokine tumour necrosis factor-alpha (TNF-a) [357] as well as increased levels of platelet activation and aggregation [41] suggest activated endothelium with accelerated proliferation, migration and adhesion of inflammatory cells which in turn may facilitate vascular dysfunction and remodelling.

The chronic abnormal release of vasoactive substances such as Endothelin-1 and VEGF in combination with hypoxia-reperfusion injury, low NO levels, increased production of free radicals, the pro-inflammatory state and associated metabolic factors such as the increased susceptibility of Low Density Lipoproteins to oxidation (Ox-LDL) [22] - known to cause injury to endothelial cells - and the increased levels of homocysteine [202] - associated with endothelial dysfunction [344] - as well as the augmented sympathetic discharge [251], fluctuations in cardiac index [136], blood pressure [75] and the increased levels of circulating catecholamines [110] are associated with the remodelling of vascular endothelium and impaired vascular compliance which in turn predispose sleep apnoeics to increased vascular morbidity [192].

The described pathophysiological, biochemical and histo-pathological changes are the cornerstone for the development of arterial hypertension [379], for the increased incidence of myocardial infarctions [213][273], strokes [270][375] and for the higher mortality rates [213][238][272] in untreated sleep apnoea patients.

The long-term consequences of the untreated OSAHS as well as current evidence in support of the latter will be discussed in detail in the next chapter.

CHAPTER 5
LONG-TERM EFFECTS OF THE UNTREATED OSAHS

In recent years, better understanding of the pathophysiology of OSAHS and its long-term consequences has encouraged clinicians to treat not only patients with moderate or severe disease but also symptomatic OSA patients with mild disease. The benefits of CPAP therapy [93] as well as the association between the OSAHS and cardiovascular disease were previously questioned by Wright et al [374]. Whilst this controversial review may have triggered larger randomised controlled trials (RCTs) which have confirmed the short-term benefits of CPAP therapy on daytime performance [92][168] and blood pressure [80][98][278] in symptomatic patients, it has in some areas of the UK resulted in funding cuts and compromised sleep service provision within the NHS resulting in many patients having had delayed diagnosis and/or being denied CPAP therapy.

In the past 10 years the adverse effects of untreated OSAHS have been undoubtedly confirmed through a number of large longitudinal cohort and case control studies. Further prospective randomised placebo-controlled study designs, as suggested by Wright et al [374], would be unethical at least in severe or moderately severe patients as randomisation to placebo treatment would mean a potentially increased risk for cardiovascular morbidity. "Non-treatment" over a longer period would also imply withholding the benefits of quality of life improvement related to the improvement in daytime performance in these patients.

I shall discuss the most important studies which confirm the association between sleep apnoea and arterial hypertension, cardiovascular and cerebrovascular disease. Studies on short-term blood pressure benefits are randomised, whereas morbidity and mortality studies are based on longitudinal observations and/or case-control studies with adequate adjustments for confounders.

5.1. ARTERIAL HYPERTENSION

Association between sleep apnoea and arterial hypertension in humans was described by Fletcher et al in 1985 [109]. The prevalence of sleep apnoea was assessed in a group of 46 male hypertensives and compared to the prevalence across 34 male normotensives. Sleep apnoea on polysomnography (AHI>10/hr) was found in 30% (n=14) of the hypertensives and was significantly more prevalent compared to the 9% (N=3) found within normotensive controls.

Brooks et al demonstrated on an animal model the causative association between apnoeas and arterial hypertension [49]. Airway occlusion was induced during nocturnal sleep in 4 dogs over a period of 1-3 months. OSA resulted in acute transient increase in nocturnal blood pressure and sustained daytime hypertension after a period of 4 weeks. Cessation of the OSA normalised the nocturnal BP immediately, whereas the daytime hypertension resolved during a period of 1-3 weeks. In contrast, sleep fragmentation without airway occlusion did not produce the same effects on daytime BP but did result in similar nocturnal hypertension.

Perturbations in blood pressure at apnoea/hypopnoea termination in humans have been described by Davies et al [74][75]. These observations have been attributed to fluctuations in blood oxygen levels, intrathoracic pressure changes as well as to EEG arousals [241], all of which may lead to sustained elevation of blood pressure via pathophysiological mechanisms that include elevated sympathetic tone [251], altered baroreceptor function [265] and endothelial dysfunction [165]. The blood pressure rise is associated with increased secretion of the Atrial Natriuretic Peptide (ANP) from atrial myocytes [195]. The overall effect of ANP is to counteract increases in blood pressure and volume caused by the renin-angiotensin system. ANP causes reduction in renal sodium reabsorption resulting in increased diuresis, reduction of blood volume and cardiac output. The increased vasoconstrictor sensitivity to angiotensin II observed in normotensive OSA patients provides an explanation for the exaggerated blood pressure response suggesting that repetitive sympathetic activation and blood pressure surges may be aggravated during the natural course of OSA [193].

The detection of blood pressure fluctuations at apnoea/hypopnoea termination has been developed by researchers into a diagnostic tool, establishing the Pulse Transit Time – described in chapter 4.3.1 – as an alternative diagnostic method for the OSA [75][281].

Two large epidemiological studies, the Wisconsin Sleep Cohort Study and the Sleep Heart Health Study, have provided robust evidence of the association between OSAHS and arterial hypertension.

Young et al conducted a cross-sectional population-based study during which 1060 men and women aged 30-60 years were studied overnight in the sleep laboratory [379]. Blood pressure was measured on the night polysomnography was performed. The authors found that after adjusting for confounding factors – age, gender, body habitus - the sitting cuff blood pressure increased linearly with increasing AHI. The odds ratio of hypertension associated with an AHI of 15 (versus 0) events per hour was 1.8. During the Wisconsin Sleep Cohort Study, a total of 709 participants were followed for a minimum of 4 years with 184 being followed for 8 years [276]. Logistic regression analysis was used to estimate the odds ratios for the presence of hypertension at follow-up according to the level of sleep-disordered breathing at baseline. The odds ratio of hypertension at follow up was 1.42, 2.03, and 2.89, respectively, for AHI values of <4.9/h, 5.0–14.9/h, and >15/h at baseline. The Wisconsin Sleep Cohort Study prospectively demonstrated a dose-response association between OSAHS at baseline and the presence of hypertension 4 years later. This association was independent of other known risk factors, such as baseline hypertension, body mass index, neck and waist circumference, age, gender, alcohol consumption and smoking. The findings demonstrate that in this cohort the presence of sleep-disordered breathing is predictive of the presence of hypertension four years later.

Nieto and co-workers found a significant association between AHI and hypertension in a cross-sectional sample of 6132 men and women from different ethnic groups aged $\geq$ 40 years who participated in the ongoing Sleep Heart Health Study, which represents the largest multi-centre prospective study to date [254]. During the study, seated cuff blood pressure was measured and unattended home polysomnography was performed on all the 6132 participants. Hypertension was defined as a blood pressure greater than 140/90 mm Hg or the use of antihypertensive medication. Logistic regression was used to calculate the odds ratio of hypertension across the different severity groups of OSA, while adjusting for known confounders such as body mass index, neck circumference, waist-to-hip ratio, alcohol intake and smoking. The odds ratio of hypertension comparing the highest AHI (>30 events/hr) with the lowest (<1.5/hr) was 1.37. Relative to an AHI less than 1.5, the odds ratios were 1.1, 1.2, 1.3, and 1.4 for AHI categories of 1.5 to 5, 5 to 15, 15 to 30 and $\geq$ 30 with increasing trend in the odds ratios and all but the lowest odds ratio were significantly greater than one. No significant association was found between blood pressure values and arousal indices. The percentage of sleep time spent in oxygen saturations below 90% showed an odds ratio of hypertension of 1.46. The results confirm the association between OSA and arterial hypertension, independent of other known risk factors.

Bixler and co-workers also found a significant association between OSA and hypertension in a Pennsylvanian cohort of 1741 men and women, aged 20-100 years [37]. The associations indicated a stronger relationship between OSA and hypertension in younger and less obese participants than in older, heavier participants. These findings correspond well with those of Young et al [379], who also found a stronger cross-sectional association of OSA and hypertension in less obese participants in the Wisconsin Sleep Cohort, and those of Nieto and co-workers, who found a stronger association in younger subjects [254].

Lavie et al examined the association between OSAHS and arterial hypertension in 2677 adults, aged 20-85 years referred to the sleep clinic over a 10 year period [204]. All subjects underwent overnight polysomnography for suspected sleep apnoea syndrome as well as morning and evening blood pressure measurements. Multiple logistic regression analysis of blood pressure levels showed that apnoeas/hypopnoeas were significant predictors of blood pressure values after adjustment for age, body habitus and gender. Each additional increase in apnoea index increased the odds of hypertension by approximately 1% whereas each 10% decrease in nocturnal oxygen saturation increased the odds by 13%. The findings confirm that OSAHS is an independent risk factor for arterial hypertension with the odds of hypertension increasing with disease severity and hypoxia gaining further support as an aetiological mechanism.

Grote et al investigated the influence of disease severity on daytime blood pressure and the association with arterial hypertension in 1190 individuals referred for suspected OSAHS [128]. The authors found a dose-related association between OSAHS and daytime blood pressure independent of age, body mass index, gender, cholesterol level, alcohol consumption and smoking. The odds ratio of hypertension increased with increasing apnoea/hypopnoea index and was more prevalent in younger ($\leq$ 50 years), less obese individuals.

Davies et al carefully matched 45 symptomatic patients with OSAHS with 45 non-apnoeic controls taken from a primary care setting with regard to age, body mass index, alcohol and cigarette consumption, hypertension treatment, and the presence of ischaemic heart disease [73]. Ambulatory blood pressure monitoring over 24 hours in both groups revealed that daytime and night time diastolic as well as night time systolic blood pressure was significantly higher in OSAHS patients than in controls. Upper versus central body fat distribution, as indicated by the waist:hip and waist:height ratios, was found to be a strong predictor of arterial pressure. The differences in blood pressure can not be attributed to differences in body fat distribution, as this was similar in both groups.

Faccenda et al conducted a randomised placebo-controlled cross-over study during which 68 normotensive symptomatic OSAHS patients underwent 4 weeks of CPAP treatment during the one study limb versus oral placebo in the other [98]. Blood pressure monitoring was performed during the last 48 hours of each study limb. Following CPAP treatment, diastolic blood pressure decreased significantly in patients who used CPAP $\geq$ 3.5 hrs per night and in those patients who had more than twenty 4% desaturations per hour. Although the improvement in blood pressure was small during the 4-week follow-up period at a mean of 4 mmHg in the systolic and 2 mmHg in the diastolic values, the findings support that treatment of the OSAHS may reduce blood pressure particularly in those patients with nocturnal oxygen desaturations.

A further randomised trial conducted by Pepperell et al, during which sub-therapeutic CPAP (1cm H_2O) was used in the placebo limb, assessed the changes in blood pressure during a 4-week period in 2 groups of 59 symptomatic OSAHS patients assigned either to the therapeutic or the sub-therapeutic group [278]. Based on a 24-hr ambulatory BP monitoring at the end of the 4-week period, a significant reduction in the mean BP was also observed in the group of patients who received adequate CPAP treatment. The reduction was independent of the baseline blood pressure but improvement was more pronounced in patients established on antihypertensive medication prior to CPAP and in patients with more severe OSAHS.

These randomised trials confirm blood pressure reduction following CPAP treatment in hypertensive, symptomatic OSAHS patients. They add to the epidemiological studies by attributing causation of hypertension to sleep apnoeas/hypopnoeas and by showing a reduction in blood pressure following CPAP therapy.

In summary, apnoeas and hypopnoeas during sleep are associated with the development of arterial hypertension, independent of other factors such as obesity, body fat distribution or baseline blood pressure at the time of the diagnosis. It can precede and predict the onset of hypertension. The risk appears to be higher in the severe spectrum of the disease and in younger less obese patients. Following CPAP treatment, diurnal and nocturnal blood pressure values improve providing significant vascular risk benefits. OSAHS is a cause of secondary hypertension and its treatment improves blood pressure control and reduces cardiovascular morbidity and mortality rates [276][278]. The prevalence of the OSAHS is approximately 3% among adults in their most productive years - 2% in middle-aged women and 4% in middle-aged men [378]. With the population in Europe being 730,000,000 and in North America 330,000,000 OSAHS may occur in around 32 million people in the Western world. It is estimated that 60% of the OSAHS patients are hypertensive, i.e. ca 19 million people [254].

Worsnop et al conducted a cross-sectional population-based study during which 93 middle-aged men and women were studied overnight in the sleep laboratory [373]. Hypertension was diagnosed following a 24-hour ambulatory monitoring prior to the sleep study. Thirty-four subjects were found to be untreated hypertensives, 34 were treated hypertensives and 25 participants were normotensive. Thirty-eight percent of the untreated hypertensives, 38% of the treated hypertensives and 5% of the normotensives had an AHI>5/hr. Logistic regression analysis confirmed that BMI, age, sex and hypertension were associated with OSA. The association between OSA and hypertension persisted after allowing for the confounders age, sex and BMI.

With the prevalence of hypertension being approximately 28% in North America and 44% in Europe [372], we extrapolate that OSA (AHI>5/hr in the presence or absence of symptoms) may contribute to hypertension in approximately 157 million people in Europe and North America. The difference between the prevalence of OSA and the number of hypertensive sleep apnoea patients suggests that a significant proportion of these patients remain undiagnosed possibly due to mild and/or asymptomatic disease. However, the outcomes of the studies depicted above suggest that hypertension and its improvement following CPAP treatment are more pronounced in the severe spectrum of symptomatic OSAHS patients. The latter is reinforced by two more recent studies. A randomised placebo-controlled study by Barbe et al compared changes in blood pressure during 24-hr recordings in 29 non-sleepy normotensive patients with an AHI>30/hr after therapeutic CPAP, to the BP changes in a group of 25 asymptomatic normotensive patients with the same disease severity following administration of sham CPAP [20]. No significant difference in blood pressure was established between the 2 groups following 6 weeks of therapeutic versus sham CPAP. Robinson et al studied the changes in blood pressure in 35 hypertensive asymptomatic OSAH patients with a mean of 28 dips/hr of >4% desaturations in a randomised cross-over trial [294]. Therapeutic versus sub-therapeutic CPAP was applied during 4-week periods. The majority of participants were established on antihypertensive medication with good levels of BP control. No significant difference in BP was found following each intervention. Although both studies are limited by the number of subjects studied and consequently the lack of adequate power to detect differences, they do suggest that improvement in BP levels following CPAP are different between symptomatic and asymptomatic patients. This may be due to the fact that non-sleepy patients do not have significant fluctuations in nocturnal BP and no sustained daytime hypertension. Non-sleepy OSA patients may therefore form a less susceptible group to the long-term cardiovascular consequences of the disease.

To further investigate the estimated prevalence of sleep apnoea among hypertensives and understand the pathophysiology and differences to normotensive sleep apnoeics, as well as the treatment benefits in sleepy and non sleepy patients with good and poor BP control, larger cross-sectional studies with subgroup analysis and long-term effects are required.

If the estimated prevalence of sleep apnoea in hypertensives is confirmed at 30% and long-term cardiovascular benefits are established, then the aims of CPAP treatment may be modified from a modality that improves sleepiness to one that prevents and improves arterial hypertension reducing cardiovascular and cerebrovascular morbidity and mortality. Consequently, the question arises at which level of severity and/or clinical symptoms the syndrome ought to be treated to prevent these long-term consequences.

5.2. CARDIAC DISEASE

5.2.1. Ischaemic heart disease

A number of case-control studies have shown increased prevalence of OSA in patients with coronary artery disease (CAD). The increased incidence, morbidity and mortality due to cardiovascular disease (CVD) among OSA patients compared to non-OSA CVD patients and to healthy individuals as well as the benefits in morbidity and mortality from CPAP therapy have been shown in prospective cohort studies with adjustment for the effect of confounding factors. The use of randomised designs in this kind of investigations, as suggested by Wright et al [374], would now be unethical. Some of the important studies are discussed below.

In a case-control study Hung et al examined the prevalence of OSA among 101 male patients, aged 37-65 years, who suffered a recent myocardial infarction (MI) and compared it to the prevalence in 53 matched healthy men [163]. Polysomnography confirmed that 36% of post-MI patients had an AI>5/hr and 14% an AI>10/hr. In the control group, 3.8% had an AI>5/hr and 1.9% had an AI>10hr. After adjusting for confounding risk factors – BMI, hypertension, smoking and cholesterol - multiple regression analysis revealed a significant association between AI and MI (OR 23.3) with a graded increase in the odds of MI with increasing AI, confirming that OSA is an independent risk factor.

Mooe et al studied the prevalence of OSA in 136 symptomatic male patients with CAD defined as >50% stenosis in at least one coronary artery on angiography, aged 35-70 years, and compared it to the prevalence in 49 age matched healthy men [238]. OSA was diagnosed based

on a lab-based sleep study and a questionnaire. The prevalence of OSA (AHI≥10/hr) was 37% among the CAD patients and 20% in the controls group. AHI was an independent predictor of symptomatic CAD in a multiple regression analysis with a graded increase in the odds of CAD with increased OSA severity; the OR of symptomatic CAD for an AHI≥14/hr was 4.5 compared to an AHI≤5/hr. Assuming a causative link between OSA and CAD, the risk ratio for CAD in male patients with OSA is 1.8 times higher compared to healthy men. The prevalence found in men with no CAD is comparable to that of 24% found by Young et al among 602 employees, aged 30-60 years using an AHI≥5/hr [378].

In another case-control study, Peker et al assessed the prevalence of OSA in a group of 62 hospitalised patients, aged 44-88 years, following myocardial infarction or angina [272]. The patients were matched for age, sex and BMI with 62 healthy controls. Following a lab-based diagnostic procedure and a completion of a questionnaire, the prevalence of OSA was 30% in the CAD group and 12% in the controls group. The diagnosis of OSA was based on a Respiratory Disturbance Index (RDI) ≥ 10/hr – RDI: change in respiratory pattern plus desaturation ≥ 4%. The prevalence of OSA found in the control group was lower than that of 21%, found by Young et al [378]among 602 employees, aged 30-60 years, based on an AHI≥ 5/hr. In this study the prevalence of OSA in CAD patients was 2-fold greater than in non-CAD individuals. Multiple logistic regression analysis confirmed that OSA, smoking and diabetes are independent risk factors of CAD but not hypertension or hypercholesterinaemia, likely due to their high prevalence in the controls group.

In a further study, Peker et al explored the incidence of cardiovascular disease (CVD) in 182 men, aged 30-69 years, during a 7-year follow-up referred to the sleep centre for possible OSA and no other co-morbidities [273]. Sixty individuals were diagnosed with OSA, 122 were healthy. The OSA patients were divided into 2 subgroups: the adequately treated CPAP tolerant patients (n=15) and the incompletely treated CPAP intolerant patients (n=37). There were 5 deaths, 2 due to CVD among the incompletely treated patients. The incidence of arterial hypertension, CAD (angina, myocardial infarction) and/or CVD event (cardiac arrhythmias, congestive cardiac failure, stroke, cardiovascular death) were the outcome measures. The incidence of at least one of the CVD manifestations was 36.7%, i.e. significantly higher in the OSA group regardless of treatment, compared to 6.6% in the healthy group. In other words, the incidence of CVD in the OSA group was 6-fold greater than in the non-OSA individuals. In a multiple logistic regression model after adjusting for smoking, BMI and BP at baseline, OSA (OR 4.9) and age (OR 23.4) at baseline were found significant predictors of CVD incidence.

Adequate CPAP treatment was associated with significant risk reduction for CVD incidence (OR 0.1) after adjusting for age and BP at baseline.

In a prospective 5-year follow-up study Peker et al compared the mortality rates due to cardiovascular disease in 16 patients with CAD and untreated OSA (mean RDI 17.5/hr), to the mortality of 43 patients with CAD but no OSA, matched for age (64-82 years), gender, BMI, BP at baseline, cholesterol levels, history of smoking and diabetes [274]. Among OSA patients cardiovascular deaths were 4 times higher than in non-OSA patients (37.5% versus 9.3%). Multiple regression analysis confirmed that the RDI is an independent predictor of cardiovascular death.

In a larger prospective observational 10 year follow-up study the incidence of fatal (MI or stroke) and non-fatal (MI, stroke, coronary artery bypass surgery and percutaneous transluminal coronary angiography) vascular events was compared in 377 male snorers, 638 OSA male patients intolerant of CPAP treatment (403 mild-moderate, 235 severe OSA), 372 OSA male patients established on CPAP therapy, all referred to the sleep unit for further investigations during a 2-year period and 264 healthy men recruited from the general population, mean age 50 years [213]. OSA was diagnosed on nocturnal polysomnography. Untreated OSA patients in the severe spectrum (AHI>30) of the disease had significantly higher incidence of fatal (1.06 per 100 person-years) and non-fatal cardiovascular events (2.13 per 100 person-years) compared to untreated mild-moderate OSA patients (0.55 and 0.89), simple snorers (0.34 and 0.58), treated OSA patients (0.35 and 0.64) and healthy participants (0.3 and 0.45). Multivariate analysis adjusted for potential confounders, confirmed that untreated severe OSA increased the risk of fatal (OR 2.87) and non-fatal (OR 3.17) vascular events compared to healthy men. Although these outcomes are moderately robust, they are limited by the sleep clinic referral bias, the non-randomised nature of treatment and the male population studied.

Worsening of the long-term prognosis in CAD patients in the presence of OSA has been confirmed in a large prospective cohort study by Mooe et al [238]. Of the 407 recruited CAD patients aged 70 years or younger diagnosed on angiography, 153 (38%) had sleep disordered breathing (SDB) based on ODI$\underline{\geq}$5/hr, 254 did not. Only 2 of the OSA patients accepted CPAP treatment; 77.5% (n=316) underwent a coronary intervention during the follow-up period. The primary end point was a composite of stroke, TIA, MI and death from any cause. During a median follow-up period of 5-years, the composite end point occurred in 26% (n=40) of CAD patients with SDB - 28% (n=32) of men and 20% (n=8) of women. The corresponding percentages among CAD patients without SDB were 15% (n=38) - 16% (n=26) of men and 14%

(n=12) of women. Kaplan-Meier graphs of the cumulative 5-year survival function revealed a clear deviation between the 2 groups/curves with a greater decline in the SDB CAD patients and a difference in the composite end point of approximately 11% at 5 years – 74% vs 85% event-free survival in the 2 groups. Risk ratio calculation revealed a 1.7-fold higher risk of death, 1.3-fold higher risk of MI and a 3-fold higher risk of cerebrovascular events among CAD patients with SDB. Multivariate analysis of hazard ratios for the composite end point of death, MI and cerebrovascular event revealed that an ODI≥5/hr, diabetes and left ventricular dysfunction all increased the risk of an end point, coronary intervention decreased the risk. The models also included the variables of age, sex, BMI and hypertension. Single end point analysis revealed that ODI≥5/hr and AHI≥10/hr were both independently associated with cerebrovascular events but neither was independently predictive of the single end points of death or MI. Diabetes was the only additional independent predictor of cerebrovascular disease. The models also included the variables of age, sex, BMI, hypertension, left ventricular dysfunction and coronary intervention.

In summary, the prevalence of OSA among CAD male and female patients has been shown to be 2-fold that of healthy individuals, whereas studies in male patients have shown up to 7-fold increase in OSA prevalence compared to healthy men. A graded increase in the odds of CAD with increasing disease severity has been shown in these studies. The incidence of CVD in male OSA patients is up to 6 times higher compared to healthy men. CPAP treatment is associated with significant risk reduction for CVD. Mortality due to CVD in OSA patients has been shown to be up to 4 times higher compared to non-OSA patients with CVD. The mortality risk in CAD patients increases with age and severity of OSA – based on ODI or AHI. Repeated nocturnal hypoxia, in conjunction with simultaneous increases in intrathoracic and transmural myocardial pressures, decreased contractility, impaired myocardial perfusion [45], increased sympathetic activity [251], depressed baroreflex sensitivity [250][265], fluctuations in blood pressure [75], accelerated inflammatory processes [90], increased ROS [166], platelet activation and aggregation [41], endothelial dysfunction [165], are proposed mediating pathophysiological mechanisms which promote vascular remodelling [286], structural arterial damage and cardiovascular dysfunction. The long-term consequences of these and other mechanisms described earlier support that untreated OSA is a potential trigger for cardiac ischemia and vascular disease.

The findings of the studies depicted above, suggest that OSA is an independent risk factor for CVD related morbidity and mortality. Although the causative pathways are not entirely

established, it is evident from the above studies that the causative link between OSA and CVD is not solely mediated by hypertension.

Although no randomised trials of the effects of OSA treatment on risk of developing coronary artery disease, myocardial infarction or risk of cardiovascular death are available, the above studies suggest a risk reduction among treated OSA patients with greater benefit in the severe end of the disease spectrum, based on either the ODI or the AHI. The use of more restrictive definitions for the diagnosis of OSA with higher AHI cut-points or an additional requirement for symptoms of sleepiness will obviously lower prevalence estimates and affect values expressing associations. It is therefore important to ascertain at which level of severity and/or clinical symptoms the syndrome ought to be treated to prevent these long-term consequences in order to integrate markers of OSA severity in models of secondary prevention of CVD.

5.2.2. Cardiac arrhythmias

Association between OSA and nocturnal arrhythmias has been demonstrated in a number of observational studies, none of which have convincingly shown an independent causative relation between OSA and nocturnal arrhythmias. The most important studies are discussed here.

A study conducted by Guilleminault et al revealed that brady-arrhythmias were the most commonly observed nocturnal arrhythmias, being found in 26% of 400 OSA patients [133]. Extreme sinus bradycardia (<30 beats/min) and sinus arrest $\geq$2sec occurred in 11%, second-degree atrio-ventricular block (Moebitz type I and II) in 8%, whereas ventricular arrhythmias occurred in 20% of the 400 OSA patients. All supra-ventricular arrhythmias resolved following tracheostomy. Similarly, Becker et al reported reversal of brady-arrhythmias in the majority of the 17 patients studied, following effective CPAP treatment [26].

During apnoeas/hypopnoeas, the reduction in intrathoracic pressure in association with increased venous return and hypoxic chemoreceptor stimulation result in increased peripheral sympathetic activity parallel to the reduction in heart rate induced by cardiac vagal activation. The mechanism behind the apnoea-related brady-arrhythmias is known as the Diving Reflex (DR). Features of the DR are peripheral vasoconstriction which preserves blood flow to the brain and heart vessels and bradycardia as a means of limiting myocardial oxygen demand.

The prevalence of nocturnal arrhythmias was studied in two age-, sex-, race- and BMI-matched groups of participants from the Sleep Heart Health Study (SHHS) cohorts [228]. A group of 228 individuals with an AHI$\geq$30/hr, mean age 71 years and, was compared with a group of 338

healthy participants, mean age 69 years and AHI<5/hr. Significantly more prevalent nocturnal arrhythmias in the OSA group were atrial fibrillation (AF) (4.8% vs 0.9%) and complex ventricular ectopy including bigeminy (14% vs 8%), quadrigeminy (11.8% vs 5.9%) and non-sustained ventricular tachycardia (5.3 vs 1.2%). When adjusted for age, sex, BMI and CAD, the odds of complex arrhythmias among OSA patients were 2- to 4-fold that of healthy controls. The prevalence of bradycardias and conduction delays was similar in both groups.

Kanagala et al monitored the recurrence rate of AF during a 12-month period following electrical cardioversion in a group of 12 effectively treated compared to 27 not effectively treated OSA patients (25 untreated, 2 noncompliant), mean age 67 years [176]. There was no significant difference in the mean BMI, the prevalence of hypertension or diabetes in the two groups. Effective CPAP treatment was associated with significantly lower recurrence rate of AF during the first year following cardioversion: 82% in the untreated group versus 42% in the treated group. Effectively treated OSA patients had a recurrence rate similar to that of the general population [206], whereas untreated OSA patients had a 2-fold higher risk of AF recurrence following successful cardioversion compared to treated OSA patients. CPAP treatment is associated with a significant reduction in arrhythmia recurrence which appears to be independent of age, BMI or co-morbidities such as hypertension or diabetes. The prevalence of AF in the middle aged population is 1%, i.e. the commonest arrhythmia; it becomes more prevalent among older individuals [206]. The application of CPAP in untreated OSA patients with AF is a rewarding therapeutic strategy not only for the treatment of the OSAHS but also as a modality likely to reduce AF recurrence. Although a causative association between OSA and AF has not been established, the increased prevalence of AF among untreated OSA patients would consequently suggest that obese patients presenting with AF should be assessed clinically and if appropriate investigated for OSA.

In a group of 10 patients with nocturnal angina studied by Franklin et al, 9 patients were found to have OSA [112]. Following CPAP treatment both the symptoms and the electrocardiographic appearances improved. Nocturnal myocardial injury was assessed by Gami et al in 15 male OSA patients, mean age 62 years, with an AHI>15/hr and established CAD [116]. Troponin T was used as a marker of myocardial injury. In the absence of any troponin rise in serum, nocturnal myocardial injury was excluded in this group of patients.

In a further study, Gami et al reviewed the time of sudden death from cardiac causes in 112 previously diagnosed OSA patients [115]. Sudden death from cardiac causes between midnight and 6 am occurred in 46% of those with OSA. The likelihood of sudden cardiac death in the

general population and in patients free of OSA is at its highest between 6 and 12 am, the window of cardiovascular vulnerability [64]. The peak in sudden deaths from a cardiac cause among OSA patients is during sleeping hours and therefore in striking contrast with the time of sudden cardiac deaths in the general population. These findings indicate that OSA may affect the timing of cardiac death possibly due to the associated increased nocturnal sympathetic activity and/or the increased prevalence of nocturnal dysrhythmias. However, no objective measures of CPAP compliance among the diseased were available in this study.

In conclusion, OSA is associated with increased prevalence of atrial fibrillation, non-sustained ventricular tachycardias, bigeminy, quadrigeminy as well as brady-arrhythmias and conduction delays. The prevalence of these arrhythmias decreases following CPAP treatment. Recently published data from the European Multi-Center Polysomnographic Study demonstrate a very high prevalence of OSA (59% of the 98 participants) among patients fitted with pacemakers [118]. The vast majority of the OSA patients were asymptomatic (mean ESS 7/24). The most common arrhythmias among OSA patients leading to the pacemaker insertion were atrio-ventricular block (68%) and sinus node disease (58%). These observations suggest that OSA should be considered as a potential triggering factor of arrhythmias prior to pacemaker insertion. Current knowledge would suggest that effective CPAP treatment may improve arrhythmias. Whether CPAP can reduce the rate of pacemaker insertions remains to be seen. In order to minimise the long-term consequences of OSA, physicians should have a low threshold of suspicion and treatment in paced patients, as OSA prevalence is high in this subgroup. However, effective CPAP treatment in asymptomatic patients can be challenging.

Pro-arrhythmogenic mechanisms in the context of OSA include haemodynamic, neurohumoral and metabolic factors. During apnoeas the increased after-load in combination with the transmural pressure rise lead to dimensional atrial changes and stimulation of atrial stretch-responsive channels [40]. Apnoea-induced hypoxia has direct adverse effects on cardiac electrical stability [1]. The synergistic effect of these factors together with increases in circulating catecholamines and inflammatory mediators may reduce the pro-arrhythmogenic threshold. The prospective observational study by Marin et al has confirmed that OSA is a risk factor for fatal and non-fatal cardiovascular events [213]. Gami et al established in a cross-sectional analysis that OSA may affect the timing of cardiac death [115]. Although the latter study does not confirm a causative association between OSA and sudden cardiac death, the outcomes of both studies demonstrate that cardiac death in the presence of OSA is more

common, possibly due to its effects on vascular and myocardial remodelling and its pro-arrhythmogenic influences.

As randomised trials of the effects of OSA treatment on the risk of developing arrhythmias would be unethical, further longitudinal cohort studies and outcome-based interventional case-control trials are needed to establish whether CPAP treatment would reduce the risk of further sustained arrhythmias as well as future cardio- and cerebro-vascular events, in particular those associated with atrial arrhythmias.

5.2.3. Heart Failure

Heart failure is a major public health problem with 500,000 newly diagnosed patients per year and a prevalence of 1.5% [164]. Hypertension [205], obesity [183] and nocturnal oxygen desaturations to <70% [114], factors common to OSA patients, have been identified as independent factors for the development of ventricular dysfunction. The longitudinal Framingham Heart Study has established that hypertension is the most common non-ischaemic risk factor for the development of heart failure. Two large epidemiological studies, the Wisconsin Sleep Cohort Study and the Sleep Heart and Stroke Study have provided robust evidence of an association between OSA and arterial hypertension [254][379]. In the Sleep Heart Health Study cohort of 6424 men and women, aged 40 years or older, OSA was a significant predictor of heart failure (OR 2.3) [315].

The most direct mechanism by which OSA may be associated with heart failure is by causing arterial hypertension, ventricular hypertrophy and myocardial remodeling. Increased sympathetic activity [72][251][322], down regulation of adrenoreceptors [72][127], reduced baroreceptor sensitivity [72][250][265], increased vascular resistance in combination with the increased preload, the associated raised transmyocardial pressure and reduction in myocardial perfusion [45][72] are pathophysiological mechanisms common to both HF and OSA patients and are fundamental to the progression of myocardial remodeling and dysfunction [65][72][164]. The prevalence of OSA in HF patients and its effect on mortality have been investigated by Wang et al in a prospective observational study of 169 consecutive patients, mean age 55 years, referred to the heart failure clinic [361]. Five of the patients were lost at follow-up, 164 patients (mean BMI 30 kg/m^2,120 males) completed the 7-year follow-up period. OSA (AHI$\geq$15/hr) was present in 26% (n=51) of the HF patients. Unfortunately, there was no control group but the prevalence found in this study among HF patients was higher than the prevalence of 9% in men and 4% in

women of similar age and using the same diagnostic cut-off (AHI≥15/hr), selected from the general population by Young et al [378]. Young et al found that obesity and male sex were strong risk factors for OSA.

Of the 51 HF patients diagnosed with OSA by Wang et al, 14 were CPAP compliant, 37 were not [361]. Multivariate proportional hazards analysis confirmed a significantly higher mortality among untreated OSA HF patients (24%) compared to the non-OSA HF patients (12%) (hazard ratio:2.8, p=0.029) after adjusting for the following significant confounders: ejection fraction, NYHA functional class and age. No deaths occurred in the treated OSA HF group. This trend to reduced mortality in the treated versus the untreated OSA group was not statistically significant (p=0.07), possibly due to the small numbers in each group. These results agree and complement the study outcomes by Marin et al, who established that untreated severe OSA (AHI>30/hr) significantly increases the risk of fatal (MI or stroke) and non-fatal (MI, stroke, coronary artery bypass surgery and percutaneous transluminal coronary angiography) cardiovascular events in 377 male snorers, 638 OSA male patients intolerant of CPAP treatment (403 mild-moderate, 235 severe OSA), 372 OSA male patients established on CPAP therapy, all referred to the sleep unit for further investigations during a 2-year period and 264 healthy men recruited from the general population, mean age 50 years [213]. The study by Wang et al is free from the sleep clinic referral bias, as the patients - male and female- were recruited from an unselected HF population and were included regardless of the presence or absence of sleepiness [361]. This study therefore suggests that untreated OSA has adverse effects on survival in diverse populations of HF patients regardless of the degree of their sleepiness (mean ESS: 8/24).

In a prospective randomised placebo-controlled double-blind crossover study, Arias et al assessed and compared the changes in diastolic ventricular function following 3 months of therapeutic versus sham CPAP in 27 normotensive OSAS male patients (AHI≥ 10/hr, ESS≥ 10/hr), mean age 52 years, and 15 healthy controls, mean age 48 years [11]. At baseline, diastolic dysfunction was found in 15 OSAS patients (56%) versus 3 healthy controls (20%) (p=0.02). In OSAHS patients, 3 months of effective CPAP therapy significantly improved left ventricular diastolic function compared to sham CPAP. These findings suggest an association between OSA and ventricular dysfunction in the absence of arterial hypertension. Prior to the onset of remodelling and the development of advanced structural myocardial changes, CPAP can improve or even reverse left ventricular dysfunction.

Cardiac remodelling is the determinant of the clinical course of heart failure [65][72]. In heart failure patients with coexisting OSA, sympatho-adrenergic activity, BP and consequently

disturbances of circulatory and haemodynamic control are exacerbated during sleep but also during the day [326], placing these patients at greater risk of worsening ventricular function, arrhythmogenesis and increased morbidity and mortality [213][238].

Large cross-sectional case-control studies may be able to confirm a significant association between OSA and HF in the future. Longitudinal studies are needed to address the questions of worsening morbidity and increased mortality in HF patients in the presence of OSA and consequently, whether CPAP treatment reduces hospitalization rates and improves prognosis.

5.3. PULMONARY HYPERTENSION

In 1947, Motley and colleagues [245] demonstrated that breathing a gas mixture containing 10% oxygen induced a rise in pulmonary arterial pressure (PAP). This hypoxic pulmonary vasoconstriction is an autoregulatory mechanism important in maintaining an appropriate ventilation /perfusion relationship [358]. Over time, hypoxic vasoconstriction may result in pulmonary vascular remodeling, which may or may not be reversible, potentially contributing to the development of secondary pulmonary hypertension (PH).

OSAHS is a recognised cause of mild pulmonary hypertension (PH). Chaouat et al studied the pulmonary haemodynamics in 220 consecutive OSA patients (AHI >20/hr), mean age 53 years, mean weight 32 kg/m^2 [59]. Thirty seven of the patients (17%) had PAP >20 mmHg, 2 patients in this subgroup had PAP>35mmHg. Pulmonary artery wedge pressures were less than 13mmHg - cardiac causes of PH were therefore excluded. Patients in this subgroup tended to be more obese, more hypoxic and 16 (57%) of the 37 patients had coexisting COPD. Thus, mild PH is common in OSA patients, often in the presence of daytime hypoxia due to COPD (overlap syndrome) or obesity hypoventilation. Consequently, sleep studies are part of the recommended diagnostic process in patients diagnosed with PH [16].

The most likely mechanisms of PH in OSA patients are hypoxia [358] and vascular remodelling resulting in increased pulmonary vascular resistance [308]. Sajkov et al measured the pulmonary haemodynamics before and after CPAP therapy in a cohort of 20 otherwise healthy OSA patients, mean age 50 years [308]. The study design did not include a control group. Following 4 months of CPAP treatment, PAP and pulmonary vascular reactivity to hypoxia decreased significantly ($p<0.05$). The decrease was more pronounced in a subgroup of 5 patients with PH at baseline (PAP>20mmHg). The findings support the hypothesis that CPAP can reverse

endothelial dysfunction prior to established vascular remodelling and improve pulmonary haemodynamics. Larger randomised placebo-controlled trials are required to ascertain the effect of CPAP on pulmonary haemodynamics and establish its role in the treatment of pulmonary hypertension.

5.4. CEREBROVASCULAR DISEASE

OSA is a prothrombotic [41], proinflammatory [90] state associated with endothelial dysfunction [165] resulting in vascular remodelling and atherogenesis [286]. There is now robust evidence that OSA is an independent risk factor for arterial hypertension [254][276] and cardiac disease [213]. Stroke is the second most common cause of death after cardiovascular disease and a major cause of disability worldwide [6][248]. The recognition of predisposing factors are therefore extremely important for primary or secondary prevention. OSA and stroke patients have many shared risk factors, including male sex, increasing age, obesity. Consequently, the hypothesis that OSA is associated with increased risk of cerebrovascular disease as a result of generalised atherogenesis has been explored by several investigators. The most important studies are discussed here.

Parra et al assessed the prevalence of OSA (AHI>10/hr) in a case series of 161 patients (82 males, mean age 72 years, mean BMI 27 kg/m^2) 2-3 days and 3 months after a cerebrovascular event (transient, ischaemic or haemorrhagic) [270]. The study design did not include a control group. Seventy-one percent (71%) of the patients had OSA (mean AHI 21/hr) during the acute phase and 61.6% continued to have OSA (mean AHI 17/hr) 3 months later suggesting that in the latter subgroup, OSA may not have been the result of the acute event but it might have preceded the cerebrovascular accident. The findings also indicate that stroke may result in pharyngeal dysfunction and OSA with an improvement of the symptoms in approximately 10% of the patients following the acute post-stroke period. The prevalence of 61.6% is significantly higher than the prevalence of 15% found in men and 5% in women randomly selected from the general population, aged between 30 and 60 years, using the same diagnostic cut-off (AHI$\geq$10/hr) [378]. Although there are clear differences in the demographics and the methodology used for the selection of the 2 study populations and therefore no direct comparisons can be made, the higher prevalence of OSA in patients with a history of strokes and TIAs suggests an association between OSA and cerebrovascular disease. The findings also demonstrate that strokes may result

in pharyngeal dysfunction and OSA, which improves in a large proportion (10%) of stroke patients after the acute period.

In order to minimise the effects of neurological deficit during the acute post-stroke period on the OSA prevalence, a case control study by McArdle et al compared 86 individuals with a history of TIA (symptoms < 24hrs) but no previous history of stroke, mean age 66 years, mean BMI 27 kg/m^2, with 86 healthy individuals matched for age and BMI [224]. The post-TIA patient group had no residual neurological deficit and consequently the diagnosis of OSA would clearly not be related to the acute event. Arterial hypertension, cardiovascular disease, hypercholesterinaemia and history of smoking were significantly more common among the post-TIA patients. No significant difference was found in the OSA prevalence (AHI$\geq$ 15/hr) across the 2 groups, with a mean AHI of 21/hr in both groups. In contrast to the study outcomes by Parra et al [270], McArdle et al did not demonstrate an association between OSA and TIAs. The majority of the 86 patients studied at 3 months by Parra et al had established strokes – 69% ischaemic, 5% haemorrhagic. With only 26% (n=23) being post-TIA patients, the differences in the study outcomes may suggest a dose-response relation between OSA and cerebrovascular disease - the latter is supported by the age difference in the 2 studies, with the McArdle group being 6 years younger. However, the different outcomes of the 2 studies are more likely related to the fact that a significant proportion of the OSA patients in the Parra group had sustained neurological impairment which may account for the increased prevalence of OSA.

A study by Sahlin et al, examined the survival rate during a 10-year period in a cohort of 132 consecutive stroke patients, mean age 78 years, referred to a rehabilitation unit 3 weeks after the acute event [307]. Seventeen percent (17%; n=23) of the patients were found to have OSA, only 4 patients were compliant to CPAP until death. With the primary outcome measure being death, the group assessed whether the diagnosis of OSA (AHI>15/hr) among stroke patients influenced mortality. During the 10-year period 88% (n=116) of the patients died, 74% of a cardiovascular cause; all 23 OSA patients died during follow-up. Using a multivariate analysis model, OSA was found to increase the risk of death by 75% and independent of age, gender, BMI, smoking history, cognition, dependency during daily living or the presence of arterial hypertension, atrial fibrillation, diabetes mellitus. Adjusted hazard ratios were also calculated for AHI of 5/hr and 10/hr. An AHI>10/hr demonstrated a weaker but independent association with death. The findings suggest that OSA in stroke patients is associated with worse prognosis.

These studies suggest a dose-response association between OSA and cerebrovascular disease. This is also supported by the study outcomes of Yaggi et al who performed a proportional-

hazards analysis in a cohort of 1022 consecutive individuals, mean age 70 years, to determine the independent effect of OSA (AHI>5/hr) on the composite outcome of stroke or death from any cause during a 3-year follow-up period [375]. Sixty-six percent (n=697) of the subjects studied had OSA. Stroke or death from any cause occurred in 88 individuals (9%), 77 of them had OSA (88%). The Kaplan-Meier estimate of the probability of event-free survival was significantly lower in the OSA compared to the control group. After adjustment for age, gender, smoking status, alcohol consumption status, BMI and the presence or absence of diabetes mellitus, hyperlipidaemia, atrial fibrillation and hypertension, OSA retained a statistically significant association with stroke or death (hazard ratio: 1.97; P=0.01). A trend analysis revealed a stepwise increase in the risk of stroke or death from any cause as a function of increased OSA severity (P=0.005). The risk of stroke or death in patients in the most severe quartile of OSA was three times that in the controls.

Mechanisms which may predispose OSA patients to cerebrovascular disease in addition to the prothrombotic [41], proinflammatory [90] processes leading to vascular remodelling, atherogenesis [286] and increased risk of arterial hypertension [254][276] include the strong association with atrial fibrillation [176][228] as well as the altered cerebral perfusion resulting from repeated nocturnal hypoxia, increased sympathetic activity [251], reduced baroreflex sensitivity [250][265] and fluctuations in intrathoracic and mean arterial pressures [75].

Although the causative pathways are not entirely established, it is evident from the above studies that the causative link between OSA and cerebrovascular disease is not mediated through hypertension. Further longitudinal cohort studies and outcome-based interventional case-control trials are needed to establish whether CPAP treatment would reduce the risk of developing cerebrovascular accidents and whether it can improve outcome.

With approximately 4% of the population being affected by stroke [6] and 4.4 million deaths worldwide as a result of a stroke [248], the clinical implications of the recognition and management of a further causative factor for stroke in terms of risk stratification and primary or secondary prevention would be of great importance.

Although no RCTs of the effects of CPAP therapy on the risk of developing coronary artery disease, myocardial infarction, strokes or the risk of cardiovascular or cerebrovascular death are available due to ethical reasons, studies referring to cardiovascular disease suggest risk reduction among treated OSA patients with greater benefit in the severe end of the disease spectrum [238].

In light of the associations described above (figure 5.1) and potential long-term benefits through early treatment, review of the OSA definition and consequently the treatment threshold may

become necessary, as the use of more restrictive definitions with higher AHI cut-points or an additional requirement for symptoms of sleepiness would reduce prevalence and affect values expressing associations.

It is therefore important to ascertain at which level of severity and/or clinical symptoms OSA ought to be treated to prevent these long-term consequences. Integration of markers of OSA severity into models of primary and secondary prevention of cerebrovascular and cardiovascular disease may contribute to further reduction of chronic disease burden.

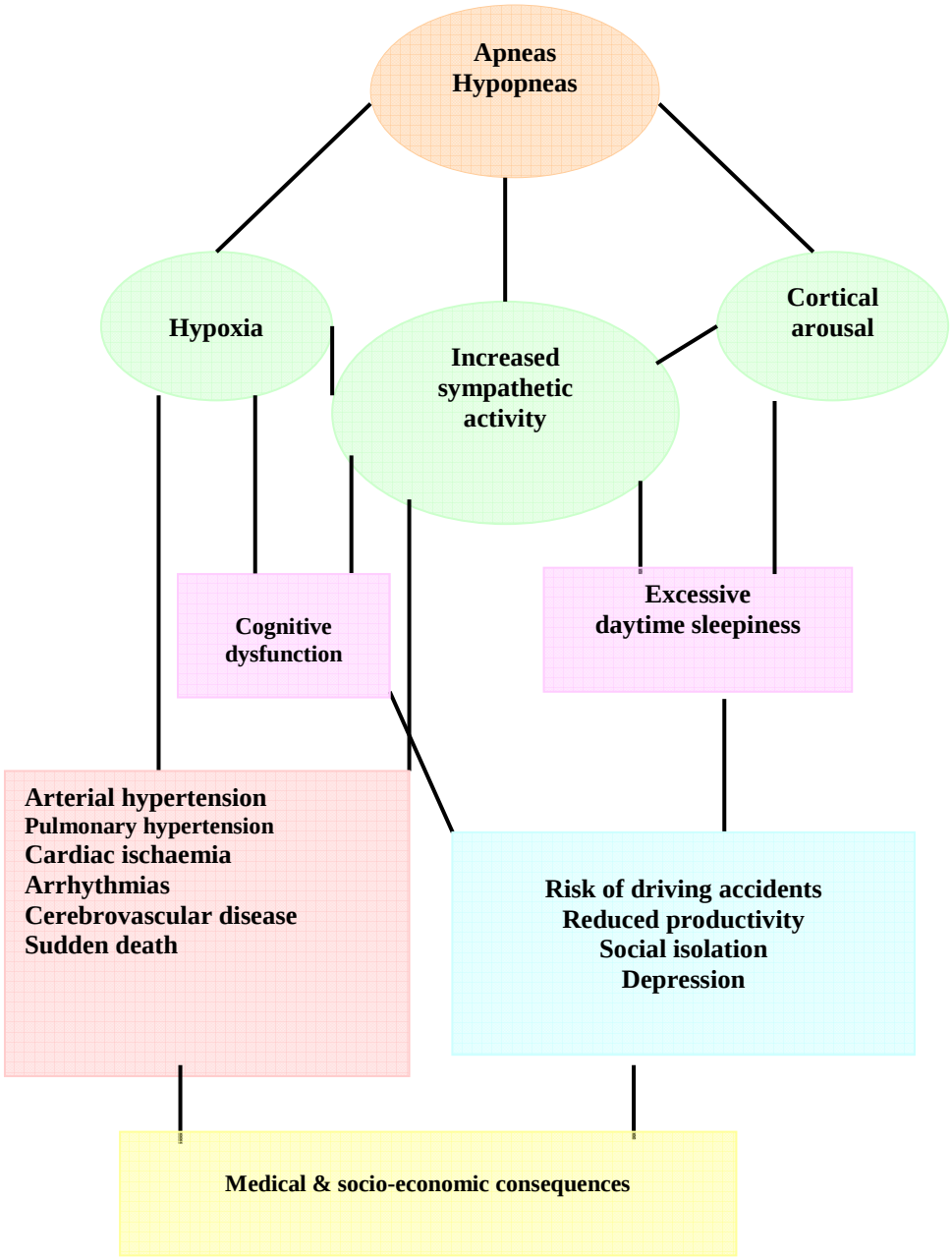


Figure 5.1. Symptoms and potential long-term consequences of the untreated OSAHS.

CHAPTER 6
TREATMENT AND SOCIO-ECONOMIC ASPECTS

6.1. TREATMENT

In the early ’70s the diagnosis of the OSAHS was limited to severe cases with excessive sleepiness, as in the Pickwick syndrome. Lacking other treatment methods at the time, these patients were tracheostomised in order to bypass the pharyngeal area of recurrent nocturnal narrowing/occlusion, improve the breathing pattern, sleep quality and long-term prognosis.
The improved diagnostic methods have expanded the disease spectrum from mild, moderate to severe. In 1981, the introduction of CPAP therapy [338] enabled the treatment of symptomatic patients from all severity groups, aiming to achieve maximum benefit whilst making best use of resources.
In the following section, the most common treatment options are discussed and classified into non-surgical and surgical.

6.1.1 Non-surgical treatment options

6.1.1.1 Weight loss, supportive measures

Before the introduction of CPAP treatment, obese OSAHS patients who did not wish to undergo tracheostomy were encouraged to lose weight, avoid alcohol, sedatives and supine position during sleep, as these reduce muscle tone and increase upper airway narrowing.
This conservative approach is presently offered to obese snorers, asymptomatic patients with mild disease or CPAP intolerant patients. The effectiveness of this treatment is limited due to problems losing weight and maintaining the ideal weight. Weight loss should be strongly recommended as an adjunct to CPAP treatment, as this may improve or even cure the syndrome in motivated, CPAP adherent patients. Weight control can prevent the development and reduce the incidence of the OSAHS [277].

6.1.1.2. Medication

Different groups of agents have been used across the disease severity spectrum including ventilatory stimulants such as theophylline and progesterone and REM suppressants such as tricyclic antidepressants or clonidine but they have been largely ineffective and are no longer indicated for OSAHS patients [162]. Recent studies have tried to identify neurotransmitters, involved in sleep-related suppression of pharyngeal muscle activity. The serotonin reuptake inhibitor paroxetine increases serotonin levels in the hypoglossal nerve and induces increased upper airway dilator activity. The numerous side effects and suggestions of association with suicidal ideation have limited its application. Modafinil improves alertness in patients who remain sleepy despite effective CPAP therapy [190]. The role of this agent in the treatment of OSAHS and its effect on CPAP compliance remain to be established.

6.1.1.3. Continuous Positive Airway Pressure (CPAP)

In 1981 Sullivan and colleagues introduced a physical treatment to OSAHS patients which succeeded in preserving upper airway patency during sleep [338]. During this first CPAP ventilation study the authors described resolution of apnoeas and improved sleep quality with less light sleep, more deep and REM sleep and reduced sleep and REM onset latencies.

The treatment consists of an air compressor – the CPAP machine - which blows a gentle, high stream of continuously positive compressed air into the airways at relatively low pressure. The pressure functions as a pneumatic splint to maintain upper airway patency throughout all phases of sleep breathing. The flow generator delivers pressure through air tubing to a nasal or face mask worn overnight. The system is open - expiration is performed against the stable pressure and through a venting device on the mask. The degree of air compression – the CPAP pressure - is calibrated individually during a titration night and is used as a pneumatic splint to preserve the diameter of the upper airway in all postures and sleep stages, aiming a reduction of the AHI< 5/hr slept. A long-term effect on airway patency is the decrease of upper airway oedema. Preserved upper airway patency restores sleep quality and nocturnal autonomic physiology with associated long-term cardiovascular benefits. The diagnosis and pressure titration in patients with an AHI>20/hr during the first 2 hours of sleep can be achieved during a split-night study. This study design is associated with a reduction in waiting times as well as in diagnostic and

treatment costs [222]. Once the optimal pressure is adjusted, continuous nocturnal use is required to maintain therapeutic benefit. Most patients require lifelong treatment and therefore long term access to a CPAP machine. Relative contradictions to CPAP treatment are bullous lung disease and recurrent sinus and/or ear infections [7]. In these cases, other forms of treatment should be considered.

Meta-analyses and systematic reviews [123][252][271][313] - level I evidence - confirm that CPAP therapy is effective in improving daytime alertness, mood and cognitive performance and is therefore recommended as the main form of treatment for the OSAHS by the American Thoracic Society [7], the European Respiratory Society [66] and the Australian National Health and Medical Research Council [14]. The recommendations are based on a number of studies, the most important of which are discussed below.

In a randomised single blind placebo-controlled crossover study design using therapeutic CPAP versus oral placebo, Engleman et al confirmed objective (MSLT) and subjective improvement in daytime alertness, mood and cognitive performance (mental flexibility, vigilance, attention) following 4 weeks of CPAP treatment in a group of 32 symptomatic patients with mild to severe disease [93]. The ESS was not used in this study.

In a randomised prospective parallel study design, Jenkinson et al confirmed the benefits of CPAP on daytime alertness using the ESS and the MWT and self reported well being based on the outcomes of the SF-36 questionnaire in 107 symptomatic men with mild to severe disease following 4 weeks of treatment [168]. During this study, subtherapeutic CPAP (1cm H_2O) was used in the control group.

In contrast to the symptomatic population, there is evidence that asymptomatic patients with severe disease do not benefit from treatment, despite good level of treatment adherence. Barbe et al conducted a multicenter parallel RCT during which the effects of CPAP treatment on objective (MSLT) and subjective (ESS) measures of sleepiness, quality of life (SF-36), cognitive function (vigilance, memory, information processing) and blood pressure were assessed across 54 asymptomatic (ESS$\leq$10) patients with severe OSA (AHI> 30/hr) assigned to receive optimal (n=29) or sham (n=25) CPAP over a 6 week period [20]. None of these variables changed significantly with optimal CPAP treatment, despite good levels of treatment adherence. The findings indicate that asymptomatic OSA patients do not benefit from treatment.

While there is strong evidence to support the use and demonstrate the benefits from CPAP in patients with moderate to severe OSAHS, the evidence is not strong enough to provide a definitive threshold above which treatment is clearly indicated and below which it is not.

Clinicians are often faced with the dilemma as to the level of disease severity at which CPAP treatment should be offered and consequently, as to what type of treatment should patients with mild to moderate disease receive. In particular, uncertainty still exists about the effectiveness of CPAP treatment among patients with mild disease, partly due to limited acceptance and treatment compliance. To help define the role of CPAP treatment in mild OSA, few RCTs have been conducted, the most important of which are discussed below.

A randomised controlled cross-over trial conducted by Engleman et al compared CPAP versus oral placebo across 32 symptomatic (ESS>8) patients with mild disease ($5 \leq AHI < 15$/hr) [92]. Following 4 weeks of treatment in each study limb, CPAP was found to improve subjective (ESS) but not objective (MWT) measures of sleepiness but only 41% (n=14) of the patients accepted CPAP at the end of the 8-week period.

A cross-over RCT conducted by Barnes et al in 28 OSA patients with mild to moderate disease (AHI 5-30/hr) and symptoms of snoring, daytime sleepiness and witnessed breathing pauses assessed the benefits following 8 weeks of CPAP versus oral placebo [24]. No significant improvement in objective (MSLT) or subjective (ESS) measures of sleepiness, wellbeing (SF-36) or mood scores were found with CPAP treatment compared to placebo. However, in contrast to the participants of the study conducted by Engleman et al [92], in this study only 65% (n=24) had an $ESS \geq 10$ at entry.

A meta-analysis of 12 RCTs trials - level I evidence - conducted by Patel et al [271] confirmed significant improvement in objective (MSLT, MWT) and subjective (11 RCTs used the ESS, one didn't [93]) measures of daytime sleepiness across diverse populations of symptomatic patients with moderate to severe disease during a mean treatment period of 6 weeks. A systematic review conducted by Giles et al which included 36 RCTs and 1718 OSA patients (AHI>5/hr), compared CPAP therapy with an inactive control or oral appliance during a minimum intervention period of 2 weeks [123]. Compared with a control, CPAP showed significant short-term improvements in objective (MWT) and subjective measures of sleepiness (ESS), well being (SF-36, FOSQ) and blood pressure values.

In 1997, the benefits of CPAP on daytime performance were questioned by Wright et al [374]. In response to this criticism, which fuelled a series of RCTs, the effectiveness of CPAP in symptomatic OSAHS patients has been undoubtedly confirmed.

To date, there is no clear evidence that asymptomatic patients benefit from CPAP. The presence of excessive daytime sleepiness is therefore currently the indication for CPAP therapy [7][252]. Although an Epworth Sleepiness Score greater than 10 is one criterion to establish the presence

of sleepiness, we must be careful that in using this criterion, we do not deny patients, who do not fully perceive their sleepiness but have performance impairments as a result of OSA, the benefits of a potentially highly effective therapy.

CPAP AND DRIVING

OSAHS patients have impaired vigilance, concentration and reaction time [60](Cheshire92), all of which are important attributes of good driving performance. Using a driving simulator, George et al assessed the driving performance of untreated OSAHS patients and found that it was markedly impaired compared to healthy individuals - 50% of the patients performed worse than any of the control subjects and some performed worse than subjects under the influence of alcohol [120]. A number of studies confirm the beneficial effect of CPAP therapy on driving performance. The most important studies are discussed here.

CPAP treatment has been shown to improve steering performance and reaction time on a driving simulator during a randomised single blind placebo controlled trial, using sub-therapeutic CPAP as placebo [145]. Horstmann et al used an anonymised questionnaire to ascertain the prevalence of RTAs in a group of 156 OSAHS patients before and after CPAP therapy and compared it to the prevalence in 160 age- and gender-matched controls [159]. Prior to treatment, the prevalence of people who suffered a RTA in the OSAHS group was 4-fold that of the healthy individuals (12.4% vs 2.9%) and more frequent among patients with severe disease (AHI>34/hr). Following CPAP therapy, the rate of RTAs dropped from $10.6/10^6$ km to $2.7/10^6$ km, whereas the RTA rate in the healthy controls group was $0.78/10^6$ km. In a cohort of 50 consecutive OSAHS patients, Findley et al studied the patients' driving records during the periods of 2 years before and 2 year after the diagnosis [104]. The authors found that in the CPAP compliant group the accident rate decreased significantly following diagnosis and CPAP therapy (0.07 vs 0 accidents/driver/yr). CPAP adherent patients had significantly fewer RTAs than did CPAP non-adherent patients (0 vs 0.07 accidents/driver/yr).

The findings confirm that CPAP therapy reduces the risk of RTAs in OSAHS patients and strongly support prompt treatment in groups of professional drivers with moderate to severe disease.

CPAP AND CARDIOVASCULAR BENEFITS

Arterial hypertension is common among OSAHS patients [109][373]. The association has been identified in a number of cross sectional studies [37][73][128][204][254][379] as well as in a

large longitudinal follow-up study of 709 patients – the Wisconsin cohort - over a period of minimum 4 and up to 8 years [276]. A number of case control studies have demonstrated the association between OSAHS, cardiovascular [163][239][272] and cerebrovascular [375] disease. Two important longitudinal studies assessed morbidity and mortality in untreated OSAHS patients compared to patients on CPAP treatment: the study by Marin et al extended over a 10-year follow-up period [213], whereas the study by Peker et al was a 7-year follow-up [273]. Both studies confirmed worse outcome in terms of morbidity and mortality in untreated OSAHS patients. Effective CPAP therapy has been shown to reduce nocturnal and diurnal sympathetic activity [322], increase nitric oxide concentrations and improve endothelially mediated vasodilatation [166]. Consequently, CPAP has been shown to reduce pulmonary artery pressures [308], left ventricular diastolic function [11] as well as nocturnal apnoea-/hypopnoea-associated bradyarrhythmias [26].

The short-term benefits of CPAP therapy on blood pressure have been demonstrated in a number of RCTs. A systematic review by Robinson et al of 6 RCTs provides sound evidence of improvement in blood pressure values during CPAP therapy in symptomatic OSA patients with moderate to severe disease [295]. The duration of treatment varied between 1 [80], 4 [98][278] and 9 weeks [27] with a mixture of normotensive and hypertensive patients across the 4 positive studies. Improvements in systolic and diastolic blood pressure values shortly after initiation of CPAP therapy compared to an inactive control have been confirmed in the systematic review of 36 RCTs which included 1718 OSA patients (AHI>5/hr), carried out by Giles et al [123]. The 2 negative studies included in the review, were 2 Spanish collaborative studies which assessed the effects of CPAP therapy on the blood pressure values of normotensive asymptomatic patients in the severe disease spectrum [20] and of normotensive patients with mild symptoms in the mild to moderate disease spectrum [233]. The randomised placebo-controlled study by Barbe et al compared the changes in blood pressure during 24-hr recordings in 29 non-sleepy normotensive patients with an AHI>30/hr following 6 weeks of therapeutic CPAP to the changes in a group of 25 asymptomatic normotensive patients with the same disease severity following 6 weeks of sham CPAP and found no significant differences across the two groups [20]. The RCT by Monasterio et al compared the BP changes in 66 normotensive patients with an AHI<30/hr and mild symptoms (ESS: 12/24) following 6 months of CPAP therapy to the changes in a group of 59 patients with the same disease severity following 6 months of conservative measures consisting of weight loss, adequate hours of sleep and avoidance of alcohol, sedatives and of supine position [233]. Both studies had negative outcomes concluding that there is no evidence

to support that CPAP therapy improves blood pressure values in asymptomatic patients with moderate to severe [20] or mild disease [233]. In a further RCT, Robinson et al studied the changes in blood pressure in 35 hypertensive asymptomatic OSA patients with moderate to severe disease using therapeutic CPAP over a 4-week period followed by 4-weeks of sub-therapeutic CPAP. The majority of participants were established on antihypertensive medication with good levels of BP control. No significant change in blood pressure was found following treatment [294].

In summary, the studies indicate that short-term cardiovascular benefits are most prevalent in symptomatic patients with moderate to severe disease [27][80][98][278]. Based on current evidence, CPAP therapy shows no short-term cardiovascular benefits in asymptomatic patients [20][294] or patients with mild disease [233].

Future longitudinal studies may establish the long-term effects of CPAP treatment and its potential to alter cardiovascular risk. Consequently, these studies may ascertain the role of CPAP in the management of asymptomatic patients or patients with mild disease with the potential to extend treatment beyond the group of patients with an ESS greater than 10 if treatment benefits are confirmed and adherence to treatment is maintained.

CPAP ADHERENCE

CPAP is widely used, with estimated prescription rates in Germany, France and the United States exceeding 100 units/million population/yr [122].Treatment benefits depend upon regular CPAP use, defined as more than 4hrs per night and more than 5 nights per week [194]. Adherence to treatment is measured either through an in-built “run time” clock or through a “pressure time” clock. The latter is a more accurate measure of the time the treatment is received at the prescribed pressure and is not affected if the machine is switched on (accidentally or deliberately) when not used. As CPAP therapy is relatively obtrusive, patients have been shown to use CPAP on average 4.7 hrs/ night [94]. Symptomatic patients are more likely to adhere to treatment, whereas patients with no significant clinical symptoms are less likely to notice significant benefits and may consequently have low long-term treatment compliance [92].

The latter has been confirmed in a retrospective analysis of 1155 patients’ records, carried out by McArdle et al [220]. The authors found that 86% of the patients with AHI$\geq$ 30/hr and ESS>10 were using their CPAP after 3 years and concluded that long-term CPAP use is related to disease severity and subjective sleepiness with independent predictors being the history of snoring, AHI and ESS. Based on their observations, the authors furthermore concluded that long-term CPAP

use can be predicted within the first 3 months of treatment. In a prospective study of 62 patients, Kingshott et al assessed the best predictors of CPAP use [189]. The authors found that improvements in self-ratings of daytime function were significantly associated with CPAP use. In contrast, polysomnographic variables – AHI and arousal frequency – were found to be poor predictors of the response to CPAP therapy. This may in part be due to inter-individual differences in the perception of symptoms. A further explanation is that conventional polysomnographic measures fail to detect changes contributing to the patients' daytime symptoms [189][215]. Detection of those measures that will best correlate with the patients' symptoms may be of importance for the selection of patients who will benefit and adhere to treatment and may consequently explain differences in the perception of symptoms and treatment adherence across patients.

Consequently, this raises the following question:

What are the reasons for treating OSA patients and how can we identify patients who will benefit and adhere to treatment?

Current evidence suggests that CPAP should be offered to symptomatic patients with mild, moderate or severe disease [7][252]. The perception of symptoms contributes significantly to treatment adherence [189][220]. There is currently no evidence to support any benefits in daytime symptoms or blood pressure values in asymptomatic patients [20][233]. However, the outcomes of the 18-year follow-up of the Wisconsin sleep cohort (n=1522) confirm that all-cause mortality risk increases with increasing AHI in untreated OSA patients after adjusting for age, sex, BMI and irrespective of symptoms of sleepiness [377]. Future studies may establish whether CPAP therapy can reduce cardiovascular risk in asymptomatic patients - that is, if they adhere to therapy despite the absence of symptoms.

CPAP SIDE-EFFECTS, MANAGEMENT AND SUPPORT

The most common side-effects of CPAP therapy are dry or congested nasal mucosa and mouth leaks. A dry or congested nose results from the flow of dry, cold air causing increase in mucosal blood flow. Mouth leaks are common cause of nasal congestion as they induce high uni-directional nasal airflow and increase in nasal resistance. Mucosal congestion and mouth leaks may result in airflow flattening, a condition also known as flow limitation - a less apparent form of sleep disordered breathing commonly associated with sleep disruption [290]. Flow limitation during CPAP ventilation results in suboptimal CPAP pressure which fails to maintain airway patency with no improvement in patients' symptoms. As symptoms persist, adherence to CPAP

tends to decrease [94]. Effective management of the side-effects during the early stages of treatment may therefore preserve adherence, improve symptoms and prevent longer-term consequences. Full face masks and heated humidifiers have been shown to reduce the impact of these side-effects [216]. Chin straps may also minimise mouth leaks.

Further side effects are conjunctivitis resulting from mask leaks, local allergies and skin abrasions. One or more of the mentioned side effects occurs in up to 65% of CPAP users [275]. Regular follow-up and early intervention usually preserve CPAP adherence. A RCT by Hoy et al compared adherence during the first 6 months of treatment in two groups of OSAS patients (AHI>15/hr): one group was offered intensive CPAP support – 3 nights of CPAP titration and frequent home visits during the first month of treatment -, the other group was offered standard support – 1 night of CPAP titration and clinic follow-ups after 1, 3 and 6 months [160]. The study outcomes confirmed that intensive nurse-led support improves CPAP adherence. The study furthermore established that CPAP adherence was better among patients who initiated their own referrals to the sleep clinic as opposed to those patients whose referral was initiated by their partner. Although it would be logistically difficult to offer intensive support to all OSAHS patients, a subgroup of patients with mild to moderate symptomatic disease would benefit from this nurse-led intervention.

An important development in CPAP technology is the “intelligent” Auto-titration CPAP (APAP) which adjusts the delivered pressure to the patient’s inspiratory pressure requirements throughout the night. The pressure adjustments are based on parameters detected during the preceding inspiratory cycle with pressures remaining the same during inspiration and expiration. Within-night pressure requirements may vary during the different sleep stages and postures. Between-night variability may be linked to alcohol consumption and/or upper airway infections. APAP can accurately identify the fixed CPAP pressure requirements in an outpatient setting [314]. Due to the pressure adjustments and the convenience of ambulatory pressure titration, APAP’s potential in improving treatment adherence has been explored [33]. However, a meta-analysis of 9 RCTs carried out by Ayas did not confirm significant benefits in treatment adherence with APAP compared to CPAP [15]. APAP continues to have an important role in home-based pressure titration and in the diagnosis of mouth leaks. Future studies may establish cost-effective treatment applications of APAP or BPAP (bilevel positive airway pressure) by identifying subgroups of OSA patients in whom these devices may improve adherence with associated long-term benefits.

6.1.1.4. Mandibular Repositioning Splint (MRS)

The Mandibular Repositioning Splint (MRS) is an oral appliance which is individually formed by orthodontists to fit the dental arch. It uses the back upper molar/wisdom teeth to keep the mandible protruded during sleep, bringing the tongue base forward and resulting in an increase of the upper airway diameter. The device reduces upper airway collapsibility with consequent reduction in the AHI [253]. In a prospective crossover RCT, Ferguson et al demonstrated that MRSs may constitute a promising alternative in CPAP-intolerant patients with mild to moderate disease [100]. A further prospective crossover RCT by Engleman et al compared MRS to CPAP in mild, moderate and severe OSAHS patients and found MRSs to be less effective in controlling AHI and sleepiness in all severity groups [96]. A systematic review by Giles et al which included 9 RCTs comparing CPAP therapy with oral appliances concluded that CPAP therapy is more effective in reducing AHI, oxygen desaturations and in restoring sleep [123]. Participants were more likely to withdraw on oral appliances than on CPAP therapy. Patients who are offered MRS treatment should therefore be carefully selected and followed up. MRSs constitute the best second-line treatment option in patients with mild to moderate disease who are CPAP-intolerant. Drawbacks include temporo-mandibular joint pain, dental damage, occlusion changes and OSAHS relapse [263]. Data on MRS long-term treatment adherence are not available. Other oral appliances such as tongue retaining or soft palate lift devices failed to demonstrate a satisfactory level of AHI improvement in patients with severe OSAHS [25].

6.1.2. Surgical treatment options

Based on the ASDA recommendations, surgical treatment should be considered in patients who have an underlying surgically correctable abnormality or where non-surgical treatment options have failed or have been declined. The choice of a surgical intervention should be based on satisfactory level of evidence supporting treatment benefits [8]. A decision in favour of a surgical intervention should result from cephalometric measurements as well as a fiberoptic examination in order to determine the areas of narrowing or obstruction.

Maxillo-Mandibular Advancement Osteotomy (MMO) is currently the only surgical procedure with extensively validated therapeutic benefits among the different OSAHS severity groups. The

indication for MMO is limited to patients with OSAHS due to skeletal deformities such as micro- or retrognathia or following trauma. The long-term (2-4 years) post-operative success rate of this intervention - based on an AHI<10/hr - varies between 80-90% across different centres [68][293]. Surgery can be performed in one session or stepwise, combined with genioglossal advancement and hyoid myotomy and suspension.

Procedures aiming to increase the nasopharyngeal space are nasal-septal reconstruction, polypectomy and reduction of turbinates. These interventions may be carried out prior to CPAP therapy in order to improve tolerance and adherence. Procedures aiming to enlarge the hypopharyngeal airspace are mainly retro-lingual operations such as Midline Laser Glossectomy (MLG) [113]. These procedures can be useful in a subset of carefully selected OSAHS patients. Procedures which aim to reduce the soft palate tissue and enlarge the oropharyngeal space are the Uvulo-Palato-Pharyngo-Plasty (UPPP) [317], the Laser-Assisted Uvulo-Palatoplasty (LAUP) [359] and the Radio-Frequency Volume Reduction – tissue shrinkage linked to protein denaturation induced by low frequent energy provided by the RF generator [284]. Long-term follow-up of patients who underwent UPPP failed to demonstrate an improvement in AHI, snoring index or snoring intensity up to 45 months post-operatively [231]. Following UPPP, CPAP therapy may become necessary in a significant proportion of patients. Higher pressures may be required to maintain airway patency due to mouth leaks following resection of the soft palate resulting in discomfort and challenging CPAP adherence in these a priori CPAP-naïve patients. Further side-effects are nasopharyngeal insufficiency with regurgitation of food or liquids, pharyngeal dryness, changes in voice, loss of taste, problems with Eustachian equilibration and should be carefully discussed with patients whishing to undergo this surgical intervention [144].

6.2. SOCIO-ECONOMIC ASPECTS: COST/BENEFIT ANALYSIS

The association between the OSAHS and arterial hypertension has been identified in a number of studies [37][73][128][204][254][276][379]. The association of the OSAHS with cardiovascular [163][239][272] and cerebrovascular [375] disease but also the increased morbidity and mortality among untreated OSAHS patients [213][273] are important observations supporting the early diagnosis and treatment of the syndrome. A number of studies confirmed that undiagnosed OSAHS is associated with higher utilization of health care services resulting in

higher long-term costs in untreated OSAHS patients. The most important studies in support of the cost-effectiveness of early diagnosis and treatment of the syndrome are discussed here.

A Canadian study compared the health care utilization of 97 OSAHS patients with 97 age- and gender-matched controls during a 2-year period [196]. The study found that OSAHS patients spent significantly more time in hospital and had higher expenditures related to physician claims. During the 2-year period the 97 OSAHS patients utilized between $100,000 and $200,000 Canadian dollars more in services than did the control group. A further North-American cost analysis of undiagnosed OSAHS compared the medical costs of 238 OSAHS patients 1 year prior to the diagnosis, with 476 age, gender and BMI matched controls [178]. The information was gathered retrospectively from a private healthcare insurance database. The authors found that the annual medical costs of the OSAHS patients prior to diagnosis were 2-fold the costs of the control subjects ($2720 vs $1384/person/year). Disease severity was correlated with the magnitude of medical costs. With an OSAHS prevalence of 3% in middle-aged adults [378], the authors estimated that undiagnosed OSAHS may cost health care providers in the USA up to $3.4 billions additional annual medical costs. The additional costs were expenditures from physician claims most commonly related to arterial hypertension (38% of the OSAHS patients; n=90) and depression (20%; n=48) and did not include expenditures from RTA related injuries. Although the study outcomes by Kapur et al may not be applicable to all OSAHS patients due to methodological issues related to selection bias of the population studied, they provide an estimate of the potential costs of untreated OSAHS related to chronic disease burden.

To establish whether CPAP therapy reduces the utilization of healthcare services and the associated medical costs, a Canadian prospective cohort study compared differences in physician claims and hospital stays between patients and age, gender matched controls 5 years before and 2 years after initiation of CPAP therapy [17]. In this study, data were collected from a government funded health care provider which facilitated unrestricted access to physician services and hospitals for all citizens. During the 5-year period prior to diagnosis and initiation of CPAP therapy, medical costs among the 344 recruited male OSAHS patients were 2-fold the costs of their controls. Following 2 years of CPAP therapy OSAHS patients had significantly less contacts with physicians, in-hospital days and associated medical tests. Patients adherent to CPAP therapy (n=282) had a greater reduction in medical costs than did non-adherent OSAHS patients (n=62), who maintained the same level of costs 2 years after diagnosis of the syndrome. The study outcomes support the findings by Kapur et al [178]. The outcomes also demonstrate a measurable reduction in medical costs following CPAP therapy, supporting its cost effectiveness.

Early diagnosis and treatment of the syndrome is therefore not a burden on healthcare providers but in the long-term, it results in significant cost savings.

The discussed cost-benefit analyses do not include costs arising from accident-related injuries. RTAs are becoming the third leading cause of death and disability worldwide [248]. Daytime sleepiness and impaired concentration are the most common daytime symptoms, reported by 87% of OSAHS patients [370]. Sleepiness is becoming acknowledged as the most common cause of accidents on highways. Sleepiness is involved in up to 2.5% of all RTAs with potentially devastating implications for the patients and their families and billions of dollars in related costs annually for healthcare providers [365].

Recent studies have shown that OSAHS patients are at higher risk of RTAs than age and sex matched controls [21][121][345]. OSA is more prevalent among long-haul commercial truck drivers [331]. Commercial drivers diagnosed with OSAHS have a 2-fold higher accident rate per mile than non-OSAHS drivers [333]. These associations are of great importance not only because of the potential devastating outcomes related to RTAs in particular where trucks are involved, but more importantly, because when related to undiagnosed OSAHS, they are preventable. These observations along with the long-term effects of the undiagnosed OSAHS on health and professional quality of life with potential social and financial implications, underline the importance of prompt and cost-effective diagnosis and treatment.

Guest et al applied the Markov model, a statistical model for process description, for purposes of long-term cost-benefit analysis of CPAP therapy among severe OSAHS patients (AHI>30/hr, ESS>12) [130]. In order to ascertain the long-term cost-effectiveness of CPAP therapy, the parameters used in the model in terms of likelihood or hazard ratios were based on a systematic literature review which included data from longitudinal morbidity and mortality studies [213], data on RTAs in OSAHS patients [104] as well as data from studies on adherence to CPAP therapy [220]. Based on the outcomes of this economic model which extended over a 14-year period and included a cumulative database of 6,000 patients from 19 sleep centres across the UK, CPAP was found to be clinically more effective than no treatment and the most cost-effective strategy from the perspective of the UK's NHS, after a minimum of 2 years' treatment. The data confirm that any intervention that improves long-term adherence, such as better patient education, is worth employing.

Chronic disease burden is a major public health issue due to impairments in the quality of life, the utilization of healthcare resources and the associated increased costs on healthcare providers. In order to prevent chronic disease and improve the quality of life, health economists have

invested in early primary and secondary prevention through the application of risk prediction models in primary care. I believe that to date, there is enough evidence to support the cost-effectiveness of the early diagnosis and treatment of the OSAHS. Markers of OSAHS severity should be therefore integrated into risk prediction charts for the prevention of cardiovascular and cerebrovascular disease.

CHAPTER 7
AIMS OF THIS THESIS

As I have outlined in the introductory part of this thesis, OSAHS is a common problem with a number of short- and long-term consequences. In the past 2 decades, the understanding of the disease pathophysiology has increased enormously. There is now greater understanding of the concept of the hierarchical arousal model, the factors contributing towards brainstem versus cortical arousals and the importance of the different sleep stages on autonomic and cortical activity. The acquisition of new knowledge and the further understanding of the interactions between pathophysiology and symptoms have raised questions regarding the groups of patients that may benefit but also adhere to treatment. A significant proportion of patients in the community remain undiagnosed and untreated. In many areas of the UK, this is due to previous funding cuts and compromised sleep service provision within the NHS resulting in long waiting lists and many patients having delayed diagnosis and therapy [122][374].

Non-conventional methods of analysis, based on novel pathophysiological indices, can potentially improve diagnostic sensitivity whilst facilitating the implementation of a remote, simplified, cost-effective diagnostic tool in order to reduce waiting lists and diagnostic costs.

The aims of this thesis, therefore, were:

1. Firstly, to establish potential factors that may contribute towards the generation of visually scored cortical arousals, in particular the influence of sleep stages on cortical EEG responses (chapter 8).
2. Secondly, to explore whether power spectral analysis of the EEG improves detection of cortical arousal responses (chapter 9).
3. Thirdly, to investigate the nocturnal autonomic activity in OSAHS patients using the power spectral analysis of RR-intervals, the Heart Rate Variability (HRV), and to compare them to the nocturnal HRV of healthy individuals (chapter 10).
4. Finally, to validate a portable diagnostic device, the Embletta (Flaga) (chapter 11).

The next 4 chapters present the studies which address these aims.

CHAPTER 8
AROUSABILITY IN SLEEP APNOEA/HYPOPNOEA PATIENTS

8.1. INTRODUCTION

Sleepiness is a major feature of OSAHS and a key factor in the increased risk of road traffic accidents. Previous studies have found only weak correlations between polysomnographic findings and both objective and subjective measures of daytime sleepiness in OSAHS [28][188]. This may partially be because the recurrent arousals, which modelling studies have shown to be central to the daytime dysfunction [214][297], are poorly identified [215]. Approximately 30% of apnoeas/hypopnoeas are not terminated by visible cortical arousals on the EEG [291].
However, there may be differences between events which predispose to more marked arousal reactions. These might include event duration and severity of oxygen desaturation. Furthermore, there may be differences between sleep stages in arousability or in the electrophysiological visibility of arousals.

We therefore investigated the hypotheses that

- arousability varies between light sleep (NREM1&2), Slow-Wave-Sleep (SWS) and Rapid-Eye-Movement sleep (REM)
- visible cortical arousals are more common with longer apnoeas/hypopnoeas and with more severe desaturation.

The study also aimed to examine the effects of respiratory event type, apnoeas or hypopnoeas and the time of their occurrence during the night on cortical responses.

8.2. METHODS

8.2.1. Primary endpoints

The association of:

1. sleep stage during which the respiratory event occurred
2. event type: apnoeas versus hypopnoeas
3. event duration
4. oxygen desaturation
5. time of night

with arousals was investigated.

8.2.2. Study Population

Fifteen consecutive patients, who were newly diagnosed with the OSAHS (AHI $\geq$10 /hr) were randomly selected from the database of the Sleep Centre at the University Hospital Charité in Berlin.

All study patients referred to the sleep centre with possible OSAHS had either self-reported daytime sleepiness (Epworth Sleepiness Scale >10) not explained by other factors or two other major symptoms of the OSAHS [370]: chocking or gasping during sleep, recurrent awakenings, unrefreshing sleep, daytime fatigue and/ or impaired concentration.

A detailed medical history and examination was carried out in the outpatient department. All patients underwent a chest x-ray, an ECG and a lung function test.

Study exclusion criteria were autonomic, endocrine, cardiac or pulmonary disease, Periodic Limb Movement Disorder, other neurological disorders or the intake of medication which affected sleep or autonomic function. Two patients were on the antihypertensive Nifedipine. All subjects were otherwise healthy and normotensive.

Fourteen of the patients were male and 1 patient was female. Their mean age was 51 years [SD 9 years] and the mean Body Mass Index (BMI) was 29 kg/m^2 [SD 2 kg/m^2].

All patients underwent an in-hospital diagnostic polysomnography.

8.2.3. Ethical Approval

All patients gave written informed consent to participate in the study, which was approved by the Ethics Committee of the Humboldt University in Berlin.

8.2.4. Polysomnography

A computerised system was used for the polysomnographic recordings, which were carried out between 10 p.m. and 7 a.m. and consisted of the following:

ELECTROENCEPHALOGRAPHY

Recording of sleep and arousals was based on following tracings:

Four unipolar EEG tracings: 2 central, the C3A2 and C4A1, and 2 occipital, the O1/A2 and O2/A1 tracings.

Two outer canthi electrodes, LEOG and REOG, for the detection of eye movements.

A submental EMG electrode for the detection of tonic muscle activity.

ELECTROMYOGRAPHY

Leg movements were detected through bilateral tibial EMG electrodes, whereas body movements were recorded through a body position detector. The body detector did not include continuous information on supine body position.

CARDIORESPIRATORY MONITORING

Monitoring of respiratory function consisted of a thermistor sensor for the oronasal airflow detection, 2 piezoelectric belts for the detection of thoracoabdominal movement, a digital microphone for snoring detection and a pulseoximeter to monitor blood oxygen saturation levels.

A 3-lead ECG was used to monitor cardiac activity.

8.2.5. Visual scoring

The visual scoring was performed by a single observer (K.D.), the author of this thesis, blind to subject details.

Every polysomnogram was scored during 3 consecutive sessions: sleep staging was followed by the scoring of apnoeas and hypopnoeas. Arousals were scored separately during the final session.

SLEEP SCORING

Sleep and arousal scoring was based on the C4A1 and C3A2 tracings. Sleep was scored according to the Rechtschaffen and Kales criteria [288], described in detail in chapter 2.

Following parameters were used to describe sleep efficiency during the total time spent in bed, which was between 10 p.m. and 7 a.m. for each patient.

Sleep onset was defined as the first scored 30-second epoch of sleep stage 2.

Total Sleep Time **[hrs]** (TST) was calculated as the total time spent asleep.

Sleep Period Time **[hrs]** (SPT) was calculated as the total time between sleep onset and the last epoch of sleep.

Sleep efficiency **[%]** was defined as the ratio between TST and SPT, multiplied by 100 and expressed in percent.

$$SE\ [\%] = [TST/SPT] \times 100$$

APNOEA / HYPOPNOEA SCORING

Apnoeas were defined as cessation of the oronasal airflow, lasting $\geq$10sec.

Hypopnoeas were defined as airflow reduction of >50%, compared to a 10sec peak amplitude during the preceding 2 minutes, lasting $\geq$10 sec and associated with either an oxygen desaturation of $\geq$3% or an arousal [9].

Oxygen saturation was based on a second-by-second measurement within a window set from the beginning of each apnoea/hypopnoea until the middle of the following apnoea/hypopnoea, or for 2 minutes, if no apnoea/hypopnoea occurred.

Apnoea/Hypopnoea Index **[hr $^{-1}$]** (AHI): the number of all apnoeas and hypopnoeas divided by the total time spent asleep.

$$AHI\ [hr^{-1}] = [Apnoeas + Hypopnoeas] / TST$$

Disease severity was based on the AHI [5]:

Mild OSA: $15 > AHI \geq 10$

Moderate OSA: $15 \geq AHI \geq 30$

Severe OSA: $30 > AHI$

An AHI<10/hour sleep was considered normal.

AROUSAL SCORING

The C4A1 and C3A2 EEG tracings were used for arousal scoring. The scoring was based on the definition of the American Sleep Disorders Association (ASDA), described in chapter 4, modified as to the time-threshold which was set at 1 second rather than 3 [9].

Arousals were scored when an abrupt shift to a faster EEG frequency (including theta, alpha and/or greater frequencies but no spindles) occurred. Arousal scoring ranged from 1 to 15 seconds, irrespective of sleep recurrence or prolonged awakening subsequent to the arousal; hence, Rechtschaffen and Kales awakenings [288] were also included in the scoring.

The scored arousals were sub-divided into the following groups:

Spontaneous Arousals (S-Arousals) were arousals with no preceding apnoeas/hypopnoeas.

Respiratory Arousals (R-Arousals) were arousals overlapping or adjacent to apnoeas/ hypopnoeas.

The number of each arousal type per hour slept was termed as followed:

Spontaneous Arousal Index (SAI) and

Respiratory Arousal Index (RAI).

Arousal Index (ArI) was the sum of all arousals per hour slept.

$$ArI = RAI + SAI.$$

Respiratory Arousals were sub-divided into following groups, based on duration (figure 8.1.):

1. 1-3 sec of duration
2. 3-15 sec
3. Rechtschaffen and Kales awakenings: >15 sec.

Apnoeas and hypopnoeas that were not associated with visible cortical arousals were sub-divided into events causing:

1. no EEG changes
2. lightening in sleep stage, e.g. from REM sleep to stage 1 (figure 8.1.).

The AHI and ArI were calculated for each patient and sleep stage.

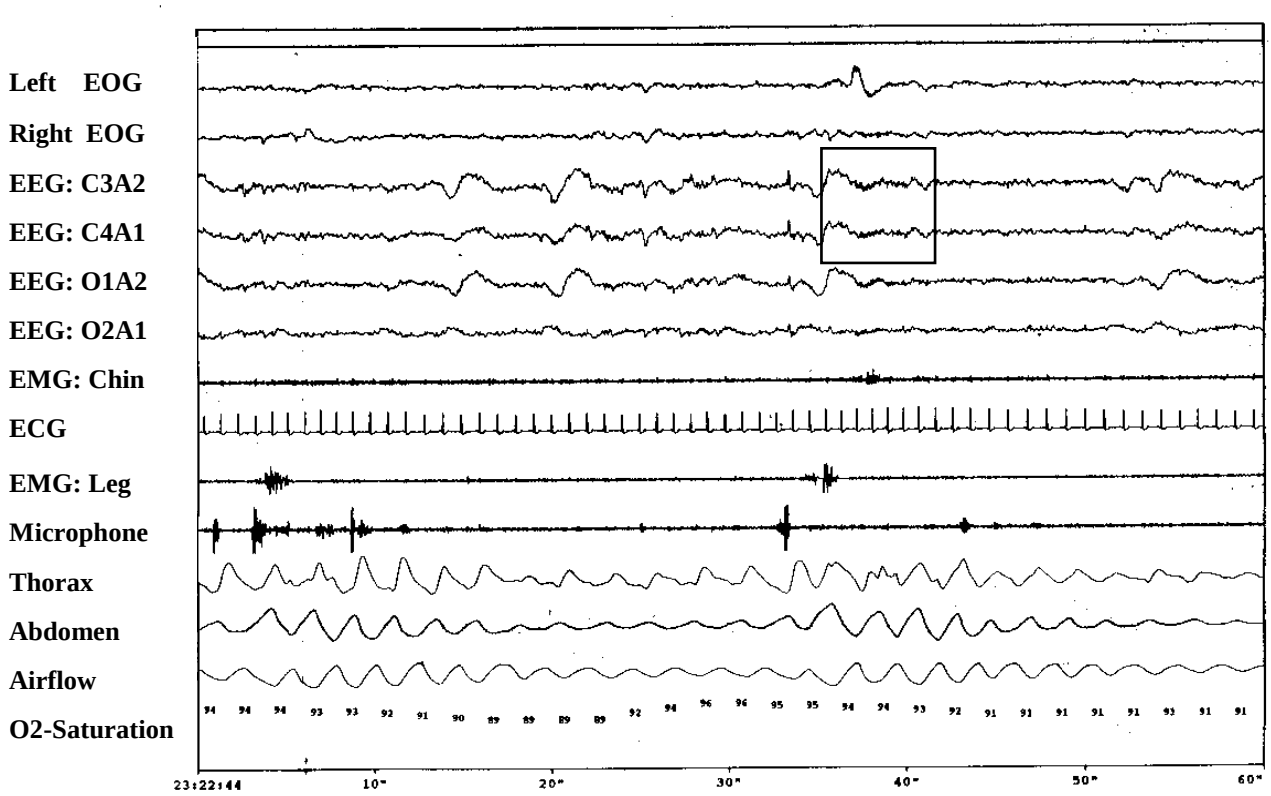


Figure 8.1.A. Hypopnoea inducing a 5-seconds arousal.

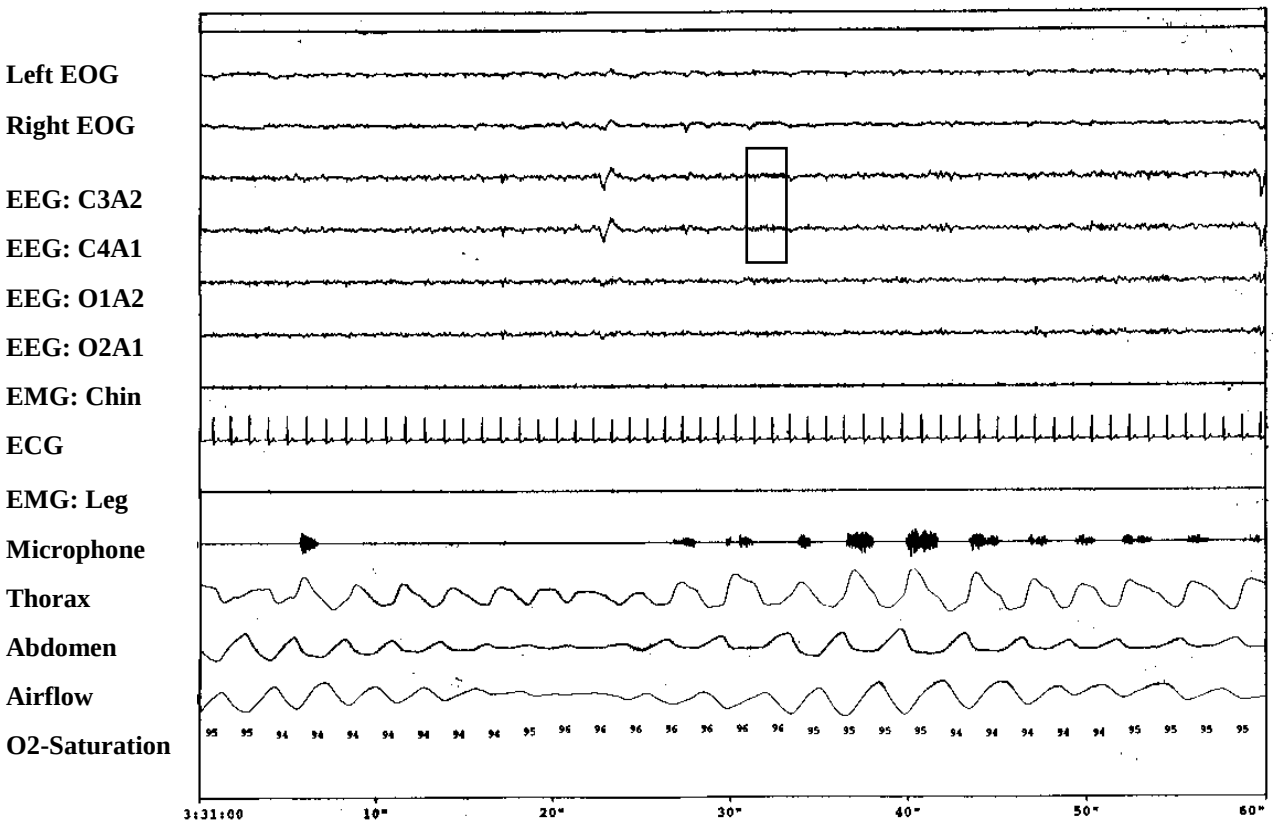


Figure 8.1.B. Hypopnoea inducing a 2-seconds arousal.

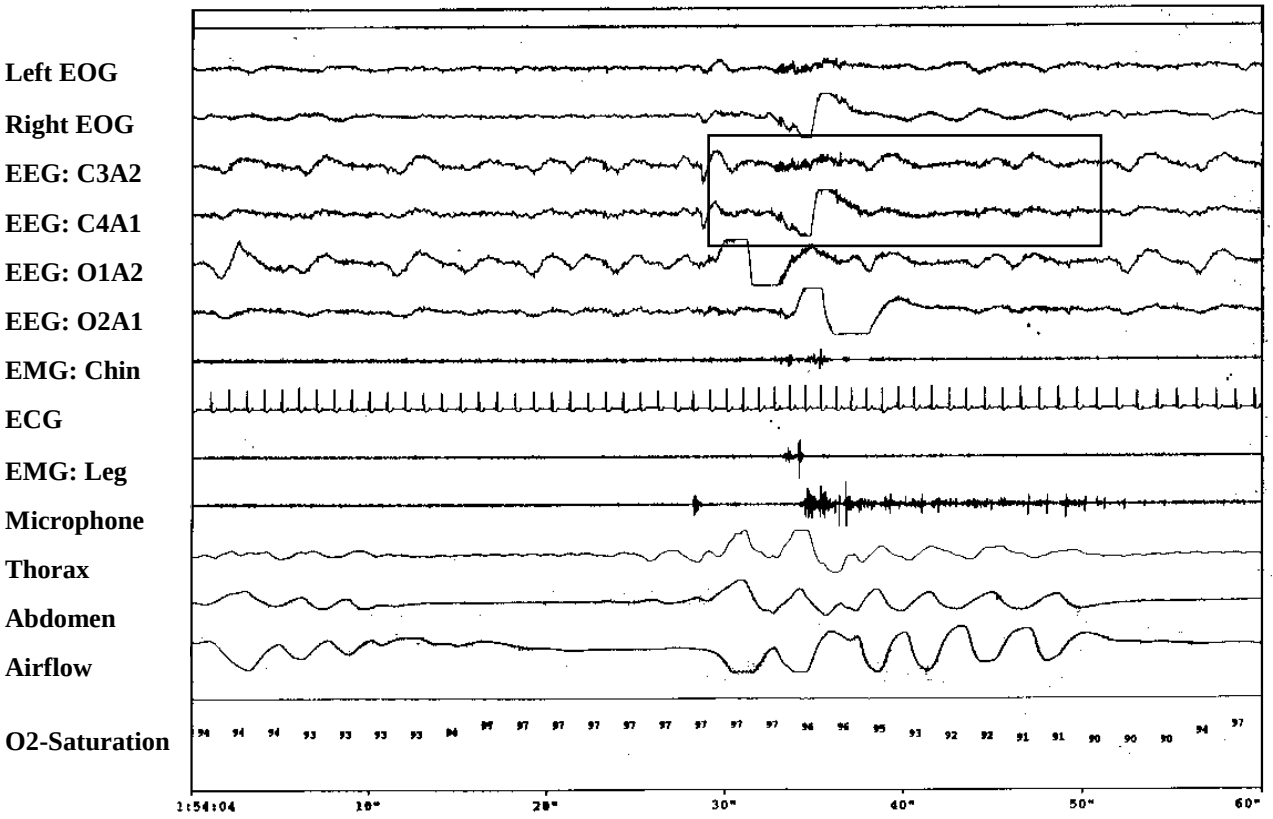


Figure 8.1.C. Apnoea causing awakening.

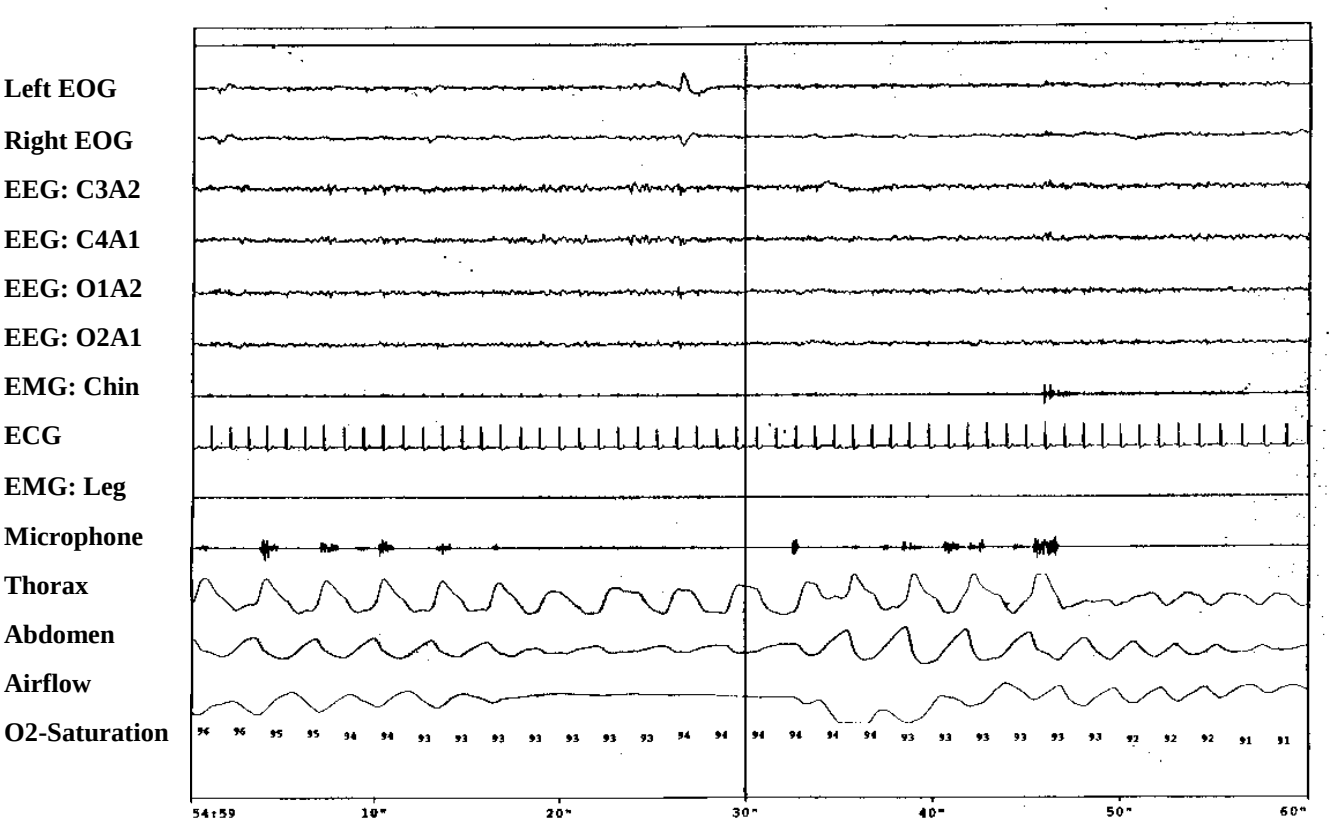


Figure 8.1.D. Apnoea which begins in REM-sleep and self-terminates in the following epoch, causing a transition from REM to stage 1 (lightening).

8.2.6. Intra-observer variability

Although visual scoring of polysomnograms is rule based, there is a degree of variability in the assessments. Variability in sleep scoring can result from variation in the quality of the sleep studies and variability in recognising EEG patterns within and between scorers.

The intra-observer variability of visual scoring was assessed as a measure of accuracy and reproducibility of the outcomes.

The 15 polysomnograms were scored twice by the primary investigator (K.D.). In these intra-rater analyses, the researcher was blinded to the patients' details and to the original results of the comparison studies. Intra-rater comparisons were made 12 months after the initial study was scored. Variability was calculated as the difference and SEM between the 2 scores in percent.

8.2.7. Statistical analysis

POWER CALCULATION

In order to design an adequately powered study with representative results, the sample size calculation was based on the study outcomes of Fietze et al [103]. The group examined the influence of sleep stage on arousal induction in 38 patients. The primary outcome measure was the respiratory (type-R) arousal index per sleep stage (RSS index), which was calculated as the number of respiratory arousals – due to apnoeas, hypopnoeas or snoring – divided by the hours spent in each sleep stage – NREM 1, 2, SWS and REM sleep – before and after CPAP treatment. Before treatment the median respiratory arousal index during NREM 1 was 23.7/hr [IQR 9.5 - 47.1], with 73-93% of all arousals occurring during NREM 1 and 2.

During SWS the median R-arousal index was 0/hr (IQR 0-6/hr), significantly lower compared to the indices of any other sleep stages ($p<0.01$). With the median value being zero and the only non-zero value being 6/hr for the upper quartile, it was concluded that at least 50% of the patients ($\geq$ 19) had zero type-R arousals in SWS. From this information in combination with the median time spent in SWS being approximately 1% of the Sleep Period Time (SPT) across the 38 patients, it was extrapolated that although the zero values may have been in part due to the low percentage of apnoea-/hypopnoea-associated arousals, this result is more likely to be due to the low apnoea/hypopnoea index during SWS and the significantly less time spent in this sleep stage.

These observations were used to calculate an adequately powered sample size in order to ascertain whether differences in the RAI across sleep stages are significant, once the bias due to differences in AHI and time spent in each sleep stage has been corrected for. In light of the findings by Fietze et al [103], we extrapolated that the RAI during NREM 1&2 is greater than during SWS in 95% of the patients, whereas the RAI during REM sleep is greater than during SWS in 85% of the patients. For a similar effect size and using the Friedman's test, a sample size of 8 patients would give at least 80% power at a 5% significance level. Assuming that each patient had a 30% chance of drop-out due to zero AHI in one or more of the sleep states studied, 15 patients were recruited allowing for a 95% chance for the final sample to be at least 8.

STATISTICAL TESTS

Statistical analysis was performed using the SPSS Inc. Chicago IL, Version 9 software.

Analysis of Variance (ANOVA) was performed for the detection of sleep state-related differences in AHI, RAI and SAI across the patients. Post-hoc analysis was performed with the Least-Significant Difference test for pair-wise multiple comparisons. ANOVA was applied to detect changes in the generation of R-arousals attributed to the time of the night.

Paired comparisons between the RAI before and after 2:30 a.m. during NREM1&2 were performed to further assess changes in arousal threshold.

Chi-squared test was performed to assess significant association between respiratory event type and the presence of arousal.

Paired t-test was performed to evaluate the influence of event duration and oxygen desaturation on the arousal generation.

Tests were 2-tailed and $p<0.05$ was accepted as statistically significant. The Standard Error of the Means (SEM) or the Inter-quartile Range (IQR) is quoted.

8.3. RESULTS

8.3.1. Sleep quality

Mean sleep efficiency was 87.6±3.5% of the Sleep Period Time. Mean time spent in NREM1&2 was 64.4±3.6%, in SWS 21.2±2.4%, in REM 14.3±2.2% of the Total Sleep Time.

8.3.2. Intra-rater variability

Across patients the highest variability between scores was 3% for arousal index, but 94% of these scores differed by < 3 arousals/hr.
The results of the intra-rater variability are shown in table 8.1.

	Intra-rater variability: Mean difference [%] (SEM)
Sleep efficiency	0.8 (0.5)
NREM 1&2 sleep	0.5 (0.2)
SWS	0.2 (0.09)
REM sleep	0.4 (0.2)
AHI [1/hour]	1.7 (0.9)
ArI [1/hour]	3.0 (0.4)

Table 8.1. The mean intra-rater variability for the sleep states, sleep efficiency, Apnoea-Hypopnoea-Index and Arousal Index. Variability was calculated as the difference between the 2 scores, multiplied by 100 and expressed in percent and their SEM, across the 15 patients.

8.3.3. Apnoeas, hypopnoeas and cortical arousals

A total of 2667 apnoeas and hypopnoeas and 2056 R-arousals were scored across the 15 polysomnograms (figure 8.2).

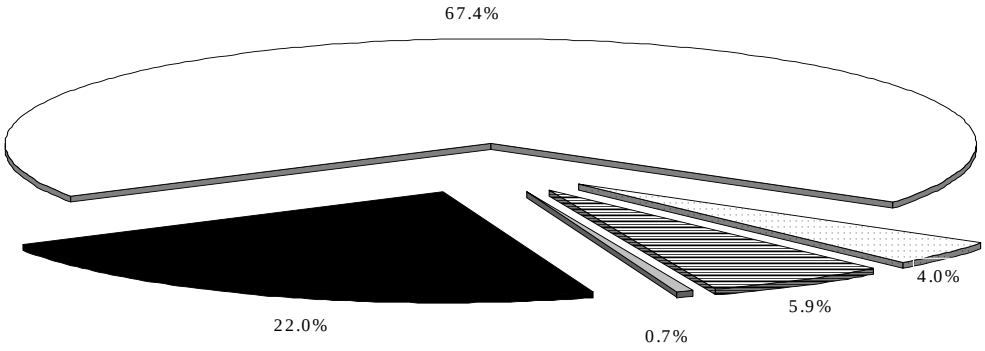


Figure 8.2. Of all apnoeas/hypopnoeas, 67.4% caused ASDA arousals, 4.0% caused 1-3sec arousals, 5.9% caused Rechtschaffen & Kales awakenings, 22.0% were terminated without any visible EEG change and 0.7% caused sleep lightening during the epoch which followed the event termination.

During NREM1&2 sleep, 2326 apnoeas/hypopnoeas were scored, 128 during SWS and 213 during REM-sleep. During NREM1&2 sleep, 1900 R-arousals were scored, 31 during SWS and 125 during REM-sleep. The mean AHI of the 15 patients was 31/h (range: 10-75/h).

Mean Arousal-Index was 34/h (range: 16-70/h). Mean SAI was 10/h (range: 4-25/h), mean RAI was 24/h (range: 8-52/h).

Hence, 80% of all respiratory events caused visible cortical arousals, 20% did not (table 8.2).

Mean ± SEM	**All night**	**NREM1&2**	**REM**	**SWS**
	15 patients			
AHI [hour^{-1}]	30.5 ± 4.5	39.6 ± 4.5	16.8 ± 4.1	11.5 ± 4.9
		38.1 ± 4.9	20.8 ± 5.4	14.9 ± 6.8
RAI [hour^{-1}]	23.4 ± 3.3	29.6 ± 4.8	11.8 ± 2.4	5.1 ± 3.3
% of all RE	79.6 ± 4.3	76.6 ± 6.8	61.6 ± 8.7	34.3 ± 8.5
SAI [hour^{-1}]	10.4 ± 1.3	13.8 ± 1.8	5.4 ± 0.6	4.9 ± 1.0

Table 8.2. Mean values and SEM across the 15 patients of: AHI: Apnoea-Hypopnoea-Index, SAI:Spontaneous-Arousal-Index as well as the RAI:Respiratory-Arousal-Index and ratio of the arousal-inducing Respiratory Events [% of all] during the whole night. In the grey field the mean values and SEM of AHI, RAI and ratio of the arousal-inducing RE [%] across the 8 patients in whom apnoeas/hypopnoeas were present in all 3 sleep states. Each patient contributed one data point to each mean.

8.3.4. Apnoea-Hypopnoea Index, Arousal Index and Sleep States

The Apnoea-Hypopnoea Index differed significantly between sleep stages across the 15 patients ($p<0.001$). Pairwise comparison showed significantly higher AHI during NREM1&2 compared to SWS ($p<0.001$) and to REM-sleep ($p=0.001$), but no significant differences between REM and SWS ($p=0.4$).

Analysis of variance (ANOVA) with the percentage of arousal-associated apnoeas/hypopnoeas in the 3 sleep states being the within subject variable, was performed between patients whose AHI was greater than zero in all 3 sleep states. Seven of the 15 patients were excluded as they failed to meet this criterion (table 8.3).

	Stage	Time [hrs]	AHI [hr $^{-1}$]	RAI [hr $^{-1}$]	Ratio [% RE]	SAI [hr $^{-1}$]
Patient 1	NREM1/2	4.4	38.7	37.6	97	13.0
	REM	1.1	5.3	5.3	100	4.4
	SWS	1.5	1.9	1.3	66	2.6
Patient 2*	NREM1/2	2.8	79.6	58.8	74	19.0
	REM	0.02	0.0	0.0	0	50.0
	SWS	0.6	52.6	21.0	40	10.5
Patient 3*	NREM1/2	3.7	57.8	42.4	73	34.5
	REM	0.3	41.4	37.9	91	3.5
	SWS	0.7	0.0	0.0	0	11.7
Patient 4*	NREM1/2	3.8	18.1	15.2	84	10.5
	REM	0.8	0.0	0.0	0	8.6
	SWS	2.2	0.9	0.0	0	3.2
Patient 5*	NREM1/2	2.7	18.7	17.5	94	15.3
	REM	0.9	5.4	4.3	80	4.3
	SWS	1.8	0.0	0.0	0	1.8
Patient 6	NREM1/2	4.5	18.5	12.5	67	8.0
	REM	0.5	14.9	10.6	71	8.5
	SWS	1.5	15.6	2.0	13	2.0
Patient 7	NREM1/2	4.1	51.8	44.8	87	4.3
	REM	0.9	43.7	20.7	47	2.3
	SWS	1.6	2.5	1.4	50	3.2
Patient 8*	NREM1/2	4.8	37.1	35.9	96	12.0
	REM	1.4	8.6	2.2	25	2.9
	SWS	1.3	0.0	0.0	0	5.4
Patient 9	NREM1/2	3.5	24.3	12.7	52	12.2
	REM	1.2	16.1	8.5	53	5.1
	SWS	1.9	2.0	1.0	50	3.7
Patient 10*	NREM1/2	4.1	33.0	28.5	86	33.1
	REM	0.8	30.5	21.9	72	11.0
	SWS	1.2	0.0	0.0	0	3.3
Patient 11	NREM1/2	5.4	50.1	47.3	94	4.4
	REM	0.8	15.0	13.7	91	6.3
	SWS	0.07	57.1	28.5	50	0.0
Patient 12*	NREM1/2	3.4	45.3	43.9	97	11.7
	REM	1.5	0.0	0.0	0	9.3
	SWS	1.1	0.0	0.0	0	4.5
Patient 13	NREM1/2	3.7	55.8	25.5	45	13.8
	REM	1.0	41.8	20.4	48	3.1
	SWS	1.7	27.4	2.4	9	3.6
Patient 14	NREM1/2	4.5	40.6	36.2	89	13.0
	REM	1.5	2.7	0.7	25	2.7
	SWS	0.9	12.5	4.5	36	13.6
Patient 15	NREM1/2	3.4	25.7	20.7	80	20.4
	REM	1.2	27.1	15.2	56	5.1
	SWS	2.0	0.5	0.0	0	4.5

Table 8.3. Time in each stage, Apnoea-Hypopnoea-Index, Respiratory-Arousal-Index, ratio of arousal inducing apnoeas/hypopnoeas [% of all RE] and Spontaneous-Arousal-Index across the 3 sleep states and patients. * The 7 patients were excluded from ANOVA comparison across the 3 sleep-states as their AHI in REM and/or SWS was zero.

All data from the remaining 8 patients including all 610 apnoeas/hypopnoeas showed significant, sleep stage-related differences in the arousal generation (F=7.0, p=0.005).

Pairwise comparison showed that a lower percentage of apnoeas/hypopnoeas were associated with arousal during SWS compared to NREM1&2 (p=0.001) and REM (p=0.02) with no significant difference between NREM1&2 and REM (p=0.2) (figure 8.3).

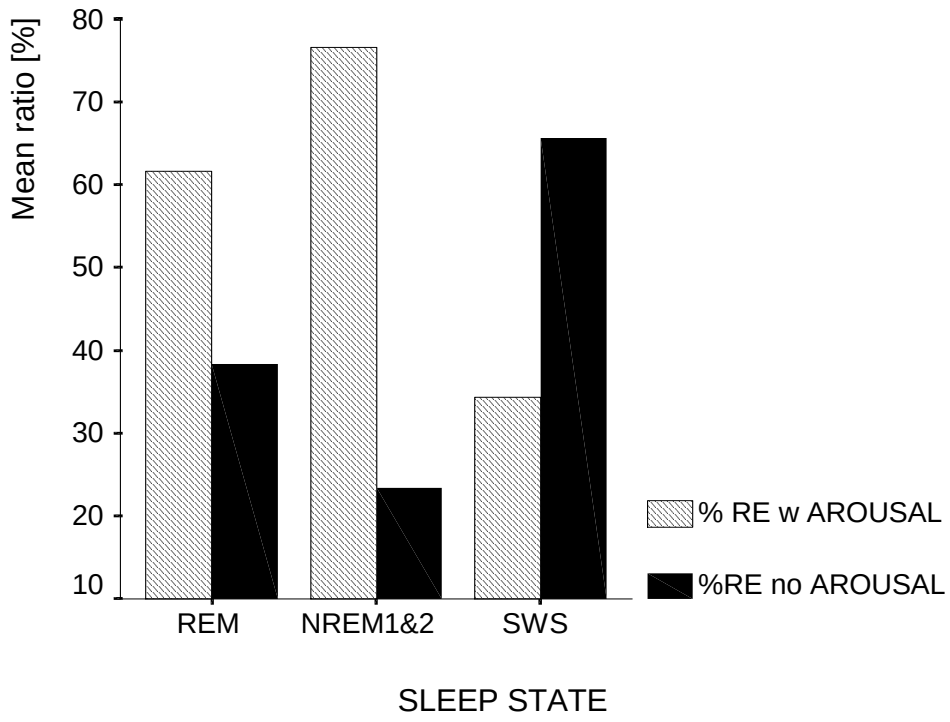


Figure 8.3. Per hour NREM1&2: 77 ± 6% of apnoeas+hypopnoeas caused arousals; SWS: 34 ± 8% caused arousals; REM 62 ± 8% caused arousals across the 8 patients.

The Spontaneous Arousal Index (SAI) differed significantly between the 3 sleep-states across the 15 patients (F=14.9, p<0.001). Pairwise comparison showed significant differences between NREM1&2 and SWS (p<0.001), NREM1&2 and REM (p<0.001) but not between SWS and REM (p=0.7).

8.3.5. Apnoeas versus hypopnoeas

Of the 2667 respiratory events, 1430 were hypopnoeas and 1237 were apnoeas. During NREM 1&2, 53% were hypopnoeas, during SWS 88% and during REM sleep 43%. Seventy-five percent of hypopnoeas and 80% of apnoeas were associated with arousals.

Chi-squared testing showed no significant difference between the respiratory event type – apnoea or hypopnoea – in the arousal generation either overall or in any sleep-state across the patients ($p>0.1$) (table 8.4).

			Apnoeas	**Hypopnoeas**	**P-value (2-sided)**
NREM 1&2	Arousals	YES	911	989	0.1
		NO	189	237	
SWS	Arousals	YES	5	26	0.7
		NO	10	87	
REM	Arousals	YES	71	54	0.8
		NO	51	37	

Table 8.4. Chi-squared test showed no significant association between respiratory event type and arousal induction

8.3.6. Apnoea / Hypopnoea duration, oxygen desaturation and arousals

The median duration of apnoeas/hypopnoeas associated with arousals was 22.5 seconds (IQR 17-30 sec), the median duration of apnoeas/hypopnoeas not associated with arousals was 20 seconds (IQR 15.5-26.0sec).

Median oxygen desaturation was 5.0% in both the apnoeas/hypopnoeas associated with arousals (IQR.0-7.0%) and in those not associated with arousals (IQR 4.0-7.0%).

Across the 15 patients, comparisons of median duration and oxygen desaturation between those apnoeas/hypopnoeas that were associated with arousal vs. those that were not associated with an arousal were not significant overall (p=0.8, p=0.07) or during NREM1&2 (p=0.9, p=0.2).

In the 8 patients in whom apnoeas/hypopnoeas occurred in all 3 sleep states, no significant differences were found in REM (p=0.3, p=0.6) or SWS (p=0.3, p=0.2) (table 8.5).

Median [IQR]	**All night**	**NREM1&2**	**REM**	**SWS**
Duration [sec]				
Hypopnoeas	21.8 [20.0-27.5]	21.5 [20.0-26.5]	37.0 [26.1-41.5]	23.0 [18.7-37.9]
Apnoeas	21.8 [17.3-22.5]	21.0 [17.0-22.5]	21.0 [19.0-25.8]	20.3 [18.0-33.1]
Desat O2 [%]				
Hypopnoeas	4.0 [3.0-5.0]	4.0 [3.0-5.0]	6.2 [5.2-9.3]	4.5 [2.2-7.7]
Apnoeas	5.0 [4.0-6.0]	5.0 [4.0-6.0]	5.0 [3.0-9.0]	3.0 [2.0-11.0]

Table 8.5. Median duration and oxygen desaturation and their IQR for apnoeas and hypopnoeas during the whole night across the 15 patients. In the grey field the median values and IQR across the 8 patients in whom apnoeas/hypopnoeas were present in all 3 sleep states. Each patient contributed one data point.

8.3.7. Time of the night

Time of the night did not influence the cortical response to apnoeas/hypopnoeas across the 15 patients (p=0.2) (figure 8.4).

Paired comparison between the mean Respiratory Arousal Index (RAI) before and after 2:30 a.m during NREM1&2 showed no significant changes across the 15 patients (p=0.6).

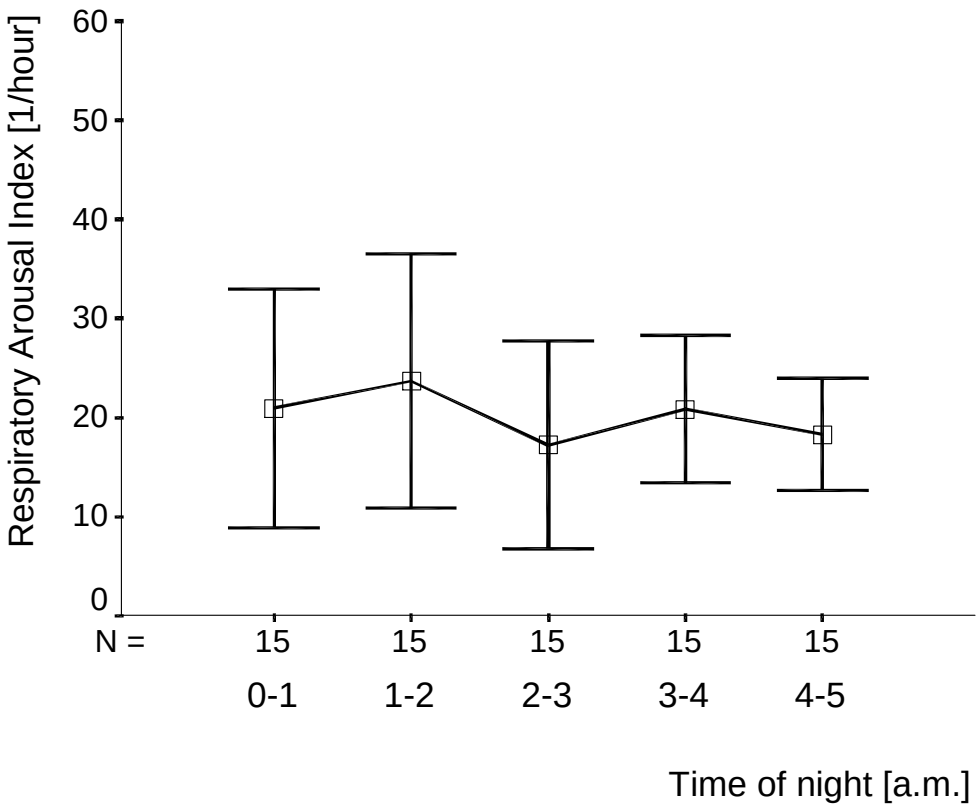


Figure 8.4. Hourly comparison of the RAI between 00:00 and 05:00 a.m. showed no significant differences in apnoea/hypopnoea-related arousability during the night across the 15 patients (p=0.2).

8.4. DISCUSSION

This study shows that apnoeas and hypopnoeas are less frequent during REM and SWS than during light sleep, that arousals associated with apnoeas/hypopnoeas are less common from SWS and that spontaneous arousals are less common from SWS and REM than from light sleep. Furthermore, the cortical arousal response is not influenced by the event type, its duration, desaturation or time of occurrence during the night.

8.4.1. Arousal threshold

This study confirms variability in the arousal threshold in response to apnoeas/hypopnoeas during SWS compared to light and REM sleep in OSAHS patients.
This is in agreement with the findings of Berry et al who, based on measurements of respiratory effort, observed a cyclical increase in cortical arousal-threshold during the night, parallel to the increase in delta power on the EEG [32].

Based on the differences found in the arousal threshold across the sleep stages, the hypothesis is proposed that all afferent impulses reach the thalamic/hypothalamic region throughout the sleep cycle but onward transmission to the cortex is dependant on the sleep state. In SWS this transmission is inhibited resulting in decreased detection of cortical arousals but preserved “non-visible” autonomic arousals [281].
The hypothesis of a higher arousal threshold during SWS is supported by the observations of Braun et al, who used Positron Emission Tomography (PET) to demonstrate dynamic changes in cerebral blood flow, reflecting changes in metabolic and functional activity throughout the sleep-wake cycle [46]. The nuclear imaging confirmed selective deactivation of fronto-parietal and sensory occipital and temporal cortical regions and their functional disconnection to thalamic nuclei during SWS.
The hypothesis of a higher arousal threshold during SWS suggests that the homeostatic drive for sleep, which cumulatively increases in sleep apnoeics as a result of repetitive arousal stimuli within and between nights [62], may be more prominent during SWS.
The frequency of spontaneous arousals also changed significantly during SWS and REM sleep compared to light sleep, supporting the hypothesis of a higher arousal threshold during these sleep states.

The difference between respiratory and spontaneous arousal frequencies during REM sleep may be related to the loss of muscle tone and the increased upper airway instability, making airways more prone to narrowing or collapse during REM sleep. Therefore, apnoeas/hypopnoeas tend to last longer [60] and are often associated with an arousal during REM sleep. In contrast, spontaneous arousals are less common in REM sleep. Some "spontaneous" arousals may be related to undetected, increased ventilatory effort [32][124].

8.4.2. Apnoeas versus hypopnoeas, arousals and AHI

The present study could not confirm the findings of Stradling et al that cortical arousal response is more commonly associated with apnoeas compared to hypopnoeas [336]. Our finding accords with the clinical impression that patients with predominately apnoeic events are not sleepier than patients with predominately hypopnoeic events [126]. Stradling et al used a different hypopnoea definition, based on a 50% thermal airflow reduction but with no desaturation or visible arousal requirement [336]. The latter study examined a mean of 18 apnoeas/hypopnoeas in 16 patients, albeit in carefully selected situations, in comparison to a mean of 178 events in 15 patients, in the present study. A further difference between the 2 studies was the mode of arousal detection. Stradling et al applied neural network analysis during a 45-seconds window and averaged to detect second-by-second changes in EEG activity as well as differences related to the type of respiratory abnormality - apnoea versus hypopnoea [336]. In the present study, visual scoring with a 1-second threshold was applied for arousal scoring.

The thermistor sensitivity in hypopnoea detection may be questioned as it is a good qualitative but relatively poor quantitative measure of airflow changes [126]. However, thermistors are widely used. To improve the precision of hypopnoea scoring and reduce the number of false positive hypopnoeas detected during visual analysis, the American Academy of Sleep Medicine modified the hypopnoea definition through the addition of an arousal and/or $\geq$3% oxygen desaturation to the 50% amplitude reduction requirement [9].

The ratio of hypopnoeas associated with arousal found in the present study is similar to that found by Gould et al [126] although the method of hypopnoea detection and the scoring criteria differed. It is therefore possible but unlikely that the hypopnoea definition used in this study has contributed to the lack of difference between apnoeas and hypopnoeas in their association with arousals.

The ratio of apnoeas to hypopnoeas varied with sleep stage, the ratio varying broadly in line with the AHI in that sleep stage. In SWS the AHI was lowest and the ratio of apnoeas to hypopnoeas was also lowest. Both presumably reflect a greater relative stability of the upper airway in SWS compared with the other sleep stages. Given that there was no difference overall, nor in any individual sleep stage, between arousal frequency following apnoeas compared with hypopnoeas, it is unlikely that the difference in arousal frequency in SWS resulted from this difference in event type.

The AHI decreased significantly from NREM 1&2 to REM to SWS in the present study.
Previous reports showed higher AHI during REM compared to NREM sleep [305].
This difference to the present outcomes may be due to:

1. previous comparisons of REM against NREM stages 1-4 and/or
2. the fact that the group studied here spent significantly more time in NREM 1&2 and 2326 of the 2667 apnoeas+hypopnoeas occurred during this state.

8.4.3. Event duration, oxygen desaturation, time of the night

The significance of apnoea/hypopnoea duration and level of oxygen desaturation in the generation of arousals was not consistent across the 15 patients.
The lack of relationship to oxygen desaturation maybe due to:

1. poor accuracy of the method to reflect chemo-receptor activation and/or
2. desaturation not being a significant cause of arousal in OSAHS, as suggested in previous studies [143].

The lack of association between apnoea/hypopnoea duration and the presence of arousals is in accordance with previous observations by Montserrat et al of apnoea-lengthening during the night [237]. The findings suggest that the more sleep is disrupted, the higher the arousal threshold and the less important apnoea/hypopnoea duration may become in disrupting sleep.
The present study could not confirm the findings of a modelling study regarding the progressive increase in arousal threshold across the night [297]. Lack of association between arousal frequency and time of night is in keeping with the findings by Rees et al [291].
Lack of progressive increase in the arousal threshold across the night may be due to the disturbed sleep cycle periodic and the sleep deprivation in OSAHS patients within and between nights,

whose circadian rhythm and homeostatic sleep dynamics are disturbed. The result is a high arousal threshold already at sleep onset in this group of patients.

8.4.4. Study limitations

Limitations of this study include power, definitions and multiple comparisons.

Fifteen patients were studied but 7 of those were excluded post hoc from the across sleep-states comparisons as they had no events in one or more sleep stages, usually in SWS although 2 patients in REM sleep and one patient only had apnoeas/hypopnoea during NREM 1&2. Therefore, it can be questioned whether these data are applicable to all OSAHS patients. The findings across the 8 patients studied are consistent in regard to the low ratio of apnoeas/hypopnoeas associated with arousals during SWS, which ranged between 0 and 66% (table8.3). The patients were randomly selected and the group studied included mild, moderate and severe sleep apnoeics with symptoms and polysomnographic findings typical of the syndrome. The low percentage of REM and SWS is common in untreated OSAHS patients and is an indicator for treatment success and adherence [221].

All apnoeas/hypopnoeas were studied. This represents a large body of data (2667 events) with which to answer the questions posed. Our results are not only robust but represent the largest body of events which have been so analysed. The study is thus adequately powered to detect clinically important differences.

There are many definitions of hypopnoeas and arousals used in the literature. The hypopnoea definition used in this study fulfils the ASDA recommendations [5]. The inclusion of arousals or desaturation in the definition may lead to a greater association with arousals than would be found using other ASDA accepted definitions, i.e. based on thoraco-abdominal movement or nasal pressure alone. The arousal definition used, has been used in previous studies and validated against respiratory events and outcomes [291].

The primary end-points were defined clearly and the aim was to keep the number of comparisons to a minimum. However, the present study involved 17 statistical comparisons. Multiple, albeit independent, comparisons would increase the chances of the study showing significant differences, whereas our results were largely negative. Thus, this does not weaken our failure to prove our a priori suggestions that event type, duration, desaturation and time of the night would predispose to arousal induction.

The author acknowledges that the present study did not look into all factors which may influence cortical response to apnoeas/hypopnoeas. These are changes in upper airway resistance, snoring, and body posture. Stradling et al have shown that the latter is not a significant contributor to cortical changes, as detected through neural networks [336]. However, the analysis of these factors in the present study was not planned and is not possible retrospectively from the data collected.

8.4.5. Implications of the study outcomes

Despite the modification in the time threshold used to score arousals, 20% of respiratory events were not terminated by cortical arousals in the present study. This suggests that the paucity of cortical changes detected at apnoea/hypopnoea termination is not due to the neurophysiological arousal definitions used but may be related to the inability of the currently applied electroencephalographic diagnostic techniques to monitor accurately sleep physiology. It is unclear what terminates apnoeas and hypopnoeas which are not terminated through visible cortical arousals. One possible explanation is that other kind of reactions, the autonomic, terminate apnoeas/hypopnoeas at thalamic/ hypothalamic level, linked to presso-/ mechano-/ chemo-receptor afferent stimuli [124][215] without inducing cortical electrical changes. Alternatively, they may be represented on the cortex but the changes cannot be detected through standard techniques and/or topology. Monitoring of frontal brain activity and of autonomic activity and/or the application of non-conventional techniques such as neural networks or Fast Fourier Transform analysis may improve the detection of changes at apnoea/hypopnoea termination and minimise the difference between AHI and RAI. There is some evidence from previous studies in support of this [259][336][360]. Whether differences between sleep stages will be minimised, needs further exploring.

The studies in the next 2 chapters will address this hypothesis.

CHAPTER 9
EEG SPECTRAL ANALYSIS:
DETECTION OF CORTICAL ACTIVITY CHANGES IN SLEEP APNOEA PATIENTS

9.1 INTRODUCTION

Cortical arousals induced by apnoeas and hypopnoeas during sleep are believed to be the primary cause of sleep fragmentation, daytime sleepiness, impaired cognitive function and concentration in OSAHS patients. However, previous studies have found a weak correlation between severity of clinical symptoms or objective sleepiness and polygraphic findings, in terms of apnoeas, hypopnoeas and cortical arousals [61][137][188]. Approximately 28% of apnoeas and hypopnoeas are not terminated by visible cortical arousals [291]. This could be due to the limitations of visual scoring to detect all arousals. This suggestion is supported by the findings of Martin et al. who showed that daytime sleepiness was increased in healthy subjects by applying stimuli which caused autonomic changes but no visible changes in cortical activity [215]. The same study showed a significant increase in alpha band power when tones induced visible arousals and also when they didn't. It has been previously suggested that differences in arousability are related to the depth of sleep [32]. Non-conventional techniques of EEG analysis such as neural networks [266][335] and power spectral analysis [32][339] succeeded in improving detection of cortical activity changes during apnoeas/hypopnoeas and at their termination, irrespective of the sleep stage during which they occurred or the arousal visibility on conventional EEG. EEG power spectral analysis during apnoeas/hypopnoeas [339] and during periods of increased inspiratory effort detected through oesophageal manometry [32], showed increase in delta power towards the end of these events. The extent of increase was found to vary in relation to the sleep cycle.

In the study reported here, we tested the hypothesis that detection of cortical activity changes at apnoeas/hypopnoeas termination may be improved through the application of spectral analysis of the EEG. The power spectrum of the EEG signal was analysed to try to improve detection of non-visible signal changes related to apnoea/hypopnoea termination. The EEG spectral power changes during sleep in OSAHS patients were compared with the EEG spectral power during sleep in healthy individuals.

9.2. METHODS

9.2.1. Primary Endpoints

To ascertain whether quantitative EEG analysis can:

1. improve detection of changes related to apnoeas/hypopnoeas
2. differentiate between physiological and abnormal sleep pattern.

9.2.2. Study population

PATIENTS

Fifteen consecutive patients, who were newly diagnosed with the OSAHS (AHI $\geq$10 /hr), were randomly selected from the database of the Sleep Centre at the University Hospital Charité in Berlin.

All study patients referred to the sleep centre with possible OSAHS had either self-reported daytime sleepiness (Epworth Sleepiness Scale >10) not explained by other factors or two other major symptoms of the OSAHS [370]: chocking or gasping during sleep, recurrent awakenings, unrefreshing sleep, daytime fatigue and/ or impaired concentration. A detailed medical history and examination was carried out in the outpatient department. All patients underwent a chest x-ray, an ECG and a lung function test. Study exclusion criteria were autonomic, endocrine, cardiac or pulmonary disease, Periodic Limb Movement Disorder, other neurological disorders or the intake of medication which affected sleep or autonomic function. Two patients were on the antihypertensive Nifedipine. All subjects were otherwise healthy and normotensive. Fourteen patients were male and 1 patient was female. Their mean age was 51 years [SD 9 years] and the mean BMI was 29 kg/m^2 [SD 2 kg/m^2].

HEALTHY CONTROLS

Seven healthy asymptomatic individuals in whom OSAHS had been previously excluded were selected to match the patients' age and BMI. Four were male and 3 were female. Their mean age was 50 years [SD 10 years] and the mean BMI was 28 kg/m^2 [SD 2 kg/m^2].

All study participants underwent an in-hospital overnight diagnostic polysomnography.

9.2.3. Ethical Approval

All participants gave written informed consent for their data to be used in the study, which was approved by the Ethics Committee of the Humboldt University in Berlin.

9.2.4. Polysomnography

A computerised system was used for the polysomnographic recordings. The system had a digital hardware Butterworth filter of 3rd order with cut-off frequencies set at 0.6 and 50 Hz at 12 dB per octave.

Recordings were carried out between 10 p.m. and 7 a.m. and consisted of the following:

ELECTROENCEPHALOGRAPHY

Recording of sleep and arousals was based on following tracings:

Four unipolar EEG tracings: 2 central, the C3A2 and C4A1, and 2 occipital, the O1/A2 and O2/A1 tracings.

Two outer canthi electrodes, LEOG and REOG, for the detection of eye movements.

A submental EMG electrode for the detection of tonic muscle activity.

ELECTROMYOGRAPHY

Leg movements were detected through bilateral tibial EMG electrodes, whereas body movements were recorded through a body position detector. The body detector did not include continuous information on supine body position.

CARDIORESPIRATORY MONITORING

Monitoring of respiratory function consisted of a thermistor sensor for the oronasal airflow detection, 2 piezoelectric belts for the detection of thoracoabdominal movement, a digital microphone for snoring detection and a pulseoximeter to monitor blood oxygen saturation levels.

A 3-lead ECG was used to monitor cardiac activity.

9.2.5. Visual scoring

The visual scoring was performed by the primary investigator (K.D.), blinded to subject.
Every polysomnogram was scored during 3 consecutive sessions: sleep staging was followed by the scoring of apnoeas and hypopnoeas; arousals were scored separately during the final session.
Approximately 7% of the data were excluded due to movement or EEG artefacts.

SLEEP SCORING

Sleep and arousal scoring was based on the C4A1 and C3A2 tracings. Sleep was scored according to the Rechtschaffen and Kales criteria [288], described in detail in chapter 2.

Following parameters were used to describe sleep quality:

Sleep onset was defined as the first scored 30-second epoch of sleep stage 2.

Total Sleep Time **[hrs]** (TST) was calculated as the total time spent asleep.

Sleep Period Time **[hrs]** (SPT) was the time between sleep onset and the last epoch of sleep.

Sleep efficiency **[%]** was the ratio of TST divided by the SPT and multiplied by 100.

Time spent in each sleep state **[%]** was the ratio of time in each sleep state divided by TST.

APNOEA / HYPOPNOEA SCORING

Apnoeas were defined as cessation of the oronasal airflow, lasting $\geq$10sec.

Hypopnoeas were defined as airflow reduction of >50%, compared to a 10sec peak amplitude during the preceding 2 minutes, lasting $\geq$10 sec and associated with either an oxygen desaturation of $\geq$3% or an arousal [5].

Oxygen saturation was based on a second-by-second measurement.

Apnoea/Hypopnoea Index **[hr $^{-1}$]** (AHI): the number of all apnoeas and hypopnoeas divided by the total time spent asleep.

Disease severity was based on the AHI [5]:

Mild OSA: $15 > AHI \geq 10$

Moderate OSA: $15 \geq AHI \geq 30$

Severe OSA: $30 > AHI$

An AHI<10/hour sleep was considered normal.

AROUSAL SCORING

The scoring of arousals was based on the definition of the American Sleep Disorders Association (ASDA), described in chapter 4, modified as to the time-threshold which was set at 1 second rather than 3 [9]. The C4A1 and C3A2 tracings of the EEG were used for the scoring. Arousals were scored when an abrupt shift to a faster EEG frequency (including theta, alpha and/or greater frequencies but no spindles) occurred. Arousal scoring ranged from 1 to 15sec, irrespective of sleep recurrence or prolonged awakening subsequent to the arousal. Hence, Rechtschaffen and Kales awakenings [288] were also included in the scoring.

Respiratory Arousal Index **[hr $^{-1}$]** (RAI): The number of arousals following apnoeas and hypopnoeas per hour slept was calculated.

9.2.6. Intra-observer variability

To assess reproducibility of scoring outcomes, the 15 polysomnograms were scored twice by the primary investigator (K.D.). In these intra-rater analyses the researcher was blind to the patient details and the results of the comparison studies. The second scoring was carried out 12 months after the initial study was scored and blind to subject name and diagnosis.

Variability was calculated as the difference between the 2 scores, multiplied by 100 and expressed in percent.

9.2.7. Quantitative analysis: EEG Spectral Power

SPECTRAL ANALYSIS

In order to monitor and analyse quantitatively both visible and non-visible electro-encephalographic changes related to apnoeas and hypopnoeas, power spectral analysis of the C4A1 tracing was performed [264][350].

The Power Spectral Density (PSD) curve was generated and the power distribution as a function of the EEG's constituent frequencies was calculated. For the PSD calculation, the non-parametric Fast Fourier Transform (FFT) algorithm was applied, using the Welch's technique [367].

The normalised power values, i.e. the absolute band power value as a fraction of the total power, were calculated for the following frequency bands:

1. delta frequency band: 1-4 Hz
2. theta frequency band: 4-8 Hz
3. alpha frequency band: 8-12Hz
4. sigma frequency band: 12-16Hz

Off line analysis was performed using a software package. The DC-free EEG signal (sampled with 100Hz) was filtered with a Finite Impulse Response (FIR) low-pass filter and a cut-off frequency at 25Hz [260].

For the calculation of the power density a 128 bin Hamming window was applied with 64 points overlap and a resolution of 0.015Hz.

For the delta, theta, alpha and sigma frequency bands, the area under the spectral curve was calculated using a 5^{th} order Gaussian integration method, yielding power expressed in $[microVolt]^2$ [232] (figure 9.1).

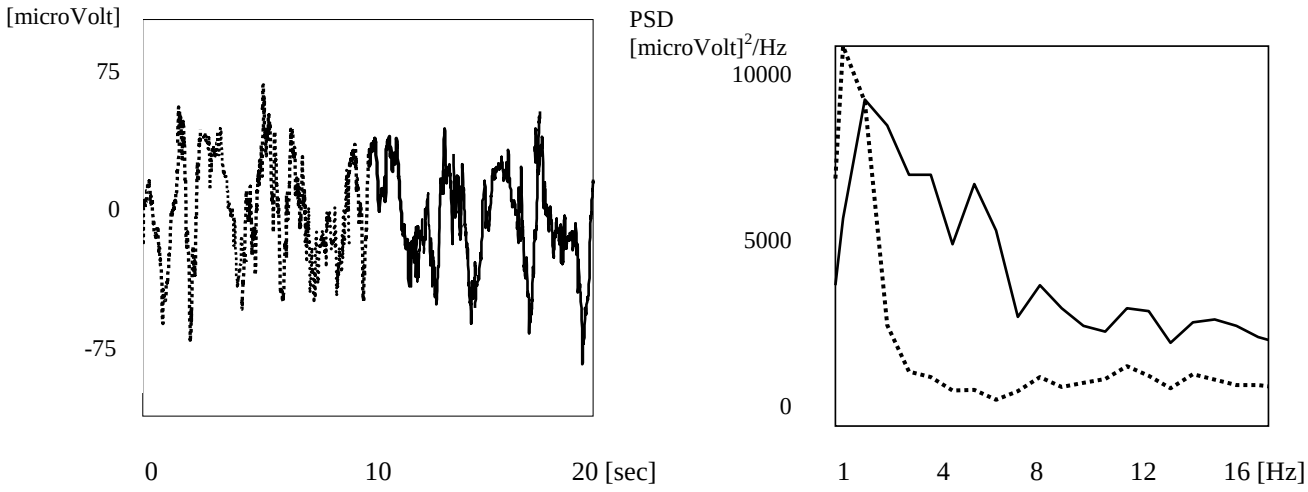


Figure 9.1. Example of the EEG spectral analysis 10sec before vs. after the end of the same hypopnoea illustrated in figure 9.2. Calculation of the PSD curves before (slashed curve) and after (black curve) the end of the hypopnoea. Calculation of area under the curves for the frequency bands delta (1-4Hz), theta (4-8Hz), alpha (8-12Hz) and sigma (12-16Hz) yield the absolute power values within each frequency band. The normalised spectral power values are the fraction of each band compared to the total power and multiplied by 100. Comparisons have been carried out between the normalised power values within the same frequency bands of the 2 PSD curves (i.e. before and after the end) in each manually scored apnoea/hypopnoea.

TIME WINDOWS

The periods analysed centred around the end of apnoeas/hypopnoeas identified as increase/recovery of the airflow amplitude. To assess power changes at apnoea/hypopnoea termination, comparisons were conducted between the synonymous frequency bands 10sec before vs. 10sec after the end of each apnoea/hypopnoea.

The time windows were chosen to maintain signal stationarity within the applied window as required for the application of the FFT algorithm. The 10-second window would in most cases avoid overlap between post-apnoeic/-hypopnoeic intervals and the following early apnoeic/ hypopnoeic periods. The further aim of the chosen windows was to maximise the signal to noise ratio by a compromise between occupying as much of the window as possible with potential arousals while at the same time allowing a reasonably wide window to cut down on random variance in the EEG signal (figure 9.2).

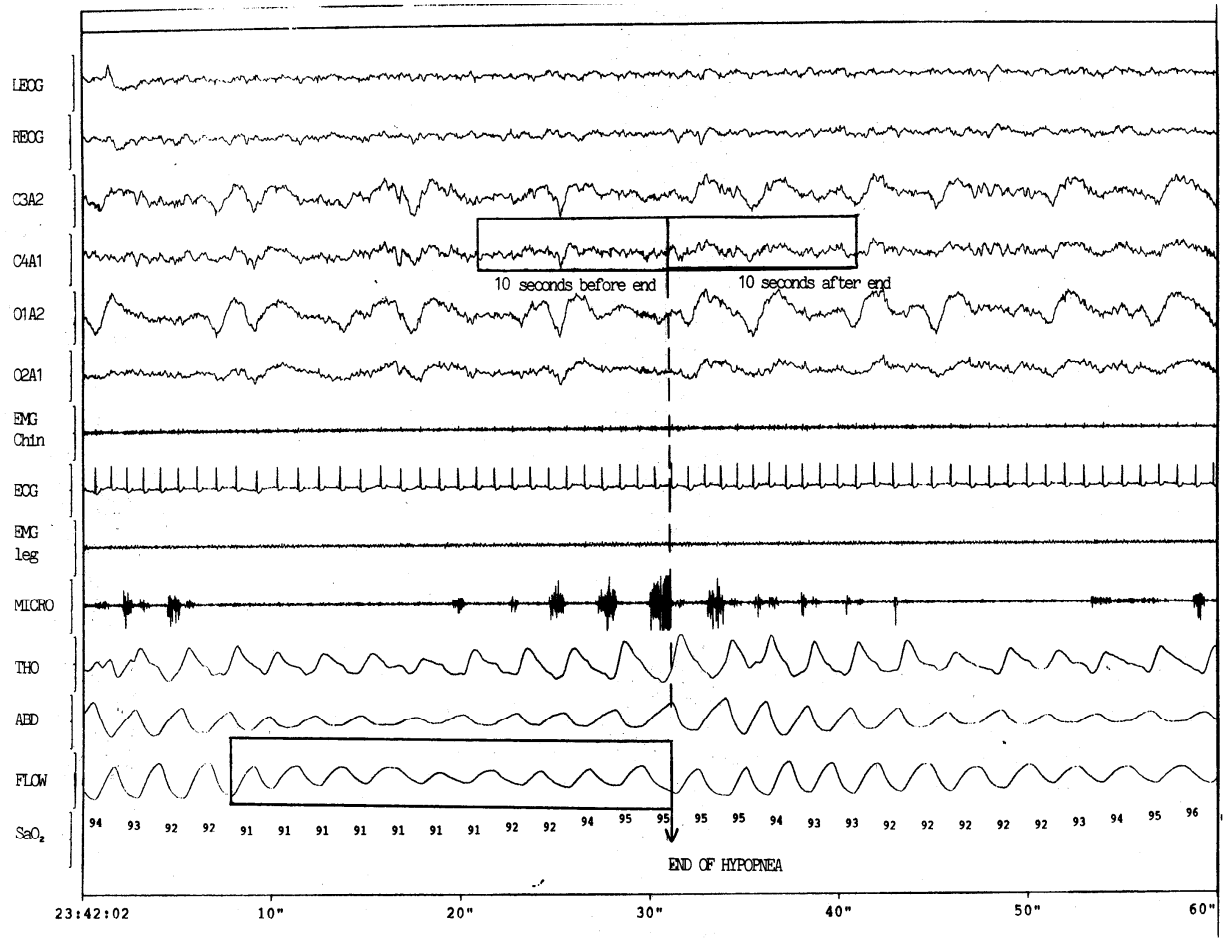


Figure 9.2. Ten second windows chosen for detection of electroencephalographic spectral power changes linked to apnoea/hypopnoea termination. The present polysomnographic window illustrates a hypopnoea during NREM sleep, terminated by a visually undetectable manner.

WITHIN PATIENTS COMPARISONS

To determine whether the observed power changes were caused by apnoeas/hypopnoeas, we compared the outcomes to periods of undisturbed sleep of the same duration in REM and NREM sleep, during which no apnoeas/hypopnoeas occurred. These periods were not necessarily free of snoring.

COMPARISONS BETWEEN PATIENTS AND HEALTHY CONTROLS

To ascertain whether the observed EEG power changes across OSAHS patients are significant, comparisons were carried out to healthy, age and BMI matched control subjects.

9.2.8. Statistical Analysis

POWER CALCULATION

To ascertain an adequate sample size, the calculation of statistical power was based on the data from a similar study by Bandla et al [18]. The authors examined 8 children and a total of 72 apnoeas. Power spectral analysis of the EEG signal was carried out for the periods of 10 seconds preceding (PRE values), 10 seconds following (POST values) the apnoeic episodes as well as for the duration of airflow obstruction, which was variable. Comparisons were made for the different frequency bands using analysis of variance (ANOVA). The authors found a significant decrease in normalised theta power at apnoea termination Theta-PRE: 27 n.u. (SD 7 n.u.), Theta-POST: 17.5 n.u. (SD 6 n.u.). From the data provided by Bandla et al [18], no information is available on the correlation between the before and after values across patients. It is therefore not possible to specify the power of these data for a paired analysis, as this would depend on how well the before and after values were correlated across patients.

However, an unpaired analysis assumes zero correlation. Using the normalised power values for the theta frequency band during the fixed, 10sec PRE and POST periods and assuming an unpaired analysis, a sample size of 11 patients would give a 90% power at a 5% level of significance. In the event of a strong positive correlation the power would increase.

In the present study, a paired analysis was carried out for the periods before and after apnoea/hypopnoea termination. For the same sample size of 11 patients, a paired analysis would have at least the power of an unpaired analysis, i.e. a paired analysis with 11 pairs should have greater than 90% power at a 5% significance level.

The study of 15 patients with each patient contributing 1 data point to each mean is therefore adequately powered, producing robust and representative results.

STATISTICAL TESTS

Statistical data analysis was performed using the SPSS Inc. Chicago IL, Version 9 software.

For the before versus after apnoea/hypopnoea termination comparisons as well as for the comparison between consecutive periods of undisturbed sleep across the patients, paired t-tests were performed. The Bonferroni correction of p-values for multiple comparisons was used and $p \leq 0.01$ was accepted as statistically significant during the paired comparisons.

The most significant band power ratio (before versus after the end) at apnoea/hypopnoea termination, as evaluated through paired comparisons, was used for the construction of Receiver-Operator-Characteristic (ROC) curves [382]. One ROC curve was constructed for each patient's polysomnogram. The ratio at the end of all apnoeas/hypopnoeas and the ratio around all points which separated 2 consecutive periods of undisturbed sleep were used for each curve. The area under the ROC curve determined how well the chosen ratio differentiates between apnoea/hypopnoea termination-related changes and undisturbed sleep (the bigger the area, the better the sensitivity).

For the comparison between OSAHS patients and healthy subjects we calculated the mean power of the same frequency band as above for each polysomnogram, using 10-second sliding windows. Wake epochs and artefacts were excluded.

Three ROC curves were constructed using the mean power values of the patients and healthy controls: one for the total sleep time, one for NREM and one for REM sleep - each subject contributed one point to the curve. The area under the ROC curves determined how well the chosen band power differentiates between OSAHS patients and healthy controls.

Independent t-test evaluated the differences between patients and healthy subjects in the band power found to be significant at the end of apnoeas/hypopnoeas.

One-tailed Pearson's correlation analysis evaluated the relation between Apnoea-Hypopnoea-Index (AHI) and mean significant band power and between AHI and time spent in SWS and REM sleep across the patients.

All comparisons and ROC curves were based on measurements within 10sec periods. $P<0.05$ was accepted as statistically significant. Mean values and the Standard Error of the Means (SEM) or median and Interquartile Ranges (IQR) are quoted.

9.3. RESULTS

9.3.1. Sleep quality

All patients showed a disturbed sleep pattern. Their mean sleep efficiency was 88 ± 3% of the Sleep Period Time. Mean time spent in sleep stages 1&2 (NREM1&2) was 64 ± 4% (range: 51-86%), in SWS 21 ± 2% (range: 1- 32%) and in REM 15 ± 2% (range: 0.6- 25%) of the TST. The healthy subjects' mean sleep efficiency was 92 ± 3%, mean time spent in NREM1&2 was 62 ± 4% (range: 49- 75%), in SWS 21 ± 3% (range: 9- 29%) and in REM 17 ± 3% (range: 4- 24%).

9.3.2. Visual scoring

Visual scoring of the patients' polysomnograms resulted in a mean AHI of 29 per hour slept (range: 10 – 75 /hr) and a mean RAI of 23 per hour slept (range: 9 – 52 /hr).

The AHI and time spent in SWS and in REM sleep were reciprocally well correlated (rho= -0.6, p=0.01) across the 15 patients (figure 9.3).

The median duration of apnoeas and hypopnoeas was 22 seconds (IQR 17- 29 seconds), their median oxygen desaturation was 5% (IQR 3-7 %).

Of all apnoeas and hypopnoeas, 21% were not associated with a visually detectable EEG arousal, 69% were associated with ASDA arousals, 4% with 1-3 second ASDA microarousals and 6% of all apnoeas/hypopnoeas induced awakenings.

During NREM sleep, 79% of all apnoeas/hypopnoeas caused visible arousals, 21% did not.

During REM sleep, 61% caused arousals, 39% did not. In 3 patients, no apnoeas/hypopnoeas occurred during REM sleep and they were excluded from REM-related comparisons.

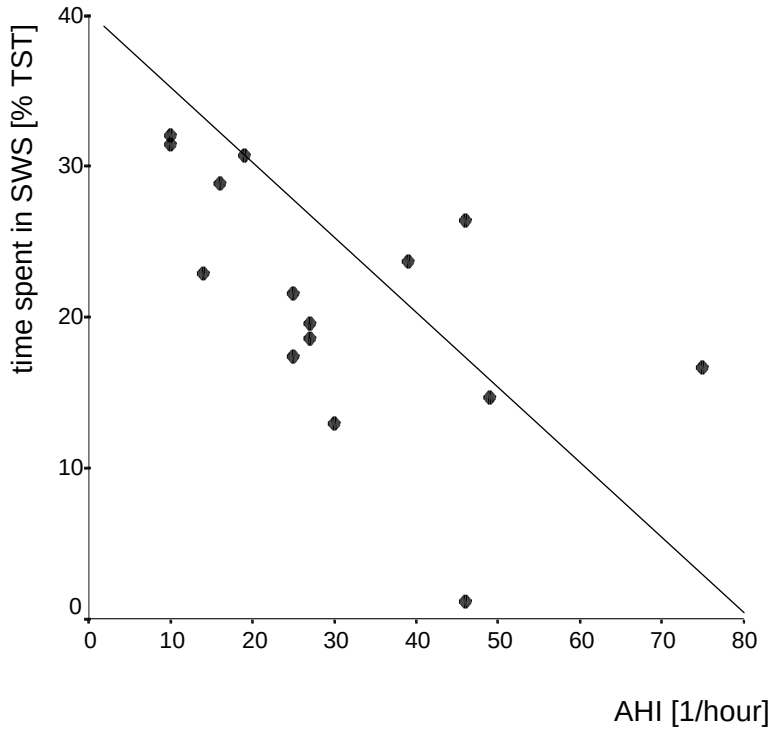


Figure 9.3.A. Reciprocal correlation between AHI and time spent in SWS across the 15 patients (rho= -0.6, p= 0.01).

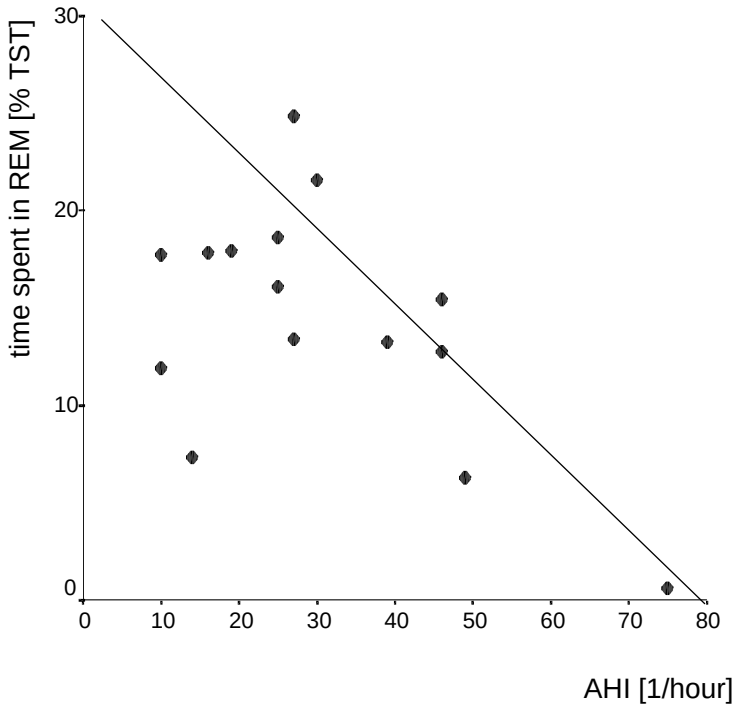


Figure 9.3.B. Reciprocal correlation between AHI and time spent in REM across the 15 patients (rho= -0.6, p= 0.01).

9.3.3. Intra-rater variability

The highest intra-rater variability was 3% (SEM 0.4) for the arousal index. The variability in sleep efficiency (awake vs asleep scoring) was 0.8% (SEM 0.5), in AHI 1.7% (SEM 0.9).

9.3.4. Across patients before and after comparisons

All apnoeas/hypopnoeas, not affected by movement and/or EEG artefacts, have been included in the comparisons. In total, 2596 apnoeas/hypopnoeas and 1690 20-second (2 x 10 seconds) "non-event" periods of undisturbed (baseline) sleep were evaluated across the patients.

During NREM sleep, 2395 apnoeas/hypopnoeas and 1363 20-second baseline sleep periods were evaluated. During REM sleep, 201 apnoeas/hypopnoeas and 327 baseline periods were compared.

Paired t-test, with each patient contributing one data point to each mean, showed that the post-apnoeic and post-hypopnoeic decrease in normalised theta power was the most significant ($p<0.003$) and consistent change during NREM and REM sleep as well as in the presence or absence of associated visible EEG arousals (table 9.1).

In addition, during NREM sleep alpha and sigma power increased significantly ($p\leq0.01$) in apnoeas/hypopnoeas associated with a visible EEG arousal, whereas in apnoeas/hypopnoeas without associated visible arousals there was a significant increase in delta power ($p\leq0.005$).

During REM sleep, no significant power changes were found around non-arousal apnoeas/hypopnoeas.

	ALL A+H		A+H & AROUSAL		A+H NO AROUSAL		BASELINE	
ALL	**BEFORE**	**AFTER**	**BEFORE**	**AFTER**	**BEFORE**	**AFTER**	**BEFORE**	**AFTER**
DELTA	41;0.006	43;0.01	41;0.01	42;0.01	41;0.01	44;0.02	42;0.01	42;0.01
THETA	28;0.003	24;0.006	28;0.005	24;0.008	28;0.004	25;0.008	28;0.004	28;0.004
ALPHA	18;0.003	19;0.005	18;0.005	20;0.006	18;0.005	18;0.008	18;0.01	18;0.01
SIGMA	13;0.002	14;0.004	13;0.04	14;0.005	13;0.004	13;0.008	12;0.004	12;0.004
NREM								
DELTA	41;0.006	45;0.01	42;0.01	41;0.01	41;0.01	48;0.02	45;0.01	45;0.01
THETA	28;0.003	24;0.006	28;0.004	23;0.009	28;0.004	23;0.009	26;0.004	26;0.004
ALPHA	18;0.004	18;0.007	17;0.006	21;0.008	18;0.007	17;0.008	17;0.01	16;0.01
SIGMA	13;0.002	13;0.005	13;0.003	15;0.006	13;0.004	12;0.007	12;0.004	11;0.004
REM								
DELTA	40;0.01	41;0.02	40;0.01	43;0.03	40;0.02	38;0.02	38;0.01	38;0.01
THETA	28;0.006	24;0.01	28;0.009	22;0.01	28;0.007	26;0.02	28;0.01	28;0.004
ALPHA	19;0.005	21;0.007	19;0.008	21;0.01	20;0.005	21;0.01	21;0.01	21;0.01
SIGMA	13;0.006	14;0.008	13;0.007	14;0.008	12;0.009	15;0.01	13;0.01	13;0.001

Table 9.1. Mean values and SEM of each frequency band power expressed as a fraction of total power multiplied by 100 before and after the end of each apnoea/hypopnoea and during undisturbed sleep, free of apnoeas/hypopnoeas (baseline). Each patient contributed one data point to each mean.

During periods of undisturbed sleep, no significant power changes ($p \geq 0.2$) were found across the patients (table 9.2).

	APNOEAS/HYPOPNOEAS			BASELINE SLEEP
P-VALUES	**ALL**	**& AROUSAL**	**NO AROUSAL**	
ALL #	2596	2001	595	1690
$DELTA_n$	0.7	0.5	0.005	0.7
$THETA_n$	<0.001	<0.001	0.001	0.3
$ALPHA_n$	0.008	0.001	0.1	0.5
$SIGMA_n$	0.05	0.01	0.5	0.6
NREM #	2395	1887	508	1363
$DELTA_n$	0.02	0.5	0.002	0.3
$THETA_n$	<0.001	<0.001	0.001	0.9
$ALPHA_n$	0.1	0.001	0.09	0.6
$SIGMA_n$	0.5	0.01	0.1	0.4
REM #	201	114	87	327
$DELTA_n$	0.8	0.3	0.2	0.7
$THETA_n$	0.003	0.004	0.2	0.2
$ALPHA_n$	0.1	0.5	0.1	0.6
$SIGMA_n$	0.1	0.5	0.1	0.9

Table 9.2. P-values, assessed through Student's paired t-tests across the patients, derived from the comparisons between the mean normalised power values within the same frequency bands 10 sec before vs. after the end of apnoeas/hypopnoeas as well as during undisturbed, apnoea-/hypopnoea-free (baseline) sleep. Each patient contributed one data point to each mean. During the whole night (ALL) and during NREM sleep, comparisons were across 15 patients; during REM sleep, comparisons were across 12 patients.

The mean normalised theta power correlated well with the patients' AHI (rho=0.6, p=0.008) (figure 9.4).

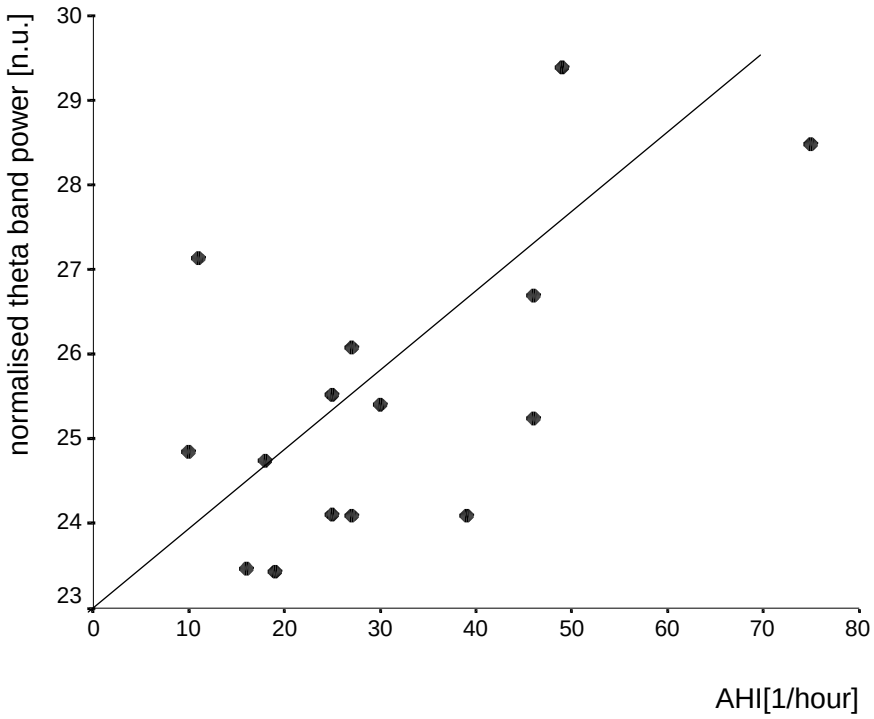


Figure 9.4. Correlation between apnoea-hypopnoea-index (AHI) and mean normalised theta band power (rho=0.6, p=0.008) across the 15 patients.

To assess how well theta power fluctuations can differentiate undisturbed sleep from apnoea- and hypopnoea-related EEG changes, 1 ROC curve was constructed for each patient.

Each curve consisted of the $theta_{normalised}$ power ratios [tn_{before_end} : tn_{after_end}] at the end of all apnoeas/hypopnoeas and around a point separating 2 10-second periods of undisturbed sleep. The areas under the ROC curves, determining the sensitivity of the method, varied between 0.6 and 0.9 across the 15 polysomnograms (table 9.3).

Patient	AHI[h^{-1}]	tn_b:tn_a (SEM; p-value)	#RE	#BL
1	75	0.7(0.04;<0.001)	256	40
2	49	0.7(0.05;0.002)	226	32
3	39	0.7(0.03;<0.001)	249	92
4	46	0.8(0.03;<0.001)	276	20
5	46	0.7(0.02;<0.001)	293	218
6	25	0.9(0.01;<0.001)	179	168
7	25	0.7(0.03;<0.001)	188	120
8	27	0.6(0.03;<0.001)	160	148
9	27	0.9(0.02;<0.001)	149	173
10	30	0.6(0.03;0.004)	198	100
11	10	0.9(0.03;<0.001)	71	32
12	10	0.7(0.05;0.002)	49	134
13	14	0.7(0.03;<0.001)	92	109
14	16	0.7(0.03;<0.001)	105	131
15	19	0.7(0.03;<0.001)	105	173
MEAN	29	0.7	173	113
RANGE	10 -75	0.6-0.9	49-293	20-218

Table 9.3. Areas under the ROC curves for each patient based on the theta normalised power ratio [tn_before_end : tn_after_end] within 20sec windows (10sec before vs. 10sec after a point marking either the end of an event or separating a period of undisturbed, baseline sleep), aiming to differentiate between apnoea/hypopnoea termination related EEG changes from baseline sleep. Abbreviations: #RE: number of respiratory events (apnoeas/hypopnoeas); #BL: number of baseline sleep periods. SEM: Standard Error of the Means.

9.3.5. OSAHS patients compared to healthy individuals

Mean normalised theta power across patients was 25 ± 0.5 n.u. and significantly lower than in healthy individuals 28 ± 1 n.u. (F=2.4, p=0.03). In the patients group the mean delta power was 46 ± 0.1 n.u., alpha power 17 ± 0.05 n.u. and sigma power 12 ± 0.02 n.u. In the healthy controls group the mean delta power was 44 ± 0.1 n.u., alpha power 17 ± 0.1 n.u. and sigma power 11 ± 0.1 n.u.; none of the three bands differed significantly between the 2 groups (p>0.2).

To assess how well theta power fluctuations can differentiate OSAHS patients from healthy controls, ROC curves were constructed. During NREM sleep the area under the curve was 0.76 (std.error: 0.1, p=0.05), during REM sleep 0.82 (std. error: 0.09, p=0.02) and 0.76 (std.error: 0.1, p=0.05) during the total sleep time (figure 9.5).

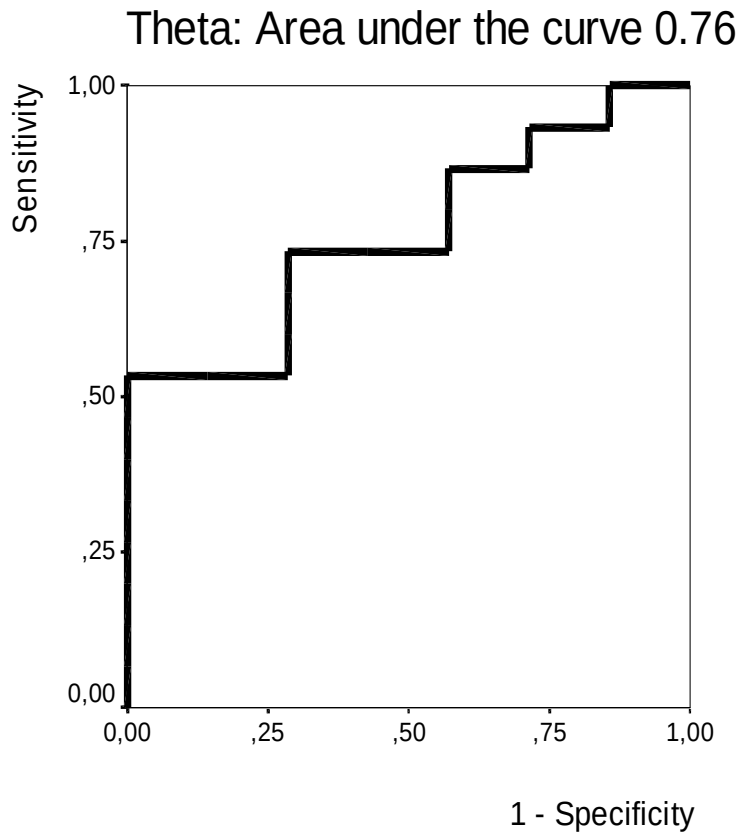


Figure 9.5. The ROC curve assesses how well the mean normalised theta power differentiates between OSAHS patients and healthy controls. The calculation of the mean theta power is based on spectral analysis within 10sec sliding windows across the whole night's EEG. Each subject contributed one point to each mean; i.e. the ROC curve consists of 22 points (15 patients and 7 healthy controls). The area under the curve is 0.76. With 1.0 being the highest possible area, mean theta power is a good marker for the differentiation between OSAHS patients and healthy subjects.

9.4. DISCUSSION

The outcomes of this study confirm the hypothesis that apnoeas and hypopnoeas are associated with significant spectral power changes even in the absence of visually apparent arousals. This finding is based on the mean body of 2596 apnoeas/hypopnoeas and 1690 “non-event” sleep periods analysed across 15 patients. Thus visual arousal scoring in OSAHS patients is less sensitive in detecting all cortical arousal-related changes. To our knowledge this is the first study which evaluates EEG spectral power changes during REM and NREM sleep, around the end of all apnoeas and hypopnoeas across patients.

The present study provides important information on cortical activity changes linked to arousal and non-arousal apnoeas/hypopnoeas during REM and NREM sleep.

9.4.1. Spectral Power, Sleep States and Arousal

The most significant and consistent change across the 15 patients was the decrease in normalised theta power at the end of apnoeas and hypopnoeas.

During NREM sleep when arousals were visible, the decrease in normalised theta derived from the increase in alpha and sigma power. When arousals were not visible, the theta fraction decreased as a result of delta power increase.

Across the 12 patients, REM-related arousals at the end of apnoeas and hypopnoeas were associated with a decrease in theta power fraction alone, which indicates a predominant decrease in the absolute theta power.

The 87 REM-related apnoeas/hypopnoeas, which were not terminated by visible arousals, were not associated with any significant spectral power changes. This may be due to the limited number of REM-related non-arousal apnoeas/hypopnoeas across the 12 patients (mean: 6, range: 1-21 per patient). It furthermore suggests that during REM sleep, the termination of apnoeas/hypopnoeas evokes a subcortical reaction but does not always evoke a cortical reaction.

Our findings are consistent with Svanborg et al who analysed the EEG power spectrum in the course of apnoeic/hypopnoeic episodes based on 2-second epochs and found an increase in delta power towards the end of apnoeas/hypopnoeas and a decrease at their termination when faster activities became more prominent [339].

Berry et al identified a cyclical delta power increase in sleep apnoeics, parallel to increased respiratory effort. The EEG spectral analysis was performed using sequential 1-second windows

[32]. Both studies identified differences between NREM and REM sleep, as there were no significant delta changes during REM-related apnoeas/hypopnoeas.

Black et al analysed the EEG power spectrum using 4-second windows around the peaks of oesophageal pressure (Pes) during NREM 2 in 15 Upper Airway Resistance Syndrome patients, and similarly identified a progressive increase in delta power preceding those peaks [39]. An increase in the power of higher frequencies was identified at Pes reversal. Although changes were more significant at arousal terminating "events", they were also present when arousals were not visible.

The present study focused around the end of apnoeas/hypopnoeas and did not look into EEG power changes in the evolution of these events. We therefore could not confirm the previously found significant delta power increase towards the end of apnoeas/hypopnoeas, identified through consecutive comparisons of short intervals prior to and during apnoeas/hypopnoeas.

Our aim was to improve detection of EEG changes at the end of apnoeas/hypopnoeas. Using longer windows, the analysis looked into changes in delta, theta, alpha and sigma power and their interaction and delivered 10sec-based power values. The most consistent finding across the 2596 events studied is the theta power decrease. In the majority of NREM apnoeas/hypopnoeas, this resulted from the increase in higher frequencies, which made these EEG changes visible. The rise in high frequency activity is consistent with the findings of Svanborg [339] and Black [39] et al. We furthermore, found that events with non-visible EEG changes and no alpha/sigma increase induced a significant delta increase. Hence, this rise in slow activity in response to apnoeas/hypopnoeas represents, as previously suggested [339], a form of arousal.

DeCarli et al support the post-event alpha and/or theta increase but not delta alone, based on the outcomes of wavelet transform, a different technique of EEG analysis [78]. The present study confirms previous observations [32][339] of power differences between REM- and NREM-related apnoeas/hypopnoeas. Visible changes in REM sleep were found to be associated with significant theta decrease alone, non-visible changes showed no significant power changes. No changes in delta power have been detected during previous studies [32][339]. Our findings furthermore provide information on differences between arousal and non-arousal REM- and NREM-related apnoeas/hypopnoeas. Previous studies did not look at these differences either because only NREM-related apnoeas/hypopnoeas were selected [39] or because differences in respect to visibility of arousals were not evaluated [339]. The increased EMG activity during visible cortical arousals could potentially alter the EEG power spectrum and induce differences between visible and non-visible arousals. Muscle artefacts (movement and breathing) would alter alpha, sigma and low delta (0.2-2.2Hz) but not theta frequency band power, as Brunner et al

have demonstrated [51]. However, the importance of this factor would be best evaluated through the elimination of EMG influences from the EEG signal, which was not performed during the present study.

9.4.2. Patients versus healthy controls

The mean theta power fraction was significantly lower in the patients group compared to healthy subjects as a result of the overall increase in sigma and delta power and despite the similar time the 2 groups have spent in NREM 1&2, SWS and REM sleep.

As the subjects were matched for age and BMI, the findings do suggest that the differences in theta power fraction between the 2 groups are related to the post-apnoeic and post-hypopnoeic power decrease and the recurrent visible and non-visible EEG changes.

These frequent fluctuations of spectral power may contribute to the patients' daytime symptoms. To examine this possibility, comparisons between objective daytime measurements – such as MSLT – and nocturnal EEG power spectrum would be necessary.

9.4.3. Sleep quality

Sleep quality was not significantly different between patients and healthy controls. This is due to:

1. the impaired sleep quality of healthy subjects during in-hospital conditions and
2. the inconsistency among patients as to their disease severity ranging from mild, moderate to severe and yielding differences in their sleep quality.

The latter is supported by the observed good reciprocal correlation between AHI and time spent in SWS and REM sleep and the good correlation between AHI and theta power across the patients. The differences in sleep quality between patients and healthy controls are therefore better preserved through the range of time spent in each sleep state.

9.4.4. Study limitations

Limitations of this study include definitions, the use of thermistor for hypopnoea detection, the unequal number between apnoeas/hypopnoeas and periods of undisturbed sleep used in the comparisons, as well as the number of subjects studied. A further limitation may be the use of only 2 bins: NREM and REM sleep.

There are many definitions of hypopnoeas and arousals used in the literature. The hypopnoea definition used in this study, fulfils the ASDA recommendations [9]. The inclusion of arousals or desaturation in the definition may lead to a greater association with arousals than would be found using other ASDA accepted definitions, i.e. based on thoraco-abdominal movement or nasal pressure alone. The arousal definition used here, has been used in previous studies and validated against respiratory events and outcomes [291].

The thermistor is a widely used good qualitative detector of airflow detection. It is unlikely that the limited accuracy of this sensor may have altered the outcomes of the EEG spectral analysis. This is supported by the fact that our findings are in keeping with the outcomes of Black et al, who used oesophageal manometry to monitor changes in respiratory effort [39].

We analysed all the 2596 apnoeas/hypopnoeas and 1690 undisturbed sleep periods, which were not affected by artefacts, across the 15 polysomnograms. This represents a large body of data with which to answer the questions posed. Our results are not only robust but represent the largest body of events which have been so analysed. The study is thus adequately powered to detect clinically important differences.

The 2 bins used, NREM and REM sleep, have been selected as a result of their different characteristics: REM sleep is a low voltage, high frequent state; its baseline –non event- power spectrum is different from the NREM spectrum.

It was beyond this study's aims to look into apnoea-/hypopnoea-related differences across all the sleep stages.

9.4.5. Implications of the study outcomes

The present study has shown that novel techniques may improve and simplify detection of electrographic changes at apnoea and hypopnoea termination. The most consistent significant change is the decrease in theta power, which during NREM sleep is either associated with an increase in high frequencies (alpha, sigma) or delta increase.

The detection of theta band changes is, as the ROC curves demonstrate, a sensitive method to pick up apnoea and hypopnoea related EEG changes and also to differentiate between OSAHS patients and healthy subjects.

Further validation, estimation of a cut-off value for the differentiation between patients and non-patients and correlation analysis between this EEG activity marker and sleepiness severity as well as cognitive function impairment are necessary.

Since part of the REM-related apnoeas and hypopnoeas is not associated with significant theta power changes, the proposed method may be only used in combination with other parameters in order to detect all apnoea and hypopnoea related changes and as part of a tool for improved and simplified diagnosis of OSAHS.
Monitoring of autonomic activity may assist towards the detection of further nocturnal changes related to apnoeas and hypopnoeas.

The study in the next chapter will address this hypothesis.

CHAPTER 10:
SPECTRAL OSCILLATIONS OF RR-INTERVALS IN SLEEP APNOEA/HYPOPNOEA SYNDROME PATIENTS

10.1. INTRODUCTION

Heart Rate Variability (HRV) – the variation of RR intervals - can be detected through power spectral analysis of RR-intervals on the electrocardiograph. The method has been widely validated in physiological and pathological conditions and used as a non-invasive measure of autonomic cardiac control within and between individuals [97][212]. This detection is based on the spectral power changes mainly within the Low (LF) and High (HF) Frequency bands of the RR-intervals.

The relation of efferent cardiac sympathetic nerve traffic to increased LF band power has been demonstrated in different conditions [97][212] and under application of excitatory and inhibitory pharmacological agents. These studies have shown that changes in normalised LF band power reflect sympathetic cardiac activation, whereas HF band power changes reflect parasympathetic, vagal outflow.

The RR spectrum of healthy subjects has been compared to their peripheral sympathetic activity measured invasively from the peroneal nerve [262], the Muscle Sympathetic Nerve Activity (MSNA).

Although the genesis of the RR-interval LF power oscillations is not clear, it has been suggested that these reflect central tonic excitatory sympathetic inputs conditional to brainstem activation [142]. This may explain the dissociation found between RR spectral power, RR duration and MSNA under certain conditions such as apnoeas or hyperventilation [355] and may reflect a common central control mechanism of respiratory and cardiac autonomic modulations.

Hypertension [319] and myocardial infarction [354] cause a reduction in HRV, i.e. an increase in LF and a decrease in total power. These conditions have been associated with the prevalence of the OSAHS [163][379]. The reduced daytime HRV found in OSAHS patients compared to healthy subjects [249] may be implicated in the development of cardiovascular disorders in these patients.

The present study investigated the nocturnal HRV in OSAHS patients through the monitoring of the RR-interval spectral oscillations and RR-duration. We tested the hypothesis that apnoeas/hypopnoeas are associated with sympathovagal changes. We compared variability characteristics around apnoeas/hypopnoeas to baseline, non-event sleep periods across the patients. The patients' RR-interval spectral oscillations and RR-duration were compared to matched healthy controls.

10.2. METHODS

10.2.1. Primary Endpoints

To ascertain whether analysis of heart rate variability can:

1. detect autonomic activity changes induced by apnoeas and hypopnoeas
2. improve detection of changes related to apnoeas/hypopnoeas
3. differentiate between physiological and abnormal activity during sleep.

10.2.2. Study population

PATIENTS

Fourteen consecutive patients, who were newly diagnosed with the OSAHS (AHI $\geq$10 /hr) were randomly selected from the database of the Sleep Centre at the University Hospital Charité in Berlin. All study patients referred to the sleep centre with possible OSAHS, had either self-reported daytime sleepiness (Epworth Sleepiness Scale >10) not explained by other factors or two other major symptoms of the OSAHS [370]: chocking or gasping during sleep, recurrent awakenings, unrefreshing sleep, daytime fatigue and/ or impaired concentration.

A detailed medical history and examination was carried out in the outpatient department. All patients underwent a chest x-ray, an ECG and a lung function test. Study exclusion criteria were autonomic, endocrine, cardiac or pulmonary disease, Periodic Limb Movement Disorder, other neurological disorders or the intake of medication which affected sleep or autonomic function. Two patients were on the antihypertensive Nifedipine. All subjects were otherwise healthy and normotensive.

Thirteen patients were male and 1 patient was female. Their mean age was 51 years [SD 9 years] and the mean BMI was 29 kg/m^2 [SD 2 kg/m^2].

HEALTHY CONTROLS

Seven healthy asymptomatic individuals, in whom OSAHS had been previously excluded,were selected to match the patients' age and BMI. Four were male and 3 were female. Their mean age was 50 years [SD 10 years] and the mean BMI was 28 kg/m^2 [SD 2 kg/m^2].

All study participants underwent an in-hospital overnight diagnostic polysomnography.

10.2.3. Ethical Approval

All participants gave written informed consent for their data to be used in the study, which was approved by the Ethics Committee of the Humboldt University in Berlin.

10.2.4. Polysomnography

A computerised system was used for the polysomnographic recordings.

Recordings were carried out between 10 p.m. and 7 a.m. and consisted of the following:

ELECTROENCEPHALOGRAPHY

Recording of sleep and arousals was based on following tracings:

Four unipolar EEG tracings: 2 central, the C3A2 and C4A1, and 2 occipital, the O1/A2 and O2/A1 tracings. Two outer canthi electrodes, LEOG and REOG, for the detection of eye movements. A submental EMG electrode for the detection of tonic muscle activity.

ELECTROMYOGRAPHY

Leg movements were detected through bilateral tibial EMG electrodes, whereas body movements were recorded through a body position detector. The body detector did not include continuous information on supine body position.

CARDIORESPIRATORY MONITORING

Monitoring of respiratory function consisted of a thermistor sensor for the oronasal airflow detection, 2 piezoelectric belts for the detection of thoracoabdominal movement, a digital microphone for snoring detection and a pulseoximeter to monitor blood oxygen saturation levels. A 3-lead ECG was used to monitor cardiac activity.

10.2.5. Visual scoring

The visual scoring was performed by the primary investigator (K.D.), blinded to subject.
Every polysomnogram was scored during 3 consecutive sessions: sleep staging was followed by the scoring of apnoeas and hypopnoeas; arousals were scored during the final session.
Approximately 12% of all data were excluded due to artefacts or abnormal R-wave morphology.

SLEEP SCORING

Sleep and arousal scoring was based on the C4A1 and C3A2 tracings. Sleep was scored according to the Rechtschaffen and Kales criteria [288], described in detail in chapter 2.

Following parameters were used to describe sleep quality:

Sleep onset was defined as the first scored 30-second epoch of sleep stage 2.

Total Sleep Time **[hrs]** (TST) was calculated as the total time spent asleep.

Sleep Period Time **[hrs]** (SPT) was the time between sleep onset and the last epoch of sleep.

Sleep efficiency **[%]** was the ratio of TST divided by the SPT and multiplied by 100.

Time spent in each sleep state **[%]** was the ratio of time in each sleep state divided by TST.

APNOEA / HYPOPNOEA SCORING

Apnoeas were defined as cessation of the oronasal airflow, lasting $\geq$10sec.

Hypopnoeas were defined as airflow reduction of >50%, compared to a 10sec peak amplitude during the preceding 2 minutes, lasting $\geq$10 sec and associated with either an oxygen desaturation of $\geq$3% or an arousal [5].

Oxygen saturation was based on a second-by-second measurement.

Apnoea/Hypopnoea Index **[hr $^{-1}$]** (AHI): the number of all apnoeas and hypopnoeas divided by the total time spent asleep.

Disease severity was based on the AHI [5]:

Mild OSA: $15 > AHI \geq 10$

Moderate OSA: $15 \geq AHI \geq 30$

Severe OSA: $30 > AHI$

An AHI<10/hour sleep was considered normal.

AROUSAL SCORING

The scoring of arousals was based on the definition of the American Sleep Disorders Association (ASDA), described in chapter 4, modified as to the time-threshold which was set at 1 second rather than 3 [9]. The C4A1 and C3A2 tracings of the EEG were used for the scoring. Arousals were scored when an abrupt shift to a faster EEG frequency (including theta, alpha and/or greater frequencies but no spindles) occurred. Arousal scoring ranged from 1 to 15 seconds, irrespective of sleep recurrence or prolonged awakening subsequent to the arousal. Hence, Rechtschaffen and Kales awakenings [288] were also included in the scoring.

Respiratory Arousal Index **[hr $^{-1}$]** (RAI): The number of arousals following apnoeas and hypopnoeas per hour sleep was calculated.

10.2.6. Heart Rate Variability

Autonomic activity during sleep and changes in activity around the end of apnoeas and hypopnoeas were monitored non-invasively, based on the RR-interval analysis [97]. Frequency domain analysis of the RR-interval time series was performed using the Fast Fourier Transform [FFT] algorithm. In the time domain, the mean RR-interval duration was evaluated.

The end of apnoeas and hypopnoeas was identified as airflow increase or recovery. To assess the fluctuations in RR-interval spectral power induced by apnoeas or hypopnoeas, comparisons were conducted between the same frequency bands within 2-minute windows centred around the end of apnoeas/hypopnoeas and 2-minute periods of undisturbed sleep.

To avoid influence on the RR-interval power spectrum through adjacent apnoeas/hypopnoeas or arousals, events were selected for the analysis only when the 2-minute window was not overlapping with any further respiratory events, arousals or wake epochs (figure 10.1).

The window was chosen following the recommendations for frequency domain analysis of RR-intervals to maximise detection of power changes related to apnoeas/hypopnoeas and maintain signal stationarity [97]. The 2-minute segments are considered as the lower bound for accurate analysis of the 0.04-0.4Hz frequency band power [97].

A concern was the influence of respiratory oscillations on the RR power spectrum (respiratory sinus arrhythmia) during this window [50].

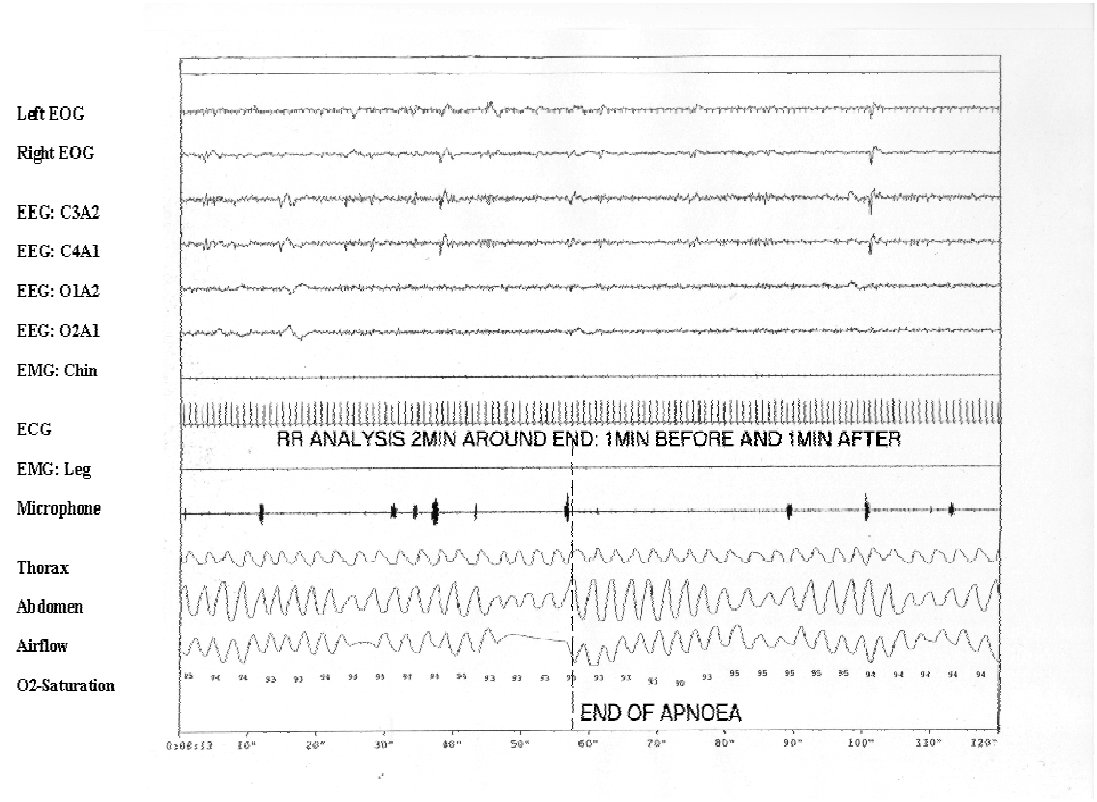


Figure 10.1. Two-minute window around the end of an apnoea (line). The spectral power of RR-intervals and their mean duration were calculated during this window and compared to 2-minute windows of undisturbed sleep.

SIGNAL PROCESSING

Heart Rate Variability [HRV] parameters were calculated according to the recommendations specified by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [97].

All data were processed with program modules from a custom signal processing framework, written in C language for Unix/Linux operating systems.

Careful editing and visual inspection of the ECG signal, sampled at 100 Hz, helped to eliminate sources of errors arising from missing QRS complexes or spurious RR-intervals. The RR-interval estimation was obtained by using a "second-order derivative with threshold" method. As this time series (tachogram) is a function of number and not of time, RR-interval series were converted to a smoothed Heart Rate time series through sampling at uniform intervals with 4Hz (ΔT=0.25s) [29]. To check stationarity of the HR series, the mean and standard deviation (SD) of all selected HR segments within each recording was calculated. The variation of the mean across the segments of the same recording was less than one calculated SD, i.e. all the segments selected fulfilled the stationarity requirement.

FREQUENCY DOMAIN ANALYSIS

For each 2-minute segment the Power Spectral Density (PSD) [ms^2/Hz] – power or energy distribution in its constituent frequencies - of the corresponding Heart Rate time series was computed by applying the Welch method [367] after removal of the DC component.

This method is based on averaging of overlapping windowed Fourier transform (sliding windows). To minimise leakage, a 50% overlapping sliding Hamming window with 128 points was used. The 2-minute Heart Rate series had a length of 480 samples. Zero padding was applied to add up to N=512 samples and was divided into seven overlapping frames.

The trade-off of spectral resolution and statistical variance is inherent to periodogram approaches. That is, by increasing the length of the Hamming window and thereby improving the spectral resolution, the number of frames to be averaged decreases and hence the statistical variance of the estimate deteriorates. Alternatively, if the number of the frames to be averaged is increased by forming shorter frames, then the spectral resolution deteriorates. The length of the Hamming window and the 2-minute analysis window in the present study was a compromise between frequency resolution and signal stationarity.

The resolution of isolated peaks was 0.0078 Hz resulting in 0.0156 Hz distinguished adjacent frequency components of unequal amplitude. The frequency bands had a width of 0.03125 Hz.

SPECTRAL POWER

The calculation of power for the low and high frequency bands was based on the Gaussian integration of the corresponding power spectral density function and was expressed in [ms^2] (figure 10.2). Frequencies below 0.04 Hz were not considered in the analysis, as these require longer data series for an accurate power calculation.

Power was calculated for the:

1. Low Frequency band [LF]: 0.04- 0.15 Hz
2. High Frequency band [HF]: 0.15 – 0.4 Hz
3. Total frequency spectrum

LF and HF power were expressed in normalised units (LFn, HFn) by calculating the percentage of each band power with respect to their sum [97][212].

$$LFn = LF / [LF + HF] \times 100$$

$$HFn = HF / [LF + HF] \times 100$$

Normalised power emphasizes the controlled and balanced behaviour of the 2 branches of the autonomic nervous system, increases sensitivity [97] and correlation with attendant changes in

MSNA [262]. The normalisation minimises the effect of the changes in total power on the values of LF and HF components [97].

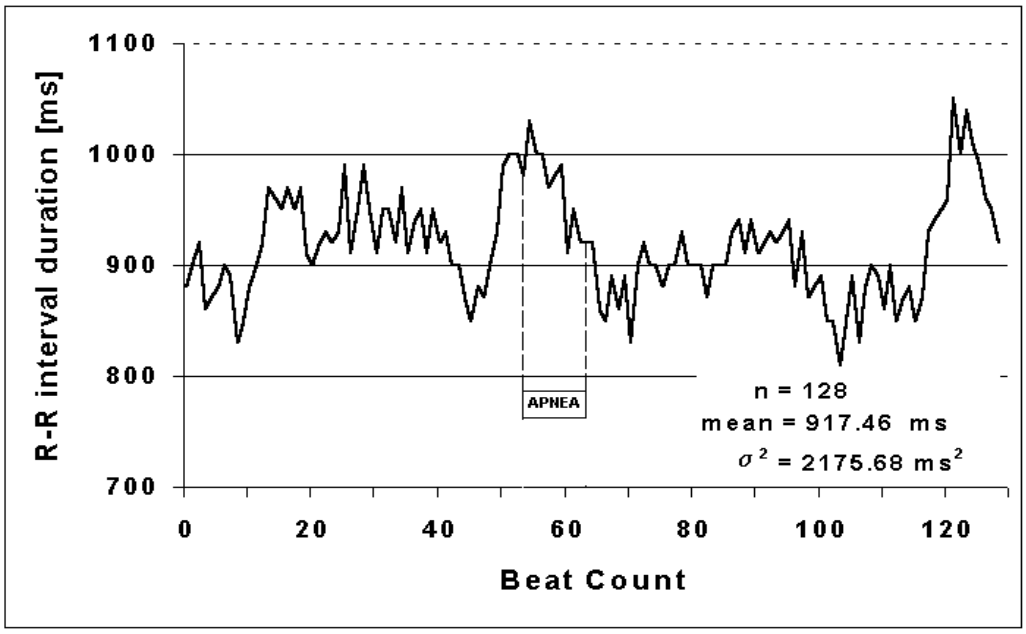


Figure 10.2.A. RR-interval time series (tachogram) 2 minutes around the end of the apnoea in figure 1. The number of RR-intervals (n=128), their mean duration [ms] and variance (σ^2) are quoted.

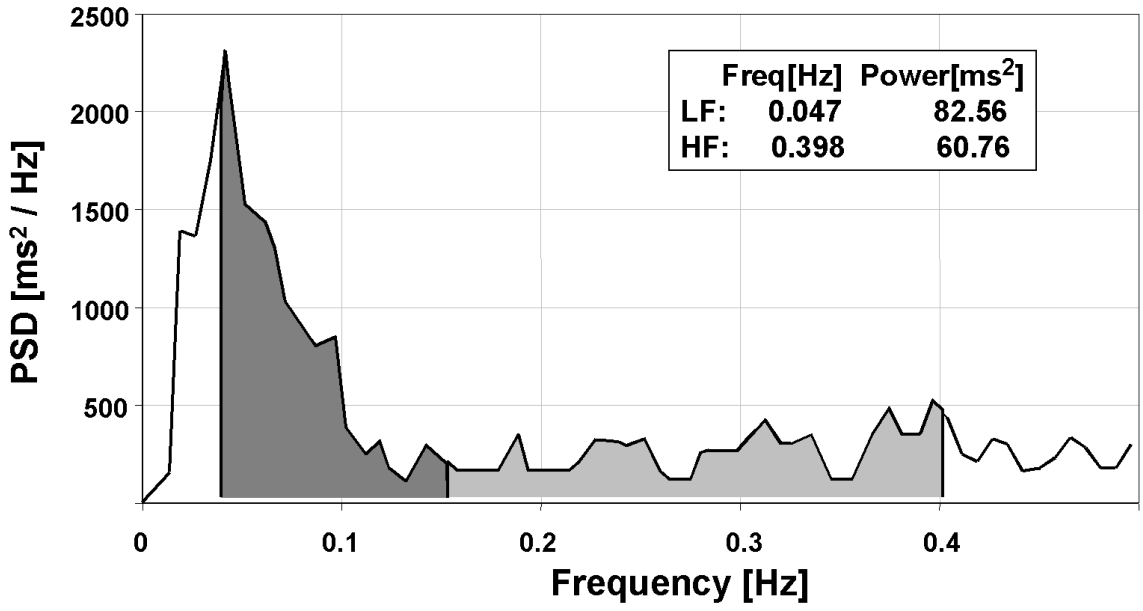


Figure 10.2.B. Power Spectral Density (PSD) curve of the same interval, calculated using the non-parametric Fast Fourier Transform algorithm. Power [ms^2] was calculated for the Low Frequency (dark grey) LF: 0.04-0.15Hz and the High Frequency (light grey) HF: 0.15-0.4Hz bands. The peak frequency in each band, is also quoted.

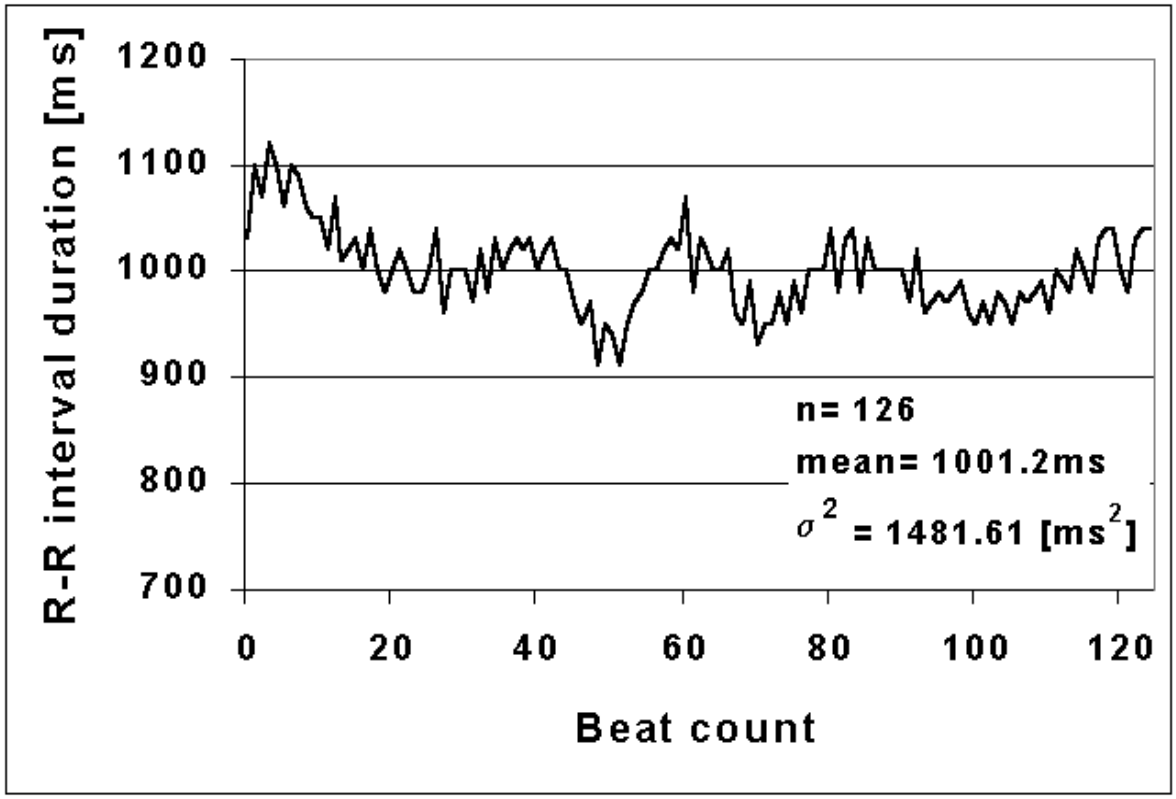


Figure 10.2.C. Interval tachogram of a 2-minute period of undisturbed sleep from the same polysomnogram.

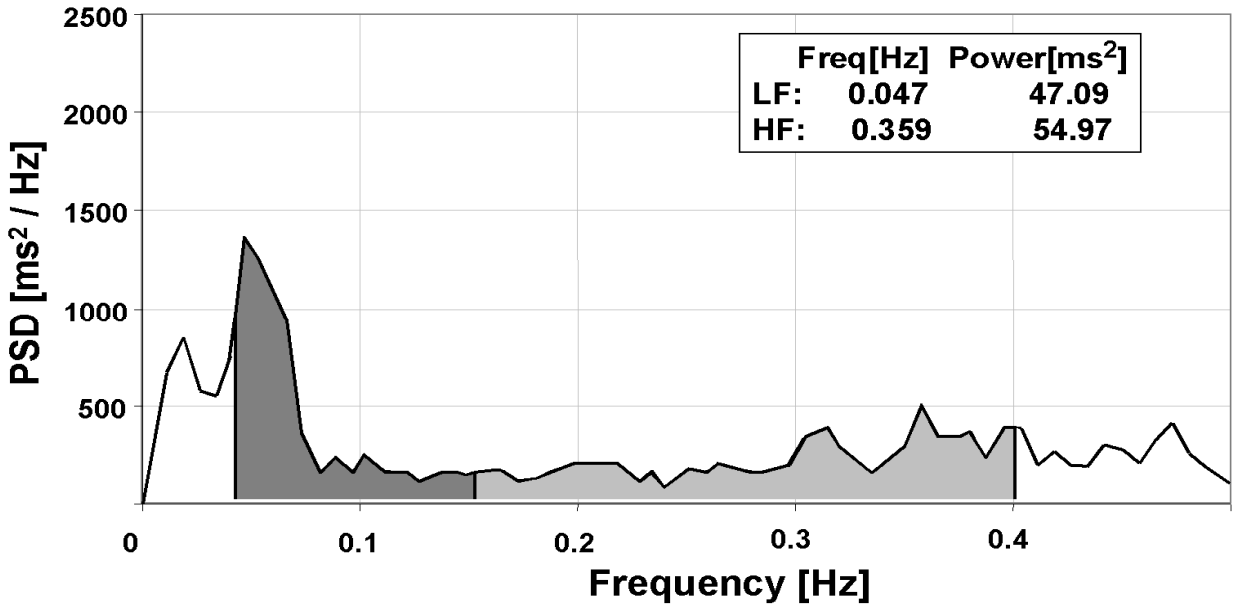


Figure 10.2.D. PSD curve of the same baseline sleep period.

TIME DOMAIN ANALYSIS

The mean RR-duration of the original tachograms was calculated in 2-minute segments.

A decrease in RR-duration is a measure of centrally mediated, phasic cardiac activation triggered by afferent inputs from the baroreflex loop [97][262].

The influence of:

1. cortical arousals and
2. event type

on heart rate variability was evaluated.

To assess the significance of the changes found in RR-duration and spectral power across the OSAHS patients, these were compared to healthy subjects.

Comparisons were conducted between the whole night recordings of patients versus healthy controls, based on the analysis of non-overlapping 2-minute sliding windows throughout the sleep periods; wake epochs were excluded. A minimum of 1.5 hours per subject was analysed. Each subject contributed one data point to the mean of each autonomic marker.

10.2.7. Statistical analysis

STATISTICAL POWER CALCULATION

To facilitate an adequately powered study with reproducible and representative results, a study with similar objectives and outcomes was used for the sample size calculation.

Roche et al studied 14 OSAS patients before and during CPAP therapy [296]. Two minute windows were used to determine the spectral power changes of the RR-interval time series during apnoeas and hypopnoeas. The outcomes were compared to the spectral power during normal breathing following CPAP therapy. Roche et al calculated the logarithmic values of the spectral power density within the different frequency bands, expressed in ms^2/ Hz, as an index of heart rate variability. Although this measure is different to the index of HRV used in the present study, the spectral power expressed in ms^2, both are comparable indices of heart rate variability. The outcome measures of Roche et al can therefore be used as a guide for the sample size calculation in the present study.

Roche et al found that the mean nocturnal LF power in untreated OSA patients was 7.12 ms^2/ Hz (SD 1.06 ms^2/ Hz). During CPAP, LF power reduced to 6.22 ms^2/ Hz (SD 1.18 ms^2/ Hz). Using the paired Student's t-test, the authors established that the reduction in LF power found during CPAP therapy was significant ($p<0.001$). With a sample size of 14, a t-statistic with 13 degrees

of freedom and a significance level at 0.001, the t-statistic calculated in the paired t test was equal to or greater than 4.22.

The t-statistic is equal to the mean difference divided by the standard error. In this study the mean difference in LF power was 0.9 (7.12 - 6.22) ms^2/ Hz, therefore the standard error was equal to or less than 0.213 (= 0.90/ 4.22). The standard deviation of the difference was less than 0.8 (= 0.213 x square root of sample size). Therefore, the effect size in the study by Roche et al was at least 0.9/0.8 = 1.12 (mean difference/standard deviation of the difference).

For a similar effect size, a sample size of 14 patients would give a 97% power at the 5% significance level.

STATISTICAL TESTS

Statistical data analysis was performed using the SPSS Inc, Chicago, IL,V9.0 program.

Paired t-test was performed for the within patients comparisons.

Independent t-test assessed differences between patients and healthy controls.

Receiver-Operating-Characteristic (ROC) curves [382] evaluated the ability of the autonomic markers to discriminate between apnoeas/hypopnoeas and undisturbed sleep in each patient.

To assess the accuracy in reproducing visual scoring, the true-positive [TP], true-negative [TN], false-positive [FP] and false-negative [FN] readings, as well as:

$$\text{Sensitivity: } TP / [TP + FN]$$

and

$$\text{Specificity: } TN / [TN + FP]$$

were calculated for each marker and polysomnogram.

The ROC curves were created by stepwise changes of the decision-threshold for the normal and pathological state for the investigated parameter. The area under the curve (the minimal value of 0.5 indicates no discrimination, the maximum value of 1.0 indicates perfect discrimination) and its standard error were calculated [149]. The area under the curve represented the probability of a randomly selected ECG interval to distinguish episodes of apnoeas or hypopnoeas from undisturbed sleep. Using the mean values of the 2-minute rolling windows across the 21 subjects, ROC curves were also constructed by stepwise changes of the decision-threshold to demonstrate the ability of the markers to discriminate between OSAHS patients and healthy subjects.

Significance was considered at $p<0.05$. Mean values and the Standard Error of the Mean (SEM) are quoted.

10.3. RESULTS

10.3.1. Sleep quality

The patients' mean sleep efficiency was 88 ± 2 % of the Sleep-Period-Time (SPT). The healthy subjects' mean sleep efficiency was 92 ± 3 % of the SPT (table10.1).

	PATIENTS	HEALTHY CONTROLS
TST [hrs]	6.4 (0.2)	4.8 (0.5)
SPT [hrs]	7.2 (0.1)	5.2 (0.3)
NREM 1&2 [hrs]	4.0 (0.2)	3.0 (0.3)
SWS [hrs]	1.4 (0.2)	1.0 (0.2)
REM [hrs]	1.0 (0.1)	0.8 (0.1)
NREM 1&2 [% TST]	63 (3)	62 (4)
SWS [% TST]	22 (2)	21 (3)
REM [% TST]	15 (1)	17 (3)

Table 10.1. Mean and Standard Error of the Mean (SEM) of Total Sleep Time (TST), Sleep Period Time (SPT), and time spent in sleep stages 1&2 (NREM 1&2), Slow Wave Sleep (SWS) and Rapid Eye Movement (REM) sleep across patients and healthy controls. Each individual contributed one data point to each mean.

10.3.2. Apnoeas and hypopnoeas

A total of 2288 apnoeas and hypopnoeas were scored. Forty-eight percent of the scored events were apnoeas, 52 % were hypopnoeas. The mean apnoea/hypopnoea duration was 25 $\pm$ 2 seconds. Across the 14 patients, 87 $\pm$ 3 % of apnoeas and hypopnoeas occurred during NREM 1 & 2 sleep, 6 $\pm$ 2 % during SWS and 10 $\pm$ 2 % during REM sleep. In 4 patients no apnoeas/hypopnoeas were scored during SWS, in 2 patients none were scored during REM sleep.

The OSA patients studied had mild to severe disease with a mean AHI of 29 /hr slept (range: 10–55 /hr) and mean RAI 20 /hr slept (range: 7- 47 /hr). Thirty-two percent of apnoeas and hypopnoeas were not associated with a visible cortical arousal.

10.3.3. Within patients comparisons

Two hundred and fourteen of the 2288 scored apnoeas and hypopnoeas (mean: 15 per patient, SEM: 2) fulfilled the “non-overlap” criteria and were selected for analysis. These events were compared to 541 (mean: 39 per patient, SEM: 8) non-event, normal sleep periods (baseline sleep).

Across the 14 patients, 77 $\pm$ 4 % of the analysed apnoeas and hypopnoeas occurred during NREM 1 & 2 sleep (Mean: 12 events, SEM: 1), 13 $\pm$ 3 % during SWS (Mean: 2 events, SEM: 1) and 22 $\pm$ 4 % (Mean: 4 events, SEM: 1) during REM sleep. In 8 patients no apnoeas/hypopnoeas were analysed during SWS, in 3 patients none were analysed during REM sleep.

Mean apnoea/hypopnoea duration was 26 $\pm$ 4 seconds.

LFn power increased and HFn power decreased 2 minutes around the end of apnoeas and hypopnoeas, compared to undisturbed sleep ($p<0.001$).

The increase in absolute LF power was also significant ($p=0.002$) and was associated with an increase in total power ($p=0.01$). No significant increase was found in the absolute HF power ($p=0.3$).

The mean RR-duration did not change significantly around the end of apnoeas and hypopnoeas compared to undisturbed sleep across the 14 patients ($p=0.9$) (table 10.2).

	RESPIRATORY EVENTS	BASELINE SLEEP	p-value
LFn	67 (1)	52 (2)	<0.001
HFn	33 (1)	48 (2)	<0.001
LF [ms^2]	229 (38)	106 (18)	0.002
HF [ms^2]	116 (17)	97 (7)	0.3
TF [ms^2]	345 (45)	203 (23)	0.01
RR-duration [ms]	904 (28)	904 (33)	0.9

Table 10.2. Mean values and Standard Error of the Mean (SEM) of the normalised low (LFn) and high (HFn) frequency power, absolute LF, HF and total (TF) frequency power [ms^2] and RR-duration [ms] during 2-minute periods around the end of apnoeas/hypopnoeas and undisturbed baseline sleep, across the 14 patients. Each patient contributed one data point to each mean. Differences between apnoea-/hypopnoea-related changes and baseline sleep were evaluated using the paired t-test across patients.

ROC curves, based on the normalised LF and HF power values demonstrated that these spectral power markers of autonomic activity can discriminate well between apnoeas/hypopnoeas and undisturbed sleep, with the areas under the curves being between 0.74 and 1.00 ($p<0.03$) across patients and standard errors between 0.0001 and 0.09 (table 10.3).

Patient	LFn/ HFn (Std.error; p-value)	Respiratory Events	Baseline Sleep
1	0.97 (0.02;<0.001)	14	86
2	0.97 (0.03;<0.001)	10	7
3	1.00 (<0.001;<0.001)	17	10
4	0.96 (0.03;<0.001)	15	36
5	0.78 (0.05;0.002)	24	49
6	0.93 (0.04;<0.001)	9	19
7	0.91 (0.03;<0.001)	11	105
8	0.74 (0.06;0.003)	7	36
9	0.95 (0.03;<0.001)	15	60
10	0.74 (0.09;0.02)	10	19
11	0.96 (0.03;<0.001)	14	28
12	0.91 (0.05;<0.001)	34	28
13	0.81 (0.07;<0.001)	18	9
14	0.81 (0.07;<0.001)	16	49
MEAN	0.88 (Std.error 0.04)	15	39
RANGE	0.74-1.00	7-34	7-105

Table 10. 3. Area under the ROC curve, standard error and significance for the normalised low (LFn) and high (HFn) frequency power in each patient. The number of 2-minute periods around respiratory events and during undisturbed, baseline sleep used for each curve is indicated in the 2 last columns. Patients 6 and 12 were on the antihypertensive Nifedipine. The areas under the curves are identical for both measures of autonomic activity, as their calculation is based on the percentage of each band power with respect to their sum:

LFn = LF x 100/ (LF+HF), HFn = HF x 100/ (LF+HF).

The ROC curves were identical for both measures of autonomic activity, as their calculation was based on the percentage of each band power with respect to their sum. High standard error values reflect outlying differences between HRV values around apnoeas or hypopnoeas and baseline sleep in some patients. The small number of samples used in some ROC curves may have contributed to the increase in standard error.

10.3.4. Apnoeas / Hypopnoeas, Arousals and HRV

Of the 214 apnoeas and hypopnoeas analysed across the 14 patients, 154 (mean: 11, SEM: 1) i.e. 72 %, were associated with a visible cortical arousal. Sixty apnoeas and hypopnoeas (mean: 4, SEM: 1), i.e. 28 % were not associated with a cortical arousal.

The absolute and normalised power values of the Low Frequency band (LF, LFn) and the absolute power value of the total estimated frequency spectrum (TF) were found to be significantly higher ($p<0.05$) around apnoeas and hypopnoeas associated with arousal compared to those not associated with arousal.

The normalised power of the High Frequency band (HFn) and the RR-duration were significantly lower ($p<0.05$) around the arousal-associated apnoeas and hypopnoeas compared to those not associated with arousals (table 10.4).

One hundred and thirty-nine events, i.e. 65 %, were hypopnoeas (mean:10 per patient, SEM:1) and 75 (mean:5 per patient, SEM:1), i.e. 35 %, were apnoeas.

No significant differences were found between apnoeas compared to hypopnoeas in any of the autonomic markers ($p>0.6$) (table 10.4).

	A+H and arousal	A+H no arousal	p-value	Apnoeas	Hypopnoeas	p-value
LFn	69 (1)	64 (2)	0.04	67 (1)	68 (2)	0.8
HFn	31 (1)	36 (2)	0.04	33 (1)	32 (2)	0.8
LF [ms^2]	260 (45)	161 (31)	0.01	228 (42)	222 (39)	0.8
HF [ms^2]	129 (22)	88 (10)	0.08	116 (21)	110 (19)	0.8
TF [ms^2]	390 (65)	249 (40)	0.02	344 (62)	332 (56)	0.8
RR-durat[ms]	903 (28)	914 (29)	0.03	909 (28)	905 (33)	0.7

Table 10.4. Mean values and Standard Error of the Mean (SEM) of frequency and time domain measures of autonomic activity around arousal-inducing and self-terminating events as well as around apnoeas and hypopnoeas. Evaluation of the influence of arousals and event type on the RR-interval power spectrum and duration. Paired t-test showed that the increase in normalised and absolute LF and in total (TF) power was higher around apnoeas/hypopnoeas associated with arousal compared to those not associated with arousal. RR-duration decreased around arousal-associated apnoeas/hypopnoeas. No differences were found between apnoeas and hypopnoeas. Each patient contributed 1 data point to each mean.

Compared to undisturbed sleep, LF and LFn band power was found to increase significantly ($p<0.02$) around both groups of apnoeas and hypopnoeas, those associated with visible cortical arousals and those which were not.

A significant decrease ($p<0.001$) was found in the HFn band power compared to undisturbed sleep, whereas the absolute HF band power did not differ significantly from baseline sleep in either of the 2 groups ($p>0.1$).

Total (TF) power was significantly higher around apnoeas and hypopnoeas associated with cortical arousals compared to undisturbed sleep ($p=0.01$), but not around non-arousal-associated apnoeas/hypopnoeas ($p=0.1$) (table 10.5).

The LF, TF, LFn and HFn power differed significantly around both apnoeas and hypopnoeas compared to baseline sleep ($p<0.04$) (table 10.5).

	A+H and arousal	**A+H no arousal**	**Apnoeas**	**Hypopnoeas**
LFn	<0.001	<0.001	<0.001	<0.001
HFn	<0.001	<0.001	<0.001	<0.001
LF [ms^2]	0.002	0.01	0.005	0.005
HF [ms^2]	0.2	0.4	0.3	0.5
TF [ms^2]	0.01	0.1	0.03	0.03
RR-durat [ms]	0.8	0.2	0.6	0.8

Table 10.5. P-values of paired comparisons between 2 minute periods of undisturbed sleep and arousal-inducing apnoeas/hypopnoeas (column: A+H and arousal), between undisturbed sleep and self-terminating apnoeas/hypopnoeas (column: A+H no arousal), between undisturbed sleep and apnoeas (column: Apnoeas), between undisturbed sleep and hypopnoeas (column: Hypopnoeas) for all autonomic markers across the 14 patients.

10.3.5. Patients versus healthy controls

Based on the 2-minute analysis throughout the sleep periods, all absolute power values were higher among patients ($p<0.03$). No significant differences were found in LFn and HFn across patients compared to healthy controls ($p=0.5$) (table 10.6).

	OSAHS patients	**Healthy subjects**	**p-value**
LFn	66 (1)	67 (1)	0.5
HFn	34 (1)	33 (1)	0.5
LF [ms^2]	111 (16)	57 (3)	0.02
HF [ms^2]	62 (6)	35 (3)	0.005
TF [ms^2]	173 (21)	91 (6)	0.01
RR durat [ms]	912 (28)	1138 (91)	0.04

Table 10.6. Mean values and SEM of the frequency and time domain measures of autonomic activity in OSAHS patients and healthy subjects during non-overlapping 2-minute sliding windows throughout the sleep periods. Differences between the 2 groups were assessed using the independent t-test.
Abbreviations: LFn: normalised Low Frequency power; HFn: normalised High Frequency power; TF: Total Frequency power.

The mean RR-duration was significantly lower in the patients group compared to the healthy controls (p=0.04) (figure10.3.A).

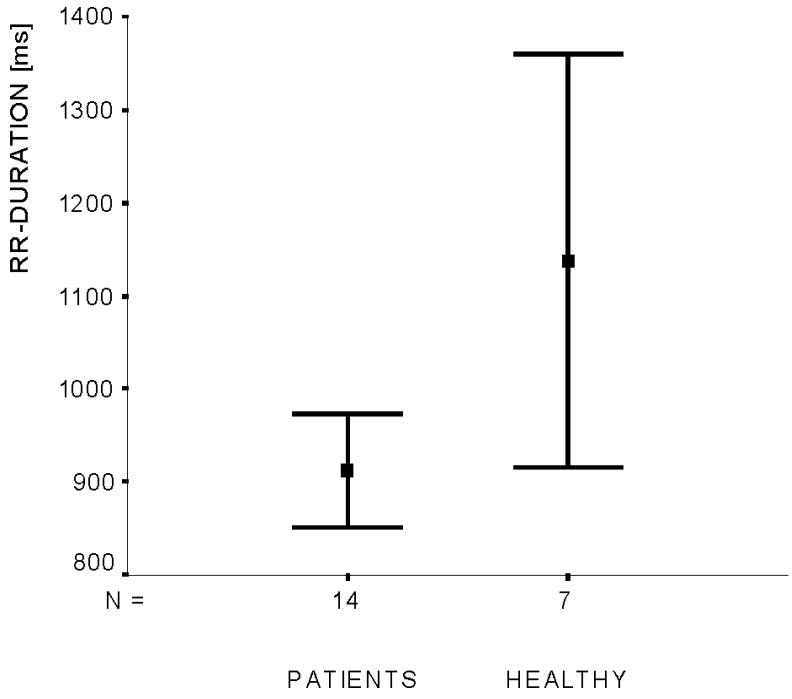


Figure 10.3.A. Error bars demonstrate the distribution (mean values and 95% confidence interval) of the RR-duration [ms] in patients and healthy controls. These values were used to construct the ROC curve.

To assess the likelihood of the measured variables distinguishing OSA patients from a healthy population, ROC curves were constructed based on the outcomes of time and frequency domain analysis. Each subject contributed one data-point to each curve.

The area under the RR-duration curve was 0.88 (SE: 0.08, p=0.006) (figure 10.3.B).

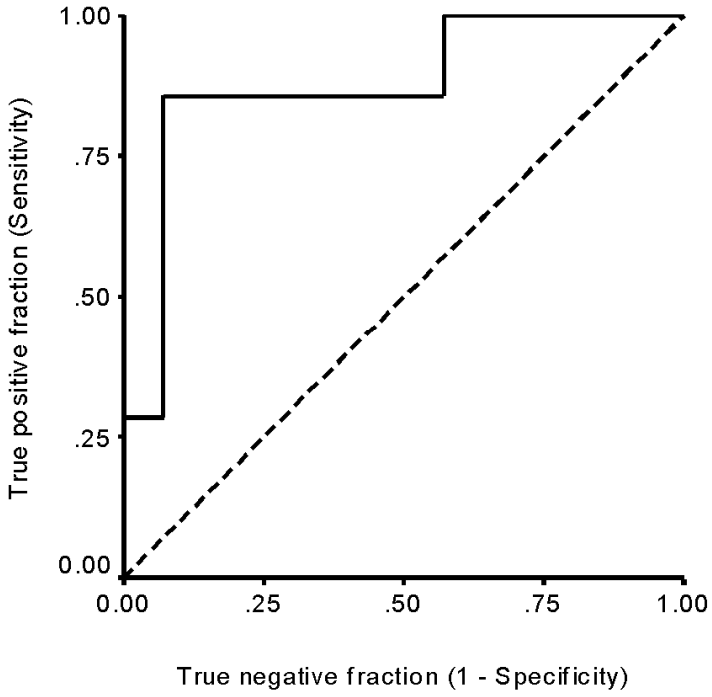


Figure 10.3.B. ROC curve based on the mean RR-duration during 2-minute sleep-related rolling windows across the 14 patients and 7 healthy controls, i.e. the curve consists of 21 points.
The area under the curve is 0.88 (std. error 0.08; p=0.006).
The mean RR-duration during this time window can therefore differentiate well between OSAHS patients and healthy controls.

The area under the absolute LF and HF power curves was identical: 0.91 (SE: 0.06, p=0.002), which indicates similar LF/ HF ratios between patients and healthy controls.

The area under the TF power curve was 0.93 (SE: 0.06, p=0.002) across the 21 subjects (figure 10.3.C).

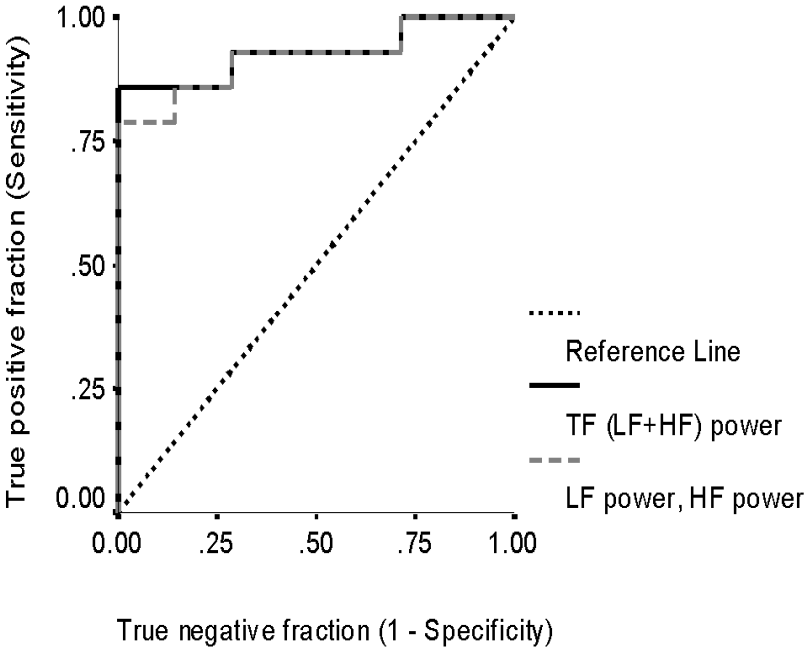


Figure 10.3.C. ROC curves of RR-interval absolute low (LF) and high (HF) frequency power values (dotted grey curve) yielded an area: 0.91 (SE:0.06; p=0.002), total (TF=LF+HF) power (solid black curve) yielded an area: 0.93 (SE:0.05; p=0.002) across the 21 subjects.

All 3 absolute power values differentiate well between OSAHS patients and healthy controls.

10.4. DISCUSSION

The novel finding of this study is the increase in Low Frequency band power around apnoeas and hypopnoeas, irrespective of association with cortical arousals or the type of respiratory event. The RR-interval analysis throughout the sleep periods, based on 2-minute rolling windows, showed a decrease in RR-duration and an increase in LF and HF and total power across OSAHS patients compared to age- and BMI-matched healthy subjects.

10.4.1. HRV, Apnoeas / Hypopnoeas and Arousals

The present study was carefully designed to maintain analysis requirements while enabling detection of autonomic activity changes during apnoeas and hypopnoeas and following their termination. To ensure accurate outcomes, only 9% of the scored events were analysed, namely those which were not adjacent to further events, arousals or wake epochs within 1 minute from their termination.

The outcomes of frequency domain analysis demonstrate a significant increase in sympathetic activity during and after apnoeas/hypopnoeas compared to periods of undisturbed sleep.

This difference could not be demonstrated in the time domain analysis of the same segments, as the segments analysed included both the bradycardia during the events and the tachycardia following their end, resulting in an overall unchanged mean RR-duration around the events compared to undisturbed sleep.

These findings demonstrate the superiority of frequency domain analysis over time domain analysis in the detection of autonomic activity changes induced by apnoeas or hypopnoeas.

The RR duration is influenced by the baroreflex loop, reflects phasic sympathetic activity and interacts with oscillations during apnoea or hyperventilation [355].

The increase in LFn band power demonstrates central tonic sympathetic activation [179][355]. This is associated with an increase in respiratory effort due to the inspiratory attempts against the occluded or narrowed airways and the gradual decrease of the intra-thoracic pressure towards the end of apnoeas and hypopnoeas. The haemodynamic consequences are the increased venous return to the right ventricle accompanied by the increased myocardial transmural pressure and a negative chronotrope attitude during the respiratory abnormalities.

During the post-apnoeic/hypopnoeic recovery period, the intra-thoracic pressure increases towards zero resulting in a decrease in the transmural myocardial pressure with a positive ino- and chronotrope effect.

This abnormal breathing pattern is associated with an increase in sympathetic activity, demonstrated through the increase in RR-interval tachogram LF band power around apnoeas and hypopnoeas and the post-apnoeic increase in heart rate and blood pressure [311].

The present study shows that the LF power increase around apnoea- and hypopnoea-termination is irrespective of event type or the presence of cortical arousals. This is in accordance with previous observations of post-apnoeic increase in heart rate and blood pressure in the absence of arousals [210]. The present study found also that the LF power increase is greater around apnoeas and hypopnoeas associated with arousals. The increase in LF power was associated with an increase in total spectral power and a decrease in RR-duration.

When compared to baseline sleep, sympathetic activity was found to be increased around both the apnoeas and hypopnoeas which were associated with cortical arousals and those that were not.

10.4.2. Apnoeas / hypopnoeas, HRV and MSNA

The present outcomes were not validated against MSNA or blood pressure, as their monitoring would disrupt sleep, influence autonomic activity and would not necessarily reflect the central tonic sympathetic traffic on the sinus node.

Some modelling studies suggest that the influence of apnoeas and hypopnoeas on peripheral MSNA may be different to the centrally mediated sympathetic activity [179][355].

In a modelling study on healthy awake individuals, Morgan et al monitored MSNA, blood pressure and heart rate during and after apnoeic episodes, based on 20-second periods [242]. Compared to baseline breathing, MNSA was found to increase during and decrease after apnoeas, parallel to the central sympathetically mediated post-apnoeic increase in blood pressure and heart rate. A further modelling study on healthy individuals used MSNA and spectral analysis of the RR-interval tachogram as markers of autonomic discharge and demonstrated dissociated activity patterns between central (spectral analysis) and peripheral (MSNA) sympathetic activity during voluntary apnoeas and hyperventilation compared to quiet, paced breathing [355].

Watanabe et al studied OSAHS patients during sleep and detected an increase in MSNA, a decrease in blood pressure during apnoeas and a further increase in MSNA and in blood pressure in the immediate post-apnoeic period [363].

The increase in LF band power observed around apnoeas and hypopnoeas compared to quiet sleep in the present study is in agreement with the outcomes of Keyl et al, which were based on 20-minute windows [184].

10.4.3. OSAHS patients and healthy controls

The present study found an increase in LF, HF and TF power and a reduction in RR-interval duration during sleep in OSAHS patients compared to age- and BMI-matched healthy controls. The decrease observed in RR-interval duration represents sympathetic activation. This finding is in agreement with previous assessments of sympathetic activity using different markers such as blood pressure and MSNA before and after treatment in OSAHS patients and compared to healthy controls [322]. Daytime HRV in OSAHS patients has been previously found to be reduced compared to healthy subjects [249]. One could postulate a causative association between nocturnal and diurnal changes in autonomic activity which result in disturbed circadian sympathovagal balance in these patients. This would be in accordance with the findings of Grote et al who demonstrated reduced vascular response to alpha and beta-2 receptor stimulation in OSAHS patients compared to controls as a result of down-regulation of peripheral vascular receptors linked to chronic circadian hyperstimulation [127].

In the frequency domain analysis, the present study found an overall increase in total, LF and HF band power across the OSA patients compared to the healthy individuals. The nocturnal increase in heart rate variability was associated with an increase in sympathetic and parasympathetic activity. None of the 2 branches of autonomic activity dominated across patients compared to healthy controls. This may be due to the 2-minute window used for the analysis, allowing the breathing pattern of OSAHS patients to influence the RR interval power spectrum. Brown et al demonstrated a strong influence of respiratory rate and tidal volume on the RR-interval HF band power but not on the RR duration, under experimentally controlled conditions in wake healthy subjects with stable cardiac sympathetic outflow [50].

According to these findings, the increase in HF band power detected in our study across the OSA patients may be the result of the periodically abnormal breathing pattern during the analysis windows.

This is supported by the parallel significant decrease in the RR interval duration found across patients compared to healthy controls, reflecting the overall increase in sympathetic activity, the detection of which in the frequency domain analysis is affected by the breathing pattern.
The importance of this factor would have been better evaluated through elimination of the respiratory oscillations from the RR-tachogram, which was not performed during the present study. Had we used shorter analysis windows to minimise the influence of the breathing pattern on the RR-interval power spectrum, these would not have been based on the standards for power spectral analysis [97] and would have required validation against objective markers of autonomic activity, such as MSNA.

10.4.4. Study limitations

Potential limitations of the study include definitions, the number of apnoeas and hypopnoeas analysed and the number of subjects studied.
The hypopnoea definition used in the present study fulfils the AASM recommendations [5]. The inclusion of arousals in the definition may lead to a greater association with arousals compared to other definitions, i.e. based on thoraco-abdominal movement or nasal pressure alone.
To avoid influence on the RR-interval power spectrum through adjacent apnoeas, hypopnoeas, arousals or wake epochs, only 9% of all the scored apnoeas and hypopnoeas were analysed. The distribution of the events analysed, was representative of the total events scored, their distribution across the sleep states, the proportion of association with cortical arousals and also the event type. Therefore, the RR-interval power changes around the apnoeas and hypopnoeas analysed are representative of the apnoeas and hypopnoeas scored across the night.
Based on the areas under the ROC curves, the differences between apnoeas and hypopnoeas and undisturbed sleep are consistent across the 14 mild to severe OSAHS patients. The patients were randomly selected based on their symptoms and polysomnographic findings, typical of the syndrome. It is likely therefore, that the outcomes are robust and represent clinically important differences.

10.4.5. Implications of the study outcomes

The present findings demonstrate sympathetic enhancement related to apnoeas and hypopnoeas during sleep. This is in accordance with the functional overlap of cerebral morphological substrates in the regulation of sleep and autonomic cardiorespiratory function [142]. The hypothesis is supported by Zinkovska et al [381] who prevented the increase of sympathetic, centrally [179] mediated input on vascular resistance in response to airway obstructions by blocking the autonomic responses to apnoeas through propranolol injection into porcine cerebral ventricles. Further evidence in support of the above hypothesis is provided through the observational study of patients with autonomic nervous system dysfunction and coexisting sleep apnoea: a "decoupling" of heart rate from the respiratory cycle was noted [132]. The present study found increased nocturnal sympathetic activity around apnoeas and hypopnoeas probably linked to increased central tonic efferent traffic in otherwise healthy OSAHS patients. This suggests an overall increase in nocturnal sinus node activity in response to neural modulatory inputs. This is supported by the nocturnal reduction found in RR-interval duration across the OSAHS patients. The nocturnal sympatho-vagal fluctuations may contribute to the previously demonstrated changes in daytime autonomic activity [249]. These observations may be of causative and prognostic importance for the development of cardiovascular disease in untreated OSAHS patients. Given the consistency of the findings despite the method's limitations linked to the stationarity requirements of the signal, it is proposed that this method may constitute part of a simplified portable diagnostic device. Further validation, estimation of a cut-off value for the differentiation between patients and non-patients and correlation analysis between this autonomic activity marker and clinical symptoms as well as long-term effects such as arterial hypertension would be necessary. None of the currently available devices for home diagnostics incorporate such analysis techniques, focussing instead largely on respiratory signals. Inclusion of more sophisticated signal analysis into the diagnostic cascade of portable devices, such as spectral analysis of the ECG RR-intervals, may improve diagnostic accuracy. It may also replace in-hospital polysomnography and make the diagnostic process more cost-effective. To ascertain the accuracy of home-based diagnosis, we validated a portable device and assessed its cost effectiveness. The validation study included comparisons of the automated software analysis to the "gold standard", the in-hospital polysomnography. In the absence of software which incorporates more sophisticated techniques of ECG analysis, we propose that such software may improve diagnostic validity and cost effectiveness of the currently available devices. In the next chapter, the results of the Embletta portable device validation study are discussed in detail.

CHAPTER 11
EVALUATION OF A PORTABLE DEVICE FOR DIAGNOSING THE SLEEP APNOEA / HYPOPNOEA SYNDROME

11.1. INTRODUCTION

The obstructive sleep apnoea/hypopnoea syndrome affects 1-4% of the population [378] causing daytime sleepiness, impaired work performance and road accidents [345] and is strongly associated with hypertension [254]. Early diagnosis and treatment of the disease may improve symptoms and prevent its long-term adverse effects.

The OSAHS is classically diagnosed by in-laboratory overnight sleep recording, the polysomnography [5]. However, the limited number of sleep centres and beds often results in long waiting lists for the diagnosis and treatment of the OSAHS. As there is evidence to support that untreated sleep apnoea is both deleterious to the patient and expensive to the healthcare budget [17][178], measures to reduce waiting lists and enable early diagnosis followed by the early initiation of treatment should be aimed at.

Home-based sleep studies have potential advantages in terms of both decreased costs [369] – including no charges for hospital accommodation - and convenience as well as, possibly, improved sleep quality for the patients.

A recent study comparing the sleep quality of in-laboratory sleep studies to home-based studies showed better sleep efficiency, more time spent in Rapid Eye Movement (REM) and Slow Wave Sleep (SWS), and significantly fewer arousals during the home-based studies [187].

The present study therefore aimed to examine the accuracy of a limited home-based diagnostic device, the Embletta (Flaga, Reykjavik, Iceland). The study also assessed the cost effectiveness of this portable device in diagnosing OSAHS patients.

11.2. METHODS

11.2.1. Primary Endpoints / Study Design

The study aimed to:

1. compare the portable Embletta device with the diagnostic "gold" standard, the in-hospital polysomnography
2. ascertain the diagnostic accuracy of the Embletta device in determining disease severity
3. ascertain the cost effectiveness of the portable device in diagnosing the OSAHS

The study had therefore two phases:

- the first consisted of synchronous recordings by polysomnography and by the portable device,
- the second was a comparison of home recording using the portable device with laboratory polysomnography on two separate nights.

The outcomes of the synchronous validation trial were applied to determine the cut-off values which defined the diagnostic groupings tested during the second, home testing sequential trial.

11.2.2. Study Population

SYNCHRONOUS TRIAL

Forty consecutive patients, 33 males and 7 females, mean age 46 years (SD 10 years) and mean BMI 32 kg/m^2 (SD 6 kg/m^2) underwent 2 simultaneous studies: an in-lab polysomnography (Compumedics S, Australia) and sleep study with the portable Embletta device (Flaga hf, Iceland). Both were applied to the patients by trained sleep technicians.

HOME TRIAL

Sixty-one consecutive patients, 47 males and 14 females, mean age 50 years (SD 11 years) and mean BMI 31 kg/m^2 (SD 6 kg/m^2) living within 50 miles from the sleep centre, were studied during 2 different nights, in random order and within a time interval of 2-40 days.

The patients were educated by a trained technician on the application of the portable diagnostic device which took on average 20 minutes. They then took the device home with written

instructions and applied the sensors unsupervised. The device was returned the next day and the data were analysed.

All study patients were referred to the sleep centre with possible OSAHS.

They had either self-reported daytime sleepiness (Epworth Sleepiness Scale >10) or 2 other major symptoms of OSAHS [370].

The only exclusion criteria were living further than 50 miles from the sleep centre and immobility, both of which excluded patients from the home study only.

11.2.3. Ethical Approval

All patients gave written informed consent to participate in the study, which had the approval of the Ethics Committee of the University of Edinburgh.

11.2.4. Recordings

11.2.4.1. Portable Device

The portable, limited sleep study performed with the Embletta device, consisted of:

Airflow / nasal pressure detection using nasal cannulae/ pressure transducer system, recording the square root of pressure as an index of flow.

Thoraco-abdominal movement detection through 2 piezoelectric belts.

Oxygen saturation recording using a finger pulse oximeter.

Body position detection.

11.2.4.2. Polysomnography

Polysomnography was performed with a standard technique [85], using a computerised recording system (Compumedics, Australia) and consisted of the following:

ELECTROENCEPHALOGRAPHY

Recording of sleep and arousals was based on the following tracings:

One bipolar central /occipital EEG tracing, the CZ-PZ and 2 frontal unipolar, unilateral tracings, the Fp1-LEOG and FP2-REOG.

Two outer canthi electrodes, LEOG and REOG, for the detection of eye movements.

A bilateral submental EMG electrode for the detection of tonic muscle activity.

ELECTROMYOGRAPHY

Leg movements were detected through bilateral tibial EMG electrodes, whereas body movements were recorded through a body position detector.

CARDIORESPIRATORY MONITORING

Monitoring of respiratory function consisted of:

A thermistor sensor for the oronasal airflow detection.

Two inductance plethysmographic belts for the detection of thoracoabdominal movement.

A digital microphone for snoring detection.

A pulseoximeter to monitor blood oxygen saturation levels, based on second-by-second measurements (Ohmeda Biox 3700, Louisville, CO, USA).

A 3-lead ECG was used to monitor cardiac activity.

11.2.5. Visual Scoring

The majority of the studies were visually scored by the principal investigator (K.D.), blinded to subject. Each patient's 2 studies were scored by the same observer. However, the observer was always blind to the patient's identity and the studies were batched to ensure the scorer could not identify the pairs of studies belonging to the same subject.

Every polysomnogram was scored during 3 consecutive sessions: sleep staging was followed by the scoring of apnoeas and hypopnoeas. Arousals were scored during the final session.

For the purposes of this study, arousal scoring will not be explored further, as no comparisons were possible between the 2 recording systems.

SLEEP SCORING

Sleep and arousal scoring was based on the CZ-PZ, Fp1-LEOG and FP2-REOG tracings. Sleep was scored according to the Rechtschaffen and Kales criteria [288], described in chapter 2.

Following parameters were used to describe sleep quality:

Sleep onset was defined as the first scored 30-second epoch of sleep stage 2.

Total Sleep Time **[hrs]** (TST) was calculated as the total time spent asleep.

Sleep Period Time **[hrs]** (SPT) was the time between sleep onset and the last epoch of sleep.

Sleep efficiency **[%]** was the ratio of TST divided by the SPT and multiplied by 100.

APNOEA / HYPOPNOEA SCORING

Apnoeas were defined as cessation of the oronasal airflow, lasting $\geq$10sec.

Hypopnoeas were defined as a reduction in thoraco-abdominal movement of $\geq$ 50%, compared to a 10sec peak amplitude during the preceding 2 minutes, lasting $\geq$10 seconds [5].

During the synchronous validation studies, hypopnoeas on the Embletta device were additionally scored during a separate session as a reduction in the nasal pressure amplitude of $\geq$50% lasting $\geq$10 seconds [255].

Two apnoea-hypopnoea measures were calculated from each polysomnogram:

Apnoea / Hypopnoea Index **(AHI) [hr $^{-1}$ slept]:** the number of all apnoeas and hypopnoeas divided by the total time spent asleep on polysomnography.

$$\text{AHI [hr}^{-1}\text{ slept]} = [\text{Apnoeas} + \text{Hypopnoeas}] / \text{TST}$$

An AHI<15/hour sleep was considered normal.

Apnoeas + Hypopnoeas **[hr $^{-1}$ in bed]:** the number of all apnoeas and hypopnoeas divided by the hours spent in bed.

As the portable device does not record sleep, study onset was marked as soon as respiration settled down to a rhythmic, stable pattern. The end of the study was set either according to the information given by the patient as to the time of morning awakening, or based on the quality of the tracings and regularity of breathing pattern.

The limited studies during the synchronous in-lab trial yielded 3 apnoea and hypopnoea measures:

1. **A+H_ma:** A+H/hr in bed based on manual scoring of changes in nasal pressure and thoracoabdominal movement.
2. **A+H_fl:** A+H/hr in bed based on manual scoring of changes in nasal pressure alone.
3. **A+H_au:** A+H/hr in bed from the automated software analysis.

The limited home studies yielded 2 apnoea and hypopnoea measures:

1. **A+H_ma**.
2. **A+H_au.**

On the basis of the synchronous, in-hospital study, the results of the Embletta manual scoring were classified into the following groups:

1. "OSAHS": $\geq$ 20 A+H / hr in bed.
2. "possible OSAHS": 10–20 A+H / hr in bed.
3. "not OSAHS": <10 A+H / hr in bed.

11.2.6. Statistical Analysis

POWER CALCULATION

The calculation of the sample size and the statistical power were based on a similarly designed study by Whittle et al [369]. Using the data from the correlation analysis between the in-lab versus home-based study outcomes across the 58 subjects (figure 2 of the paper by Whittle et al [369]), we calculated the difference between the 2 A+H measures [A+H/hr recording – A+H/hr asleep] for each subject. The mean difference across the subjects was 13.6 /hr and the standard deviation of the difference was 36.7 /hr. Based on these values, a sample size of 60 patients is required for a paired t-test to reach 80% power at a 5% significance level.

Although 61 patients were initially recruited in the present study, there were 11 drop-outs.

The sample size of the remaining 50 patients achieved a statistical power of 73% at a 5% significance level.

STATISTICAL TESTS

Statistical data analysis was performed using the SPSS Inc, Chicago IL, V9.0 program.

The diagnostic accuracy and reproducibility of the patients' classification into no OSAHS, possible and definite OSAHS based on the portable study outcomes was assessed through kappa statistics which evaluated the agreement between polysomnography and portable Embletta recording system, based on the different scoring criteria.

Bland and Altman plots were generated to demonstrate the degree of identity between the outcomes of the 2 diagnostic methods, taking into consideration the different scoring criteria of the limited study data.

Pearson's correlation analysis evaluated the significance of agreement in the A+H score between the 2 methods. Paired sample t-test was performed to evaluate differences in the outcomes across the subjects due to the different scoring criteria used.

Tests were 2-tailed and $p<0.05$ was accepted as statistically significant. Mean values and the Standard Deviation (SD) or the Standard Error of the Means (SEM) are quoted.

11.3. RESULTS

11.3.1. Recording Adequacy

SYNCHRONOUS STUDY

Of the 40 patients, one was excluded from the data comparison due to technical problems as the Embletta only recorded 3 minutes of interpretable data. The remaining 39 patients, 32 male and 7 female, had a mean age of 46 years (SD 9 years) and a mean BMI of 32 kg/m^2 (SD 6 kg/m^2).

The mean sleep efficiency in this group of patients was 76 $\pm$ 2% (mean $\pm$ SEM) of the Sleep Period Time (SPT). Mean Total Sleep Time (TST) was 5.1 $\pm$ 0.3 (mean $\pm$ SEM) hours.

The mean AHI of the patients was 35 /hour sleep (table 11.1).

Patient group	**# pat**	**PSG dur.[hr]**	**Emb. dur.[hr]**	**PSG: AHI**	**PSG:A+H/hr bed**	**Emb:A+H/hr bed**
Validation study PSG:AHI/hr sleep	39	6.8 $\pm$ 0.3	6.9 $\pm$ 0.3	35.4 $\pm$ 5.5	29.2 $\pm$ 3.7	27.2 $\pm$ 3.4
AHI<10/hr	6	6.5 $\pm$ 1.1	6.7 $\pm$ 1.1	5.3 $\pm$ 0.9	6.3 $\pm$ 1.0	6.3 $\pm$ 1.0
10$\leq$AHI<20/hr	12	6.6 $\pm$ 0.6	6.7 $\pm$ 0.6	13.8 $\pm$ 0.7	13.7 $\pm$ 0.8	14.2 $\pm$ 1.7
AHI$\geq$20/hr	21	7.0 $\pm$ 0.4	7.1 $\pm$ 0.4	56.3 $\pm$ 7.7	44.6 $\pm$ 4.7	40.7 $\pm$ 4.6

Table 11.1. Mean values and SEM of polysomnography (PSG) and limited Embletta studies duration in hours, the Apnoea-Hypopnoea-Index (AHI/hr slept) on polysomnography, apnoeas and hypopneas per hour in bed on polysomnography and Embletta during the validation study.

A further 3 patients were excluded from the comparison between nasal pressure-based hypopnoea scoring versus scoring based on thoraco-abdominal movement and nasal pressure changes due to technical problems in the nasal pressure recordings. In 2 of these patients the nasal pressure tracing did not record, in 1 patient it was not interpretable.

HOME STUDY

Inadequate recordings were obtained at home in 11 of the 61 patients. One was due to the patient not using the equipment at all, five due to technical problems with the equipment (plugging, batteries or software) and in five no reason was identified. This overall fail rate of 18% contained a learning effect; the fail rate in the latter two-thirds of the study was 12% (5 out of 42 studies). Fifty patients then studied in the sleep centre had a mean sleep efficiency of 82 $\pm$ 1 % (mean $\pm$ SEM) of the SPT. Mean TST of the in-lab recordings was 6.4 $\pm$ 0.1 hours (mean $\pm$ SEM). Mean AHI was 29/ hr sleep (table 11.2).

Patient group	**# pat**	**PSG dur.[hr]**	**Emb. dur.[hr]**	**PSG: AHI**	**PSG:A+H/hr bed**	**Emb:A+H/hr bed**
Prospective study Emb:A+H/hr bed	50	7.8 $\pm$ 0.06	6.5 $\pm$ 0.3	29.2 $\pm$ 2.7	27.0 $\pm$ 2.5	23.5 $\pm$ 2.6
A+H<10/hr	9	7.7 $\pm$ 0.1	5.5 $\pm$ 0.9	9.8 $\pm$ 1.7	9.8 $\pm$ 1.6	5.5 $\pm$ 3.0
10$\leq$A+H <20/hr	18	7.7 $\pm$ 0.1	5.7 $\pm$ 0.5	22.4 $\pm$ 2.4	22.0 $\pm$ 2.6	15.6 $\pm$ 2.5
A+H$\geq$20/hr	23	7.9 $\pm$ 0.08	6.9 $\pm$ 0.4	42.5 $\pm$ 4.0	38.2 $\pm$ 3.2	37.1 $\pm$ 3.9

Table 11.2. Mean values and SEM of polysomnography (PSG) and limited Embletta studies duration in hours, the Apnoea-Hypopnoea-Index (AHI /hr slept) on polysomnography, apnoeas and hypopneas per hour in bed on polysomnography and Embletta during the prospective study.

11.3.2. Diagnostic Accuracy

SYNCHRONOUS STUDY

The mean difference [polysomnography – Embletta] in A + H /hr in bed was 2 /hr (SD 5 /hr) (p=0.02), with a close correlation between the results of the 2 studies (rho=0.98, p<0.001) (figure 11.1.A and B).

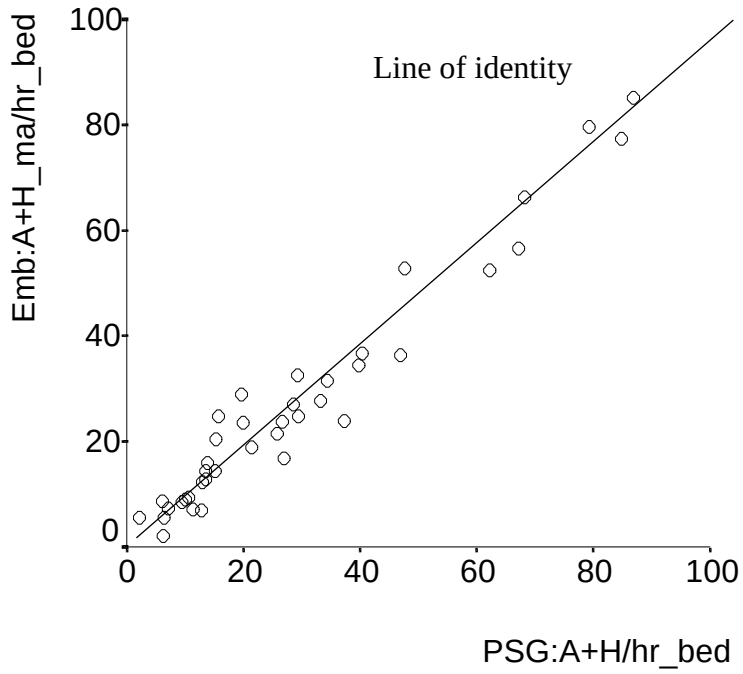


Figure 11.1.A. Scatterplot with line of identity across the 39 patients of the synchronous trial, between:
Emb: A+H ma: Manual Apnoea and Hypopnoea (A+H) Embletta score per hour in bed (hypopnoea score is based on nasal pressure and thoraco-abdominal changes) and PSG: A+H /hr bed: Polysomnographic A+H score per hr in bed.

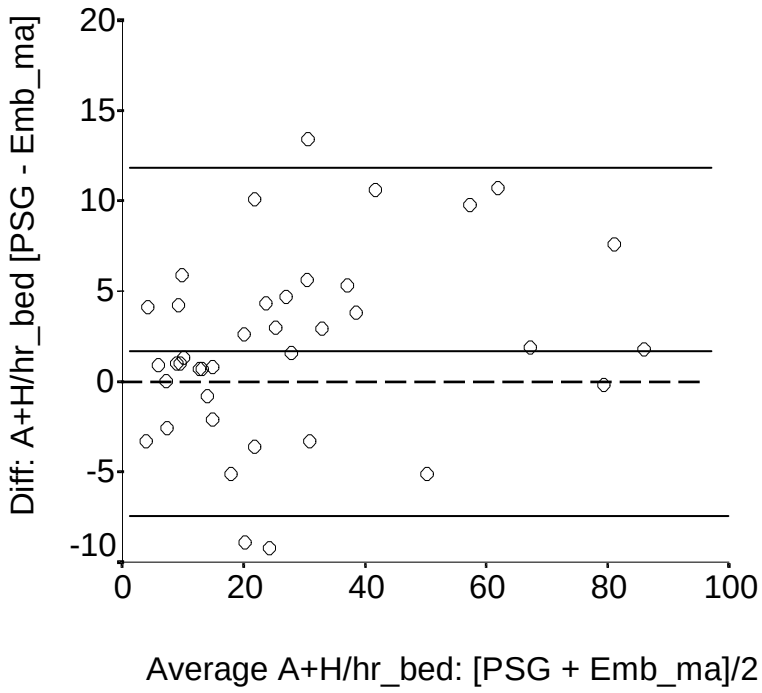


Figure 11.1.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 39 patients of the synchronous trial, between: Emb: A+H ma: Manual Apnoea and Hypopnoea (A+H) Embletta score per hour in bed (hypopnoea score is based on nasal pressure and thoracoabdominal changes) and PSG: A+H /hr bed: Polysomnographic A+H score per hour in bed.

In comparison to the polysomnographic AHI per hour slept, the Embletta A+H /hr in bed differed by a mean of 8 /hr (SD 16 /hr) (figure 11.2.A and B). Patients with an A+H > 40 /hr in bed on both methods were excluded from the comparison. The mean difference in the latter group was 2 /hr in bed (SD 5 /hr).

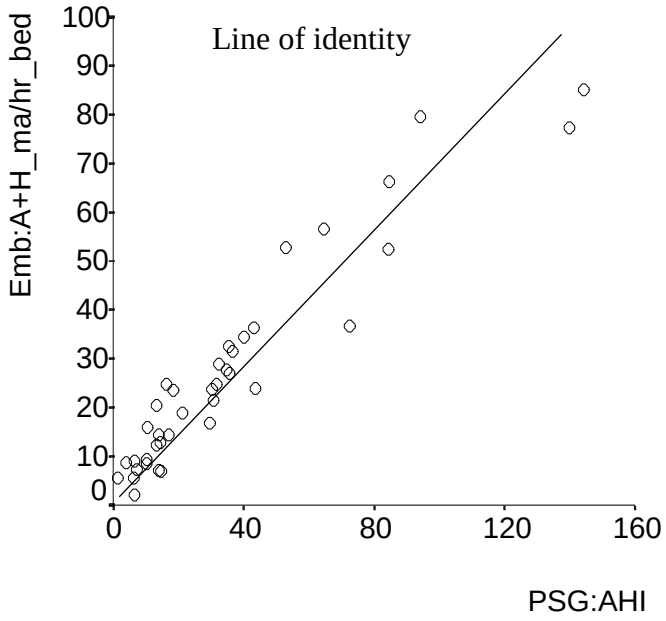


Figure 11.2.A. Scatterplot with line of identity across the 39 patients of the synchronous trial, between:
Emb: A+H ma: Manual A+H Embletta score and PSG: AHI: Polysomnographic A+H score per hr slept.

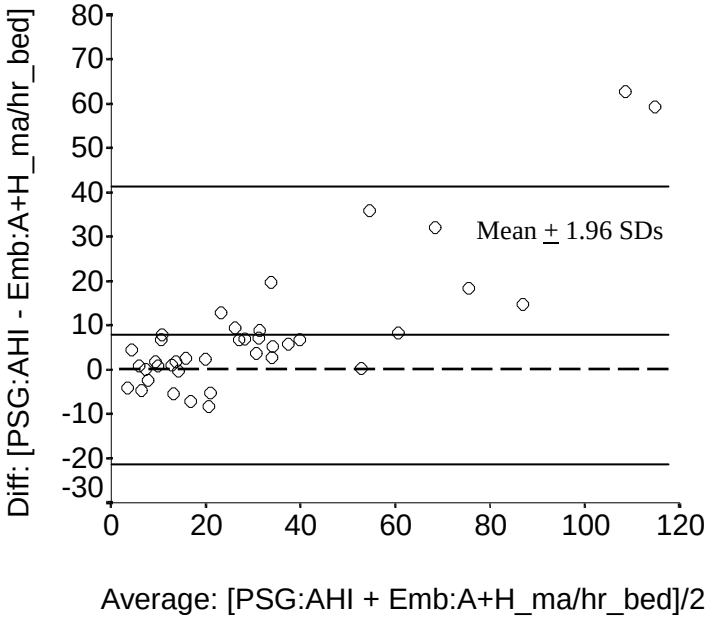


Figure 11.2.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 39 patients of the synchronous trial, between:
Emb: A+H ma: Manual A+H Embletta score and PSG: AHI: Polysomnographic A+H score per hr slept.

Hypopnoea scoring based on nasal pressure changes alone was evaluated through comparison between polysomnographic outcomes (A+H/ hr in bed) and Embletta A+H_fl outcomes. The mean difference of [polysomnography – Embletta] in A+H per hour in bed was 3 /hr (SD 9 /hr) (p=0.004) (figure 11.3.A and B).

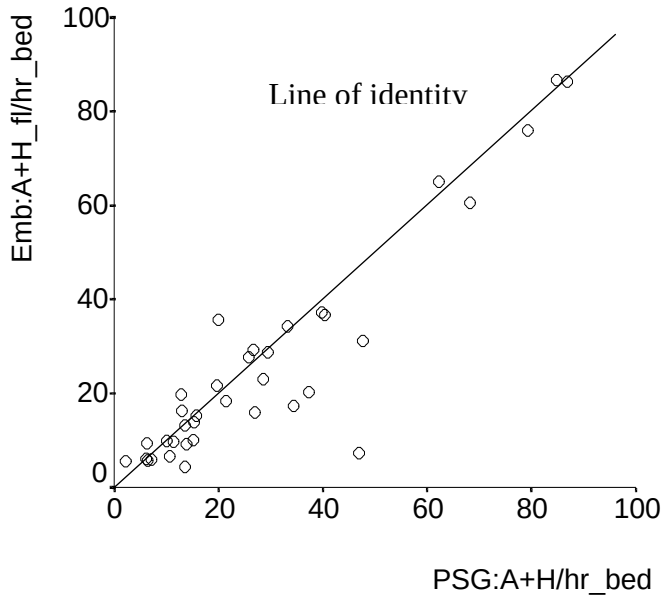


Figure 11.3.A. Scatterplot with line of identity across the 39 patients of the synchronous trial, between: Emb: A+H fl: Manual A+H Embletta score per hour in bed (hypopnoea score is based on nasal pressure changes alone) and PSG: A+H /hr bed: Polysomnographic A+H score per hour in bed.

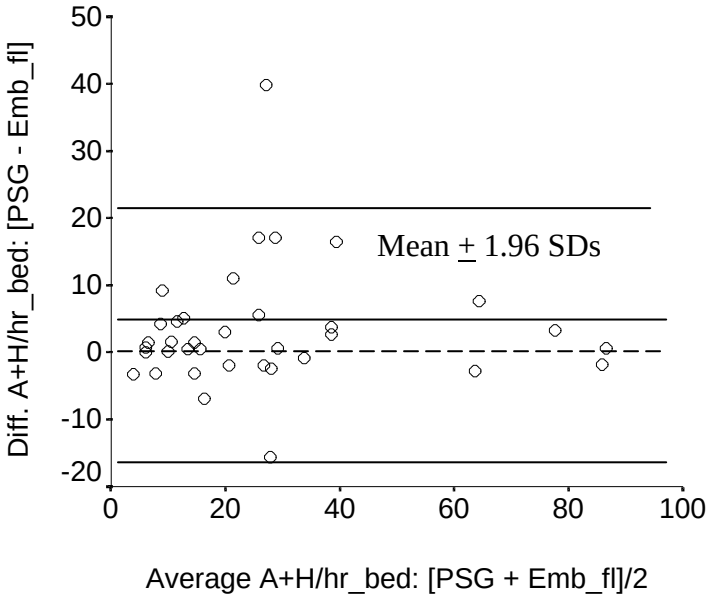


Figure 11.3.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 39 patients of the synchronous trial, between: Emb: A+H fl: Manual A+H Embletta score per hour in bed (hypopnoea score is based on nasal pressure changes alone) and PSG: A+H /hr bed: Polysomnographic A+H score per hour in bed.

The difference between outcomes based on the 2 hypopnoea definitions used (combined nasal pressure and thoraco-abdominal movement changes versus changes in nasal pressure alone) was not significant across the 36 patients (p=0.4). However hypopnoea scoring based on nasal pressure changes alone would have scored 1 patient as normal (A+H fl: 7 /hr in bed) who had an A+H > 15 /hr both on polysomnography and on manual Embletta scoring when hypopnoeas were defined on both thoraco-abdominal and nasal pressure changes.

Automated Embletta scoring produced a mean difference from the polysomnography of 0.1 /hr in bed with a larger variance (SD 15 /hr in bed) (figure 11.4.A and B) compared to the manual Embletta scoring (mean difference 2 /hr, SD 5 /hr in bed). Statistical significance was not reached between the 2 mean differences across patients (p=0.4).

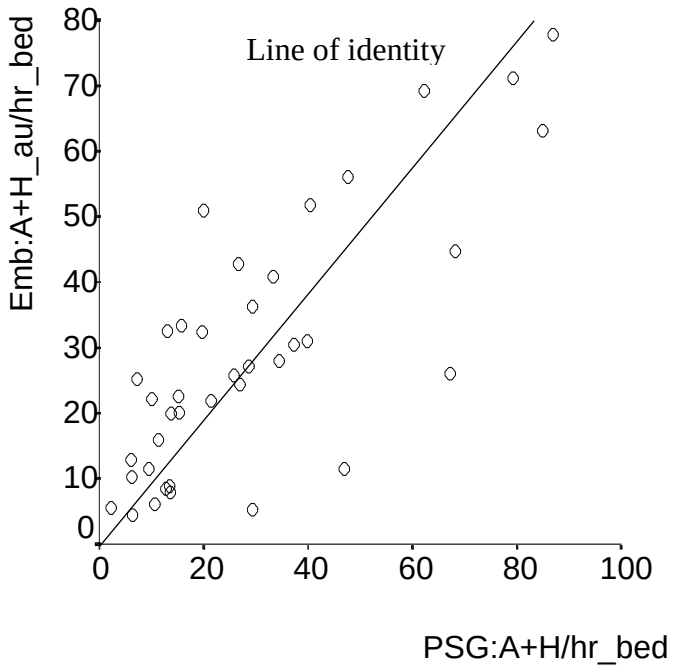


Figure 11.4.A. Scatterplot with line of identity across the 39 patients of the synchronous trial, between:
Emb: A+H au: Automated A+H score of Embletta software and
PSG: A+H /hr bed: Polysomnographic A+H score per hour in bed.

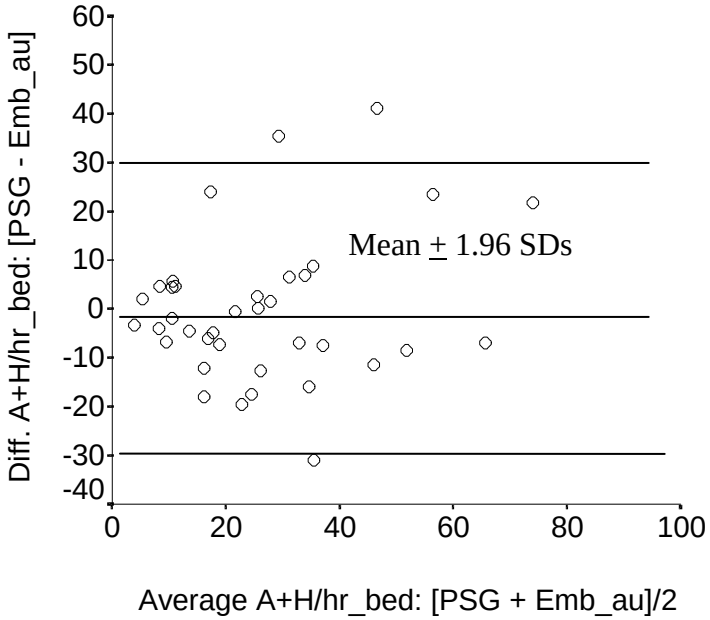


Figure 11.4.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 39 patients of the synchronous trial, between: Emb:A+H au: Automated A+H score of Embletta software and PSG: A+H/hr bed: Polysomnographic A+H score per hour in bed.

HOME STUDY

On the basis of the synchronous study, the results of the Embletta manual scoring were categorised as "OSAHS" ($\geq$ 20 A+H /hr in bed), "possible OSAHS" (10-20 A+H /hr in bed) or "not OSAHS" (<10 A+H /hr in bed). On this basis, 21 of the 22 patients with "OSAHS" ($\geq$ 20 A+H /hr in bed on manual Embletta scoring) had AHI $\geq$15 on polysomnography, the 22nd patient having an AHI of 13 /hr. All 10 patients categorised as not OSAHS had AHI <15, while three of the seven patients with possible OSAHS had AHI $\geq$15 (AHI: 17, 21 and 30 /hr slept).
This categorisation was then used for the home study results.

The mean difference [polysomnography – Embletta] in A+H /hour in bed was 3 /hr (SD 13 /hr) (p=0.06) (figure 13.5.A and B) with a good correlation (rho=0.74, p<0.001).

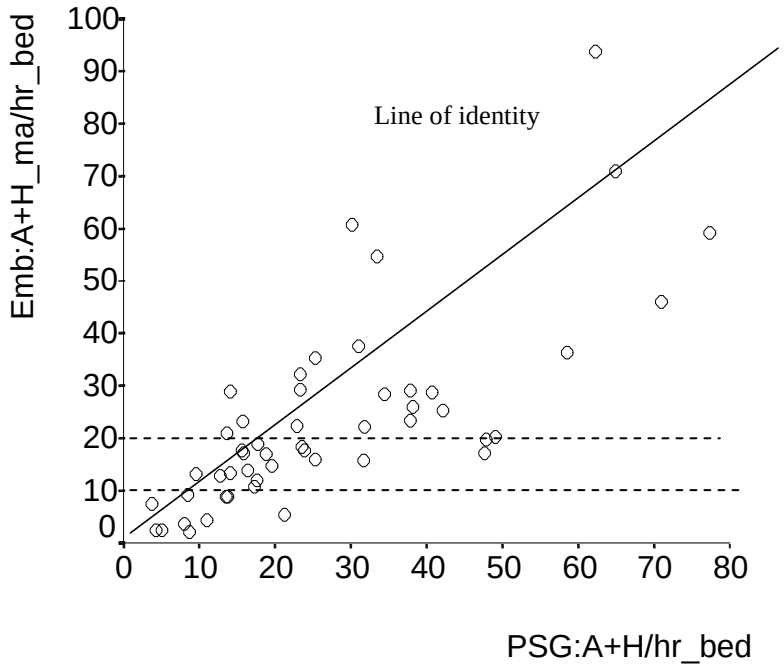


Figure 11.5.A. Scatterplot with line of identity across the 50 patients of the prospective trial, between: Emb: A+H ma: Manual Apnoea and Hypopnoea (A+H) Embletta score per hour in bed (hypopnoea score is based on nasal pressure and thoracoabdominal changes) and
PSG: A+H /hr bed: Polysomnographic A+H score per hr in bed.

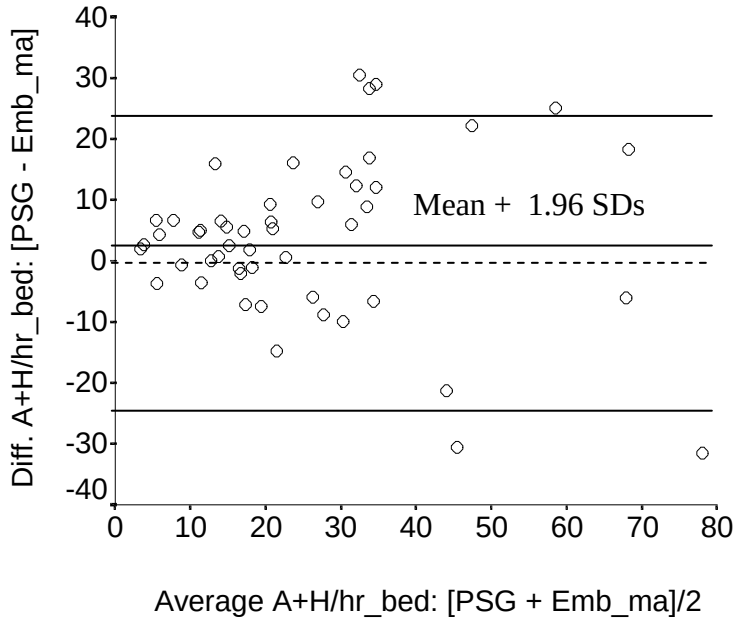


Figure 11.5.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 50 patients of the prospective trial, between: Emb: A+H ma: Manual Apnoea and Hypopnoea (A+H) Embletta score per hour in bed (hypopnoea score is based on nasal pressure and thoracoabdominal changes) and PSG: A+H/hr bed: Polysomnographic A+H score per hour in bed.

In comparison to the polysomnographic AHI /hour slept, the Embletta A+H /hour in bed differed by 6 ± 14 /hr (Mean ± SD; P=0.006) (figure 13.6.A and B).

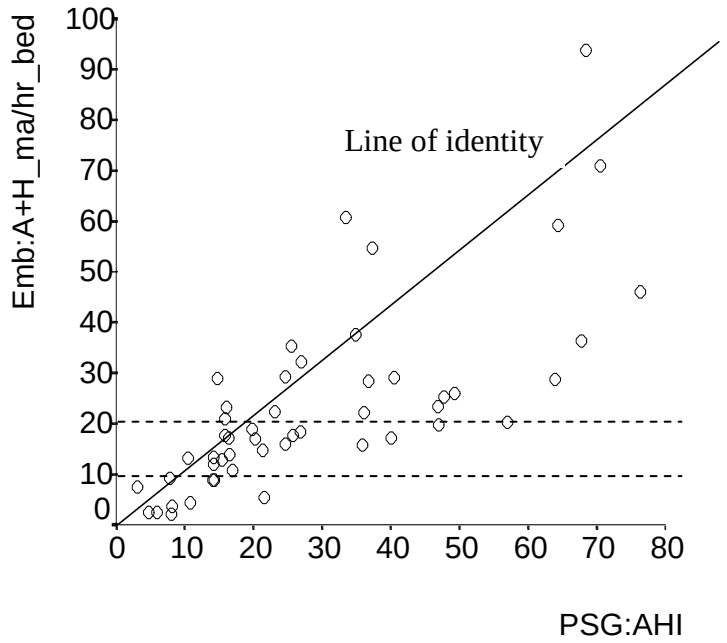


Figure 11.6.A. Scatterplot with line of identity across the 50 patients of the prospective trial, between: Emb: A+H ma: Manual A+H Embletta score and PSG: AHI: Polysomnographic A+H score per hour slept.

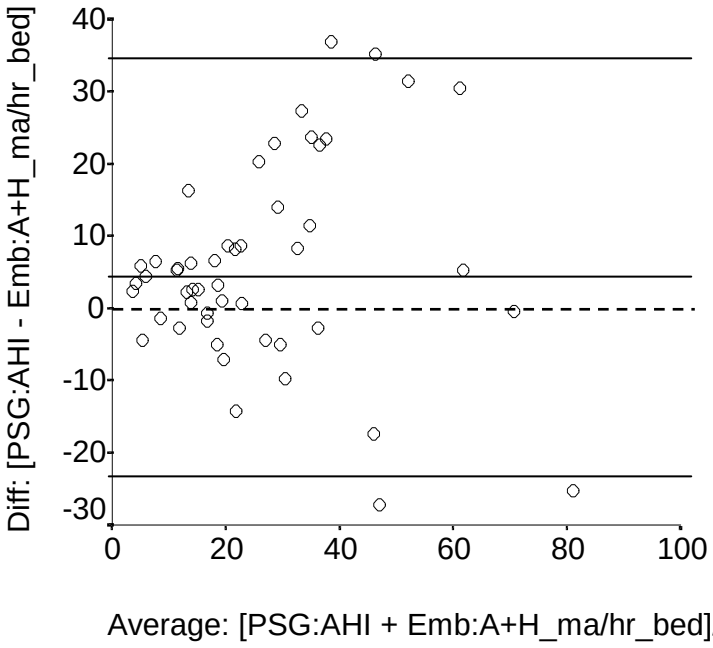


Figure 11.6.B. Bland and Altman plot with mean value and 1.96 Standard Deviations (SD) across the 50 patients of the prospective trial, between: Emb: A+H ma: Manual A+H Embletta score and PSG: AHI: Polysomnographic A+H score per hour slept.

All nine patients with home A+H <10 /hr in bed had an AHI<15 /hr slept on polysomnography (median 9 /hr, range 4-14 /hr) and all 23 with >20 A+H /hr in bed Embletta had an AHI ≥15 /hr on polysomnography.
Eighteen of the 50 patients had home studies in the range of 10-20 A+H /hr in bed, of whom three had AHI <15 /hr (10, 14 and 14 /hr slept) and 15 AHI ≥15 /hr slept on polysomnography (median 21 /hr, range 15-57 /hr). Twelve of the 50 patients had ≥ 5 periodic limb movements per hour slept (range: 5-218 /hr) during their in-lab study.

To obtain a criterion for the Embletta-based diagnosis of OSAHS, the agreement on the classification of the patients into groups was evaluated. There was good agreement between the outcomes of the limited recordings and polysomnography in the synchronous studies (kappa coefficient: 0.62, $p<0.001$). The agreement between outcomes based on nasal pressure changes only and polysomnography was also good (kappa coefficient: 0.57, $p<0.001$).
The home-based studies showed an overall good agreement with the polysomnographic outcomes (kappa coefficient: 0.54, $p<0.001$).
During both the synchronous and home studies, agreement between polysomnography and automated Embletta software analysis was poor (table 11.3).

	Kappa coefficient value	p-value
Validation study		
Emb:A+H_ma[1] & PSG:AHI[2]	0.62	<0.001
Emb:A+H_ma & PSG:A+H/hr_bed[3]	0.62	<0.001
Emb:A+H_ma & Emb:A+H_fl[4]	0.69	<0.001
Emb:A+H_fl & PSG:A+H/hr_bed	0.57	<0.001
Emb:A+H_ma & Emb:A+H_au[5]	0.35	0.003
Emb:A+H_au & PSG:A+H/hr_bed	0.28	0.01
Prospective study		
Emb:A+H_ma & PSG:AHI	0.50	<0.001
Emb:A+H_ma & PSG:A+H/hr_bed	0.54	<0.001
Emb:A+H_ma & Emb:A+H_au	0.17	0.03
Emb:A+H_au & PSG:A+H/hr_bed	0.10	0.16

Table 11.3. Evaluation of diagnostic accuracy of the portable Embletta device using kappa statistics.

[1] **Emb:A+H_ma:** A+H score of manual Embletta scoring per hr in bed. Hypopnoea score is based on nasal pressure and thoracoabdominal changes.
[2] **PSG:AHI:** Polysomnographic apnoeas and hypopnoeas (A+H) score per hr slept.
[3] **PSG:A+H/hr_bed:** Polysomnographic A+H score per hr in bed.
[4] **Emb:A+H_fl:** A+H score based on manual Embletta scoring per hr in bed. Hypopnoea score is based on nasal pressure changes alone.
[5] **Emb:A+H_au:** A+H score of automated Embletta software analysis.

11.4. DISCUSSION

This study shows that home recording with the Embletta device can produce clinically useful results when the recordings are manually analysed.

On the basis of the 3 diagnostic bands determined from the synchronous study, all 32 of the 50 patients in the home study who fell into a clear diagnostic category were correctly categorised. It must be stressed that these categorisations were determined before the home study results were examined.

However, 18 of the 50 patients fell into the "possible OSAHS" category in which further study might be indicated. As 15 of these patients had AHIs on polysomnography of $\geq$ 15, further studies might be indicated to determine whether a lower A+H /hr in bed on home studies could be regarded as diagnostic for OSAHS.

The analysis is critically dependent on the definition of OSAHS used. In the present study a conservative threshold of an AHI of 15 /hour plus symptoms was used, as there is robust evidence for the benefits of CPAP therapy in symptomatic patients with AHI$\geq$15/hr [93][95].

Had an AHI of 5 /hr [5] been used as the diagnostic cut-off, then 48 of the 50 patients in the consecutive study reached this threshold and their Embletta results ranged from 2 to 94 /hr in bed.

However, as diagnostic and treatment decisions should not be slavishly based on any single number derived from an overnight study, the above type of analysis, where absolute numbers are regarded as critical, can demonstrate "inaccuracies" in categorisation which are in fact of little importance if a more flexible approach to diagnosis is used.

Had a diagnostic cut-off of A+H >15 /hour in bed on the Embletta been applied, then all 34 patients with an A+H >15/hour in bed on the Embletta also had an AHI >15/hr on polysomnography. Of the 16 patients with A+H < 15/hour in bed on the Embletta, three had AHI >15 /hour on polysomnography (range 16-21 /hour).

Thus, post hoc alternative cut-offs could be chosen but their value would need to be tested during a further study.

11.4.1. Study Limitations

Limitations of this study include the number of subjects studied and the venue of the studies. The purpose of the synchronous study was to determine the accuracy of the device against the alleged "gold standard" of polysomnography.

Based on the power calculation, the 39 patients are sufficient to demonstrate the broad agreement and the limitations of this approach.

The main part of the study with the larger number of patients was testing the device in the location in which we believe it will be mainly used, namely at home.

It is always debatable whether to perform the synchronous study in the home or sleep laboratory environment. A new device to be used at home must be tested both synchronously with polysomnography, in either the home or laboratory setting, and then tested in the home setting against in-laboratory polysomnography. In-laboratory polysomnography is the appropriate comparator as that is the location where diagnostic studies are commonly performed and is the nearest to a gold standard that exists.

Had the study been limited to synchronous home-based polysomnography versus Embletta testing, there would have been no comparison with the standard diagnostic test of in-laboratory polysomnography. This is particularly important as there are differences in sleep quality between polysomnography at home versus in-hospital [187]. One of the causes of the bigger variance between Embletta and polysomnography results in the home versus laboratory study (SD 13 /hr) in comparison to synchronous study (SD 5 /hr) is night to night variation which can be significant in OSAHS [371].

Another limitation of the study was that the data set encompassed early experiences with the use of the Embletta devise. This is manifested by the high initial fail rate of the home studies (six of 21), as the instruction and education package for the patients was refined. The latter two-thirds of the home studies were much more successful with only five of 42 fails. Nevertheless, the overall home diagnostic fail rate of the current study was very similar to that in other studies on base recording. For example, Whittle et al reported a fail rate of 18% on home-based recordings, during the application of a different portable device [369].

While this learning effect has influenced the success of the home studies, it will not have affected their accuracy.

11.4.2. Cost Benefit Analysis and Implications

The utility of this device to sleep centres will depend on local factors, including the relative costs and availability of sleep centre diagnostic studies, the use made of "split night studies", which combine diagnostic studies with CPAP pressure titration in the same night [222], the geographical spread of the patients investigated and whether the equipment is attached by the patient or a technician.

The device is suitable for use in the patient's home or can be used in hospital to study patients admitted for investigation of OSAHS or those already admitted in whom the question of OSAHS is then raised.

In the mode used in the sequential study with the diagnostic categories chosen, it is estimated that in comparison to performing overnight sleep centre diagnostic polysomnographies in all patients, the use of home Embletta studies reduce diagnostic costs by 42% if those in the diagnostic categories went straight to CPAP titration or to no further investigation with only those in the "possible OSAHS" and failed home study groups proceeding to polysomnography. This is calculated on the basis of a cost of £250 for each polysomnography (including charge for accommodation, equipment write down, technician and nurse time and disposables) and £29 for an Embletta study, this comprising 2 hours of technician time to prepare the device, educate the patients on its application and score each Embletta recording manually, £6 for disposables and write down of the £5,800 purchase price of an Embletta over 5 years with five studies per week over that period. Thus, use of the Embletta in the mode described is cost effective.

The study outcomes also suggest that use of the nasal pressure signal as the sole indicator of hypopnoeas will result in unscorable traces in approximately 8% of studies. Thus, the use of the nasal pressure in combination with the thoraco-abdominal movement signals as indicators of hypopnoeas is recommended, at least until further data are available.

The automated scoring software did not relate closely to the manually scored results. This could perhaps be a problem with the preset criteria in the software rather than a fundamental flaw. Incorporation of more sophisticated signal analysis, such as spectral analysis of the ECG RR-intervals, may improve further the diagnostic accuracy of the automatic scoring software.

Thus, the Embletta as used in this study can save both costs and sleep laboratory usage in the diagnosis of the obstructive sleep apnoea/hypopnoea syndrome.

CHAPTER 12
CONCLUSIONS AND FUTURE WORK

This thesis has explored differences in the changes associated with apnoeas/hypopnoeas across sleep stages as well as the detection of changes in autonomic activity and their association to cortical arousals. A further important objective of this thesis was to explore whether better detection and analysis of the physiological changes associated with apnoeas/hypopnoeas in OSAHS patients may lead to alternative ways of diagnosing the syndrome. The results showed that apnoeas/hypopnoeas are consistently associated with increased Heart Rate Variability – a marker of sympathetic activity – irrespective of the presence of visually detectable arousals. A further important observation is that spectral analysis of the EEG improves the detection of changes related to apnoeas/hypopnoeas. Differences across sleep stages are less apparent through the application of these alternative methods of analysis. Therefore, integration of these non-conventional methods into automated signal analysis algorithms of portable diagnostic devices may contribute towards prompt and cost-effective diagnosis, early treatment and prevention of the long-term consequences of the syndrome.

Following the application of a modified arousal definition in terms of duration, the study in chapter 8 found that 82% of apnoeas and hypopnoeas are associated with visually detectable arousals, but 18% are not. These findings are consistent with the observations by Rees et al [291]. Differences in the cortical arousal threshold and consequently in arousal induction, but also differences in the ability to maintain airway patency and counteract the onset of apnoeas and hypopnoeas in association with the different sleep stages, have been shown to be of importance.

The importance of the arousal in the termination of apnoeas and hypopnoeas and the restoration of regular ventilation was recognised by Phillipson et al in 1978 [280]. Research into the factors and stimuli that may contribute towards an arousal response has shown that the major contributor is the increased respiratory effort rather than chemical stimuli [124][186]. The lack of association between chemoreceptor stimulation following hypoxia and arousal induction has been also demonstrated during the study described in chapter 8. This does not exclude an additive stimulative effect of the chemoreceptor afferents towards the brainstem [186].

Consequently, the following hierarchical model of arousal induction is proposed:

Afferent impulses reach the brainstem in response to mechanoreceptor stimulation from the upper airway, lungs, respiratory muscles but also from the baroreceptors and chemoreceptors. The stimulation of the brainstem may modulate an arousal response which is propagated to the

cortex. Alternatively, following interactions with neurons which control sleep stage, the arousal response may not be propagated to the Cortex but is fed back at brainstem level through the mechano- and baroreflex loop leading to autonomic and pharyngeal motoneuron activation and resulting in the restoration of breathing (figure 12.1).

This hypothesis has been explored further in chapters 9 and 10. The outcomes of these studies suggest that alternative analysis techniques as well as monitoring of autonomic activity may improve detection of changes related to disturbed sleep architecture as well as the autonomic dysfunction associated with apnoeas and hypopnoeas.

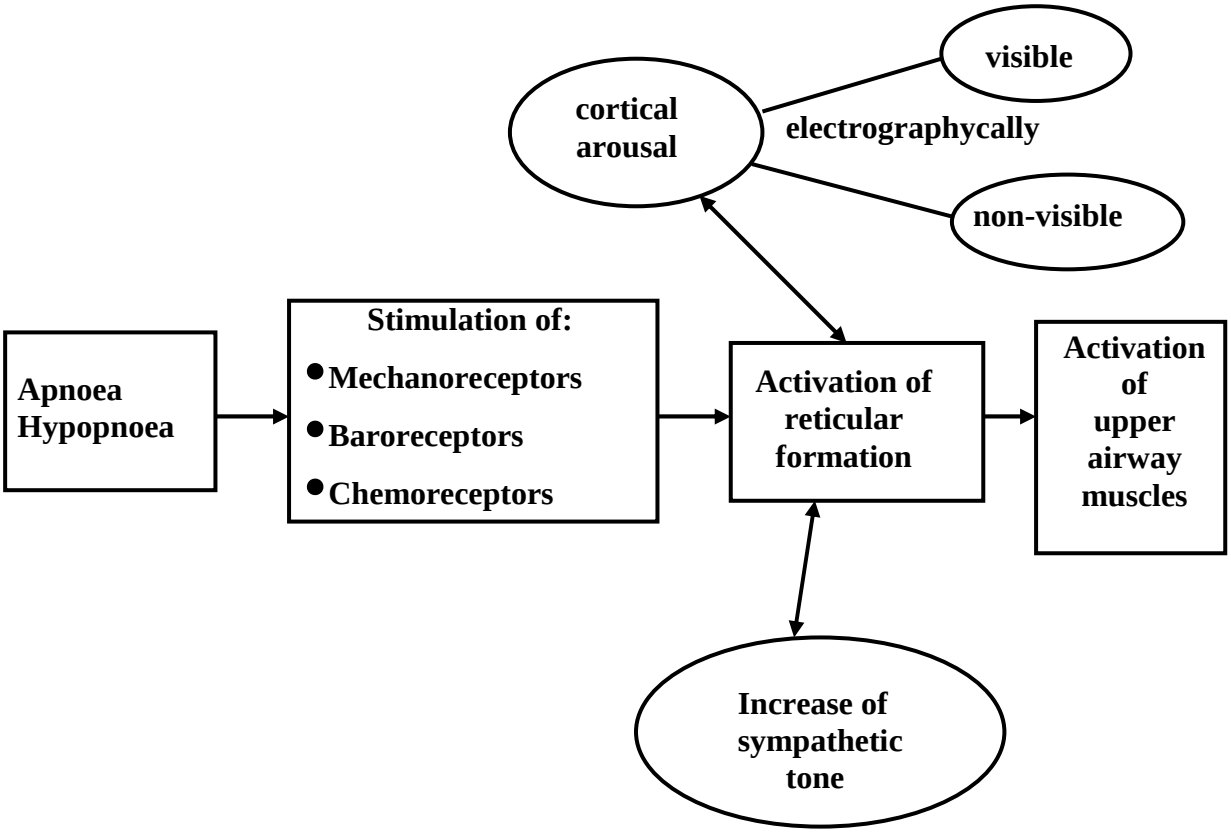


Figure 12.1: Proposed hierarchical arousal model.

In 2004, Magdu Younes published the outcomes of overnight measurements in 82 OSAHS patients, aiming to assess the causative association between apnoeas, hypopnoeas and arousals [376]. During that study, a pneumotachograph was used to measure tidal volumes, respiratory rate, inspiratory and expiratory times and consequently record apnoeas, hypopnoeas, episodes of increased respiratory effort as well as the post-arousal induced changes in flow and respiratory

effort. Arousals were recorded based on visual inspection and also based on Fourier analysis of the EEG. Across the 82 patients, 525 events were studied. In order to ascertain whether arousals triggered the increase in flow, respiratory events were classified into 3 groups: those without arousal, those followed by an arousal and events overlapping the arousal response. In 17% of the events, restoration of normal flow occurred in the absence of an arousal on visual inspection or during Fourier analysis. The remaining 83% of all events studied were associated with a form of cortical activity change which occurred before (61%) or after (22%) the restoration of normal flow. An increase in delta power was inversely related to the arousal frequency and the degree of upper airway instability – based on the measurements of tidal volume, respiratory cycle times, respiratory rate and effort. Regression analysis confirmed that delta power, minute ventilation and body mass index were independently associated with the event type. In other words, the author's findings support that interindividual differences in relation to the BMI as well as the pharyngeal collapsibility and the degree of narrowing can influence whether and when an arousal may occur.

These findings suggest that arousal represents a graded response to the degree of limitation in minute ventilation with inter-individual variability. The former is in keeping with the findings by Stradling et al who found differences between apnoeas and hypopnoeas in their association with arousal [336]. Regression analysis, performed to assess the influence of arousal type, delta power or minute ventilation on the time of flow reversal, found that the effect of minute ventilation was significant. The time of flow reversal, in other words the duration of the event, was independent of delta power and whether or not an arousal occurred. The above findings support the study outcomes in chapter 8 in regard to the proportion of apnoeas and hypopnoeas associated with arousals, the lack of association between event duration and arousal but also the variability in apnoea and hypopnoea index and the arousal index parallel to the delta wave prevalence on the EEG. The findings are also in keeping with previous observations by Tangel et al [343] in regard to differences in muscle tone across the sleep stages as well as the observations by Berry et al [32], who found that the increase in inspiratory resistance correlated with the increase in EEG delta power, i.e. the threshold for upper airway patency – either due to the arousal or the pharyngeal motoneuron threshold - during SWS was higher than in NREM1&2 or REM sleep.

The study by Younes did not confirm a consistent pattern of spectral power changes in association with the increases in respiratory effort [376]. This is in keeping with the findings in chapter 9. Younes observed that post-arousal periods were associated with hyperventilation and increased airway instability. Based on the above findings, the author concluded that arousals are not required in order to restore airway patency and flow and that the association is more likely to

be incidental rather than causative. It was furthermore concluded that arousals may lead to a flow overshoot and hyperventilation and may further destabilise airway patency either directly through the change in pharyngeal load or through the centrally mediated respiratory control system and may therefore contribute towards the pathogenesis and increased frequency of apnoeas and hypopnoeas. These findings may explain the low apnoea and hypopnoea index in conjunction with the low arousal index found during SWS compared to NREM 1&2 and REM sleep, described in chapter 8.

Magdu Younes [376] proposed an alternative pathophysiological model for the restoration of the pharyngeal airway in OSAHS patients. Based on this model, the arousal threshold and the upper airway opening threshold are independent. The 2 thresholds may coincide in OSAHS patients or they may differ. When an arousal occurs first, for example during NREM 1&2 sleep, excitatory inputs are conveyed to the upper airway motoneurons which in turn restore pharyngeal patency. When pharyngeal opening occurs first, this may provide an additional arousal stimulus increasing the chance of an arousal occurring. By increasing the upper airway opening threshold, it is likely that arousal will occur first, whereas by increasing the arousal threshold, deeper sleep increases the probability of upper airway opening to occur first. In this model, autonomic excitation is considered as part of the graded arousal response, not capable of restoring pharyngeal patency.

This model has similarities to that proposed in figure 12.1. It furthermore provides a possible explanation for the mechanisms that terminate events not associated with arousals and raises further pathophysiological questions with potential therapeutic implications for future research. Of particular interest would be to investigate whether increase of the arousal threshold in a manner that would not significantly influence muscle tone may reduce arousal frequency and consequently stabilise respiratory effort and maintain pharyngeal patency and flow.

The hypothesis of lack of a causative association between apnoeas/hypopnoeas and arousals may support the previously observed poor association between symptoms of sleepiness and arousal frequency in OSAHS patients [188]. However, questions on the arousal pathogenesis, the termination of apnoeas and hypopnoeas as well as the pathogenetic mechanisms of sleepiness in OSAHS patients, remain partly unanswered. Early modelling studies confirmed the association between sleep fragmentation due to arousal and daytime sleepiness [327]. A number of studies looked into the clinical significance of arousal frequency on daytime symptoms in OSAHS patients and found poor correlation [61][188]. There is controversy as to what extent daytime deficits such as daytime sleepiness, cognitive decrements and decreased psychological well-being are due to sleep fragmentation caused by cortical arousals or by other factors which may

disrupt the sleep physiology. Previous studies [215][291] suggested that autonomic fluctuations may be associated with disruption of sleep physiology and cause sleepiness.

During the study in chapter 10, Fourier analysis was applied to estimate heart rate variability (HRV) of the RR interval tachogram and derive its spectral components in order to detect changes related to apnoeas and hypopnoeas as a non-invasive method of monitoring autonomic activity. Markers of sympathetic and parasympathetic tone derived from the spectral power of HRV is a well established technique [97][212], which has been applied to different patient groups including patients who suffered myocardial infarction to predict outcome [354].

It has been previously suggested that changes in Low Frequency (LF) power represent central tonic excitatory sympathetic inputs conditional to brainstem activation [142] and may reflect a common central control mechanism of respiratory and cardiac autonomic modulations, which may contribute towards the regulation and restoration of flow as part of the "arousal" cascade or model. In contrast, RR interval duration represents central phasic sympathetic excitation, whereas invasive monitoring of the Muscle Sympathetic Nerve Activity (MSNA) represents peripheral phasic sympathetic activation [262]. This may explain the dissociation found between RR spectral power, RR duration and MSNA under certain conditions such as apnoeas or hyperventilation [355]. It is therefore believed that the application of this method and the observed reduction in HRV (increased LF power) following the restoration of pharyngeal patency in the presence but also in the absence of cortical arousals accurately reflect changes in central sympathetic drive related to changes in minute ventilation and may therefore represent part of the non-cortical arousal mechanism aiming to restore flow.

The differences observed between healthy individuals and OSAHS patients during sleep in all spectral components of HRV, the LF, HF and TF power, represent a persistent non-physiological increase in nocturnal autonomic activity which may contribute towards the in chapter 5 described long-term effects of the syndrome in undiagnosed, untreated patients [213][239][254][272][273][276][375].

Due to the inherent limitations of the FFT analysis requiring stationarity of the signal, alternative algorithms may be used in future studies to ascertain quantitative differences as well as significant thresholds for the diagnosis of the syndrome.

A further important area of future research would be to determine the significance of the abnormalities observed in sympathetic activity in relation to the patients' clinical symptoms. A further aim would be to assess the importance of these novel physiological parameters in the development of the long term effects of the syndrome. The latter would reset the diagnostic markers of disease severity and the therapeutic requirements and may confirm and expedite the

integration of these novel severity markers into risk prediction models for primary and secondary prevention of cardiovascular disease.

The OSAHS remains an important public health problem because of its neurocognitive sequelae and the observed associations with cardiovascular disease. Future studies into the pathophysiology of the disease may identify the importance of apneas, hypopneas, arousals, and sleep deprivation in the initiation of cardiovascular disease. An added challenge will be to disentangle the influence of multiple co-morbid conditions that are often present in OSAHS patients. On the other hand, given the observed familial aggregation of the OSAHS [289], genetic studies may delineate whether common genetic causative associations between the OSAHS and obesity, insulin resistance and/or hypertension predispose patients to multiple comorbidities. Further clarification of the pathophysiological pathways may contribute towards the development of novel mechanism-based diagnostic modalities and treatment options that interrupt or delay progression of pathophysiologic processes related to obstructive sleep apnea. A number of mechanisms is implicated in the long-term effects of the OSAHS. Sleep fragmentation plays a major role in the excessive sleepiness that patients experience [370]. Repeated nocturnal hypoxia, in conjunction with simultaneous increases in intra-thoracic and transmural myocardial pressures, decreased contractility, impaired myocardial perfusion [45], increased sympathetic activity [251], depressed baroreflex sensitivity [250][265], fluctuations in blood pressure [75], accelerated inflammatory processes [90], increased production of free radicals [166], platelet activation and aggregation [41], endothelial dysfunction [165], are proposed mediating pathophysiological mechanisms which promote vascular remodelling [286], structural arterial damage and cardiovascular dysfunction. Simulation of these events in animals have identified damage to hippocampal neurons [302] and to neurons promoting wakefulness [356]. The former may contribute to the learning problems found in OSAHS patients [61], whereas the latter may be the basis of the residual sleepiness that is found even in well-treated patients with OSA. The above findings stress the importance of early diagnosis and treatment of the syndrome in order to prevent irreversible vascular and neuronal damage.

Sleep laboratories and in-lab polysomnography have limited diagnostic capacity and are expensive. Diagnostic modalities which can provide a timely, simplified and cost-effective diagnosis will enable early treatment and prevention of the long-term consequences of the syndrome. The currently available portable diagnostic devices and their diagnostic validity have been discussed in chapter 3. One of the aims within this thesis has been to validate a portable device for the home-based diagnosis of the OSAHS. The study has shown that in 52% of the patients studied, diagnosis could be reached on the basis of the home-based study, contributing

towards time and cost savings. An important contributor towards an accurate diagnosis with a certain class of devices is the pre-test probability of each individual tested to have the disease. In individuals with a high pre-test probability, diagnosis is usually achieved with devices with low diagnostic validity and sensitivity, such as pulse-oximeters. Patients' and partners' questionnaires [223] as well as subjective reporting during the consultation [370] are good guides for clinicians to assess the pre-test probability. The consensus on the best evidence-based diagnostic approach in certain groups of patients on the basis of the pre-test probability and the diagnostic validity of the devices, published by the Task Force on Portable Monitoring of the American Academy of Sleep Medicine [4] will guide clinicians to the appropriate diagnostic modalities and make current practice across centres more uniform and comparable against set standards.

A further objective of the present work has been to contribute towards the simplified diagnosis of the OSAHS, through the recognition of surrogate markers of the pathophysiological nocturnal changes in OSAHS patients and the application of novel techniques and algorithms.

The understanding of the pathophysiology of the OSAHS is the key component for the development of cost-effective diagnostic modalities with high diagnostic validity. Such devices will expedite the diagnostic process, make it accessible to people living in rural areas and enable prompt initiation of treatment, whereby the risks but also the costs associated with an undiagnosed sleep apnoea syndrome will be reduced.

Future research may facilitate the automated data analysis of high diagnostic validity which will enable the implementation of user-friendly (for patients and practitioners) home-based diagnostic systems and support decentralised models for the early diagnosis and treatment of the disorder.

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APPENDIX

EPWORTH SLEEPINESS SCALE

How likely are you to doze off or fall asleep, in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

0	=	would **never** doze
1	=	**slight** chance of dozing
2	=	**moderate** chance of dozing
3	=	**high** chance of dozing

Situation	Chance of Dozing
Sitting and reading	.....
Watching TV	.....
Sitting, inactive in a public place (eg a theatre or a meeting)	.....
As a passenger in a car for an hour without a break	.....
Lying down to rest in the afternoon when circumstances permit	.....
Sitting and talking to someone	.....
Sitting quietly after a lunch without alcohol	.....
In a car, while stopped for a few minutes in traffic	.....
TOTAL	==========

DECLARATION

I declare that I have been the principal investigator in all the studies presented in this thesis and that the contents as well as the composition of this thesis are my own work. The present thesis has not copied, not even in parts, previous scientific work and the entire literature used, is duly listed.

The work was performed at two major centres: the Sleep Laboratory of the University Hospital Charité in Berlin and the Scottish Sleep Centre at the Royal Infirmary in Edinburgh between August 1997 and September 2001.
Excluding a period of 5 months when I undertook clinical work, I worked full time as a clinical research fellow towards completion of the work presented in this thesis for 3 years and 9 months.

I have been assisted in many aspects of the studies by various people whose contributions have been noted in the acknowledgement section.

Kyra Dingli

CURRICULUM VITAE

Due to data protection, my Curriculum Vitae is not included in the electronic version of this thesis.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS RESULTING FROM THIS THESIS

PUBLICATIONS

1. Dingli K, Fietze I, Assimakopoulos T, Quispe-Bravo S, Witt C, Douglas NJ.
Arousability in sleep apnoea/hypopnoea syndrome patients.
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3. Dingli K**,** Coleman EL, Vennelle M, Finch SP, Wraith PK, MacKay TW, Douglas NJ.
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ABSTRACTS AND PRESENTATIONS

1. Dingli K, Vennelle M, Wraith PK, MacKay TW, Douglas NJ.
Home-based monitoring versus in-lab polysomnography in diagnosing the sleep apnoea/hypopnoea syndrome.
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3. Dingli K, Assimakopoulos T, Fietze I, Witt C, Douglas NJ.
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Article in Proceedings

Assimakopoulos T, Dingli K, Douglas NJ.
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