Detection and neuromuscular response to respiratory loading in obstructive sleep apnoea

Warren Ruehland
Orcid ID: 0000-0001-9626-7460

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Department of Medicine (Austin Health)
Faculty of Medicine, Dentistry and Health Sciences
University of Melbourne
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Abstract

Obstructive sleep apnoea (OSA) is characterised by the repetitive narrowing or collapse of the upper airway during sleep. While anatomy is considered important in OSA pathogenesis it has been recognised that other factors must be involved. This thesis aimed to examine whether (i) sensory detection, and (ii) upper airway neuromuscular compensation, were impaired in awake OSA patients, in response to small negative pressure respiratory loads, close to the conscious detection threshold. It was reasoned that this may be important in OSA pathogenesis, as failure to detect and respond to minor airway patency threat may lead to worsening collapse which is difficult to remedy.

Sensory detection was measured using the early P1 component of the respiratory related evoked potential (RREP). The RREP is the average cortical response to multiple presentations of the same respiratory stimulus and the P1 component is thought to represent arrival of somatosensory information at the cortex. The neuromuscular response was measured with genioglossus muscle intramuscular electromyogram (EMGgg). No significant differences were found between control and OSA participants in the threshold or the sensitivity of relationship between P1 amplitude and stimulus intensity. Also, there were no significant differences between control and OSA participants in the threshold or the sensitivity of the relationship between EMGgg amplitude and stimulus intensity.

These results do not support the concept that a neuromuscular response deficit or a sensory detection deficit contribute to OSA pathogenesis. However, the neuromuscular results were confounded by a counterintuitive genioglossus suppression observed in a significant proportion of participants. This response was not more commonly observed in OSA participants and may be related to the centrally mediated protective reflex inhibition seen in other inspiratory muscles in response to sudden onset negative pressure.
A methodological study examined the impact of using two abbreviated signal montages, typically used in portable polysomnography (PSG) during OSA diagnosis, on sleep and cortical arousal scoring, compared to a standard reference montage. The results demonstrated that abbreviated signal montages may result in underestimation of the arousal index and poorer precision in sleep scoring, with potential consequences for OSA diagnosis. The results guided thesis methodology and have implications for future PSG standards.

A further experiment aimed to improve understanding of respiratory stimuli sensory detection. From prior sensory detection studies it has been hypothesised that there is a subcortical gating mechanism, preventing arrival of afferent information at the cortex. This concept was examined by comparing RREPs produced by consciously detected vs. undetected loads, near the detection threshold. Results obtained from ten healthy males suggested that load-related sensory information may reach the somatosensory cortex for sub-threshold loads. The findings argue against subcortical gating of somatosensory information.
Declaration

This is to certify that:

- This thesis comprises only my original work towards the Doctor of Philosophy, except where indicated in the preface.
- Due acknowledgement has been made in the text to all other material used.
- This thesis is fewer than the 100,000 words in length, exclusive of tables, references and appendices.

Signed:

[Signature]

Warren Ruehland
Preface

This thesis primarily aimed to examine whether (i) sensory detection, and (ii) upper airway neuromuscular compensation, were impaired in awake obstructive sleep apnoea (OSA) patients, in response to small negative pressure inspiratory loads close to the conscious detection threshold.

Experiment 1 (Chapter 2) is an experiment designed to answer a question that arose in the lead up to conducting the key experiments relating to the methodology used to diagnose OSA. This experiment has been recently published in a peer-reviewed scientific journal [1] and a copy of this publication is included as part of chapter 2. While this work was carried out in collaboration with other authors, with contributions outlined in more detail below, all the authors agreed that the primary author (PhD candidate) contributed greater than 50% of the work involved in the publication. It should be noted that polysomnography conducted for this experiment was recorded and scored prior to commencement of the PhD project; however data collation and analysis, and manuscript preparation occurred following project commencement. Additionally, results were used to guide subsequent PhD project procedures.

Experiment 2 (Chapter 6) seized on an opportunity recognised to improve understanding of the sensory detection of respiratory stimuli. Experiment 3 (Chapter 7) and Experiment 4 (Chapter 8) address the key thesis aims. At the time of writing Experiment 3 has been submitted for publication, and Experiments 2 (Chapter 6) and 4 (Chapter 8) are yet to be submitted for publication. The remaining chapters (1, 3, 4, 5 and 9) are supporting chapters for the four experimental chapters.

While not all experiment chapters have been submitted for publication, they are all prepared in a style suitable for publication. As a result: (i) each experimental chapter contains an abstract summarising the experiment, (ii) the experimental...
chapters generally do not refer to supporting chapters, and therefore some of the material from the supporting chapters is repeated in summarised form, and (iii) abbreviations are defined in full at first use in experimental chapters even if they have previously been defined in the thesis. The only other published data arising from this thesis are published abstracts, as outlined in the following ‘Publications, presentations and awards’ section.

Funding
The work carried out for this thesis was supported by research grants from the National Health and Medical Research Council of Australia (grant numbers 430300 and 430302), the Austin Medical Research Foundation, the Jack Brockhoff Foundation and the Institute for Breathing and Sleep. Medical equipment was supplied by ResMed Australia, Philips Respironics and Fisher and Paykel Healthcare.

Contributions
All work for this thesis was conducted by me under the supervision of Dr F. O'Donoghue, Mr P. Rochford, Prof. Rob Pierce, and Prof. John Trinder with additional contributions as outlined below:

A significant contribution to study design of all experiments was provided by me, Fergal O'Donoghue and Peter Rochford. In addition Thomas Churchward, Tristia Lakey and Linda Schachter contributed to study design of experiment 1, Kate Webster contributed to study design of experiment 3, and Rob Pierce contributed to study design of experiments 1, 3 and 4. Ethics submissions were conducted by me with a significant contribution from Paul Read. Establishment of a laboratory and equipment suitable for collection of experimental data was conducted by me with a significant contribution from Peter Rochford and additional support from Brad Hayes, Paul Read and Rebecca Bailey. Computer programming to automate stimulus delivery, data collection, data reduction and data analysis was conducted by me with a significant contribution from Peter Rochford and additional support
from Rebecca Bailey. Collection of pilot data was conducted by me with support from Melanie Anson. Recruitment of participants was conducted by me with support from Allison Collins. Respiratory function tests and polysomnography (PSG), including PSG scoring were conducted by me with the following exceptions: PSG for OSA participants was conducted in a clinical setting in the sleep laboratory at Austin Health and PSGs for experiment 1 were additionally scored by Thomas Churchward, Tristia Lakey and Natalie Tarquinio. Data collection and analysis were conducted by me with data analysis support from Jo Spong. Participant follow-up was conducted by me with support from Julie Tolson. Additionally, Laura Gainche provided assistance with establishment of ultrasound measurements of the upper airway musculature and Steve Vander Hoorn provided assistance with statistical analysis. No third party editorial assistance was provided in preparation of the thesis.
Publications, presentations and awards

The following are publications, presentations and awards that have arisen from work conducted for this thesis:

Publications


Published abstracts


Conference presentations


Awards

Shortlisted for New Investigator Award at 2013 ASA/ASTA ASM, Brisbane, for abstract entitled: Cortical response to threshold resistive loads in severe obstructive sleep apnoea.

Awarded second prize in scientific section for oral presentation at 2011 TSANZ Victorian Branch ASM entitled: Genioglossus EMG response to threshold upper airway loading.

Shortlisted for New Investigator Award at 2011 ASA/ASTA ASM, Sydney, for abstract entitled: Genioglossus EMG response to threshold upper airway loading.

Awarded third prize in scientific section for oral presentation at 2010 TSANZ Victorian Branch ASM entitled: Intact cortical response to a respiratory stimulus in untreated severe obstructive sleep apnoea.
Acknowledgments

I would firstly like to thank my supervisors, Dr Fergal O’Donoghue, Mr Peter Rochford and Professor Rob Pierce for providing me the opportunity to carry out this research, and for their significant input into project conception and design. I would like to thank Fergal and Peter in particular, for their mentorship and thoughtful advice and input throughout my candidature. They have helped me to develop into a more well-rounded researcher, with a stronger understanding of research and its foibles, how to work independently and have trust and confidence in myself and my ability. Unfortunately Rob passed away early in my candidature. He was a great loss to this project; however he continues to be an inspiration to those that knew him. I have fond memories from just before his death of support and encouragement for me as a relatively new researcher, of his input to my first ever publication, and in acknowledging its importance despite his extensive experience and publication record. I would like to thank Professor John Trinder, who stepped in as co-supervisor after the loss of Professor Pierce, for his significant and insightful input into the project and careful review of the work.

I would also like to thank Dr Kate Webster for her expertise and input in establishing the techniques and study design for experiments utilising respiratory related evoked potentials. I would additionally like to thank Dr Linda Schachter, Ms Tristia Lakey, and Mr Tom Churchward for their input into study design of experiment 1.

I am grateful to the following individuals who provided assistance during the project: Melanie Anson for assistance with equipment development and pilot testing; Julie Tolson for follow-up of participants; Allison Collins for assistance with recruitment; Laura Gainche for assistance with ultrasound measurements; Jo Spong for assistance with analysis of data and manuscript review of experiment 2; Thomas Churchward, Tristia Lakey and Natalie Tarquinio for scoring of PSGs for experiment 1; and Steve Vander Hoorn for statistical analysis. Thanks also to
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I would also like to thank the study participants who agreed to sit through a lengthy, monotonous and sometimes uncomfortable protocol, largely only for the benefit of science.

A big thank you to Rachel Schembri and Allie Peters for the valuable camaraderie, support and the study sessions that gave me a kick-start I needed for thesis completion.

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List of abbreviations

2PD Two-point discrimination
AASM American Academy of Sleep Medicine
AHI Apnoea hypopnoea index
BMI Body mass index
CAD Coronary artery disease
CHF Congestive heart failure
CI Confidence intervals
CPAP Continuous positive airway pressure
EDS Excessive daytime sleepiness
EEG Electroencephalogram
EMG Electromyogram
EMGgg Genioglossus electromyogram
EOG Electrooculogram
FER Forced expiratory ratio
FEV1 Forced expiratory volume in 1 second
FRC Functional residual capacity
FVC Forced vital capacity
LMM Linear mixed model
MNE Minimum-norm estimate
MRI Magnetic resonance imaging
NPV Negative pressure ventilation
OSA Obstructive sleep apnoea
$P_{0.1}$ Pressure change at 0.1 seconds post stimulus
$P_{CO_2}$ Partial pressure of carbon dioxide
$P_{crit}$ Critical closing pressure
$P_{epi}$ Epiglottic pressure
$P_{epi_0}$ Background epiglottic pressure
$P_{ETCO_2}$ End-tidal CO$_2$
PM  Portable monitoring  
$P_{\text{mask}}$  Mask pressure  
$P_{\text{mask}}_0$  Background mask pressure  
$P_{O_2}$  Partial pressure of oxygen  
PPV  Positive pressure ventilation  
PSG  Polysomnography  
$R_0$  Background resistance  
$R_{\text{aw}}$  Airway resistance  
REM  Rapid eye movement  
RIP  Respiratory inductive plethysmography  
RREP  Respiratory related evoked potential  
RV  Residual volume  
SD  Standard deviation  
SDB  Sleep disordered breathing  
SHHS  Sleep Heart Health Study  
$\text{SpO}_2$  Blood oxygen saturation measured using pulse oximetry  
TLC  Total lung capacity  
TST  Total sleep time  
TTL  Transistor-transistor logic  
UARS  Upper airway resistance syndrome  
VT  Vibration detection threshold  
WSCS  Wisconsin Sleep Cohort Study  
$\Delta P_{\text{epi}}$  Epiglottic pressure change  
$\Delta P_{\text{mask}}$  Mask pressure change  
$\Delta R$  Resistance change (added resistance)  
$\Delta R_{50}$  Resistive load detection threshold (The added resistive load that can be detected on 50% of occasions)
1. Introduction - obstructive sleep apnoea

The overarching goal of this thesis is to improve understanding of obstructive sleep apnoea (OSA) pathogenesis. This introductory chapter describes OSA, including the methodology used in its diagnosis. The importance of exploring pathogenesis of OSA is highlighted by outlining its consequences, prevalence, risk factors, co-morbidities and costs. Finally, the chapter describes what is currently known about OSA pathogenesis, before focussing on the key targets of the current research.

1.1. Description

OSA is a respiratory sleep disorder characterised by the repetitive narrowing or collapse of the pharyngeal (upper) airway during sleep, resulting in reduction (hypopnoea) or cessation (apnoea) of airflow, despite ongoing breathing effort. These breathing disturbances are often associated with snoring, intermittent oxygen desaturation, brief cortical arousals from sleep and surges in sympathetic activation; ultimately resulting in a cyclical breathing pattern and fragmented sleep [2, 3].

The primary symptoms reported by OSA patients are excessive daytime sleepiness (EDS) and snoring but symptoms may also include unrefreshed sleep, witnessed apnoeas, mood changes and depression, and attentional dysfunction [4, 5]. However, the symptoms may not be linked with OSA by sufferers, as snoring and apnoeas are often not self-recognised [6].

1.2. Diagnosis

1.2.1. In-laboratory polysomnography

In-laboratory polysomnography (PSG) is considered the standard method for diagnosis of OSA [7, 8]. PSG involves the simultaneous monitoring of numerous physiological variables during sleep, allowing for the quantification of respiratory disturbance and its influence on sleep quality, sleep disturbance and blood gas disturbance [9]. The signals typically recorded during PSG to quantify sleep and sleep quality include the electroencephalogram (EEG), electrooculogram
(EOG), and skeletal muscle electromyogram (EMG) (Figure 1-1). Respiratory disturbance is generally documented using techniques that estimate respiratory flow and respiratory movements. Respiratory flow is typically estimated using a thermistor to monitor temperature change at the nose and mouth and nasal prongs to monitor nasal pressure, whereas respiratory movements are typically estimated using respiratory inductive plethysmography (RIP), with bands placed around the thoracic and abdominal walls. Other parameters often monitored during PSG include sound, for monitoring of breathing sounds and snoring, pulse oximetry for monitoring blood oxygenation ($\text{SpO}_2$), body position, electrocardiography and digital video.

![Figure 1-1: Example of some of the signals collected during polysomnography. Signal from top to bottom include: central EEG, blood oxygen saturation measured using pulse oximetry ($\text{SpO}_2$), respiratory flow estimated using nasal pressure, and thoracic and abdominal movement measured using respiratory inductance plethysmography (RIP). Cortical arousals are marked in green on the EEG trace.](image)

Quantification of respiratory disturbance in OSA, in both clinical and research settings, is largely based on the apnoea hypopnoea index (AHI), which is a summary statistic derived from the analysis of PSG data. The AHI is the average number of apnoeas and hypopnoeas per hour of sleep [4]; in the most severe cases the AHI can be greater than 100 events per hour.
In 2007 the American Academy of Sleep Medicine (AASM) published the Manual for the Scoring of Sleep and Associated Events in an attempt to standardise the recording and analysis of PSG in a single document [9]. Recommendations for technical specifications, recording techniques, as well as guidelines for the analysis and quantification of sleep quality, sleep disturbance and respiratory disturbance were included, and of note, these standards were recommended for adoption in Australia in 2010 [10], which was after the commencement of the research conducted in this thesis. Additionally, there were further minor updates to standards in 2012 [11] and 2016 [12]. As such, recording and PSG quantification techniques used in the current research was based on previous standards [13-15]. One of the most significant changes in the standards adopted in Australia in 2010 [9], compared to previous standards used in thesis research [13], was in the hypopnoea definition. In effect the hypopnoea scoring criteria used in the current research was more liberal compared to criteria used in newer standards, as well criteria used in prior epidemiological studies [5], allowing for the scoring of more hypopnoeas. The impact of using different definitions was reported in an important study comparing PSG scoring and OSA diagnosis in over 300 patients investigated for obstructive sleep apnoea [16]. The most significant observation from that study in terms of the research conducted for this thesis, was that an AHI cut-off of 15 events per hour, using the criteria utilised in this thesis, was approximately equivalent to 5 per hour using criteria similar to that used to define sleep disordered breathing in large epidemiological studies that have established links between OSA and co-morbidities [5]. For this reason, control subjects included in thesis experiments were required to have an AHI of less than 15 per hour. This 15 per hour cut-off was roughly equivalent to 10 per hour using criteria recommended in Australia in 2010, which is very similar to criteria recommended in current standards [11, 12]. The AHI cut-off 30 per hour, which was selected for inclusion of severe OSA for thesis research, was reported to be roughly equivalent to an AHI of 11 per hour using criteria used in prior epidemiological studies and 18 per hour using criteria in 2010/2016 recommendations. While these comparisons have no impact on the outcome of
the current research, they do facilitate comparisons to research that have used differing hypopnoea scoring criteria.

1.2.2. Portable monitoring
Portable monitoring (PM) is an alternative to PSG that utilises devices that are able to be used in non-laboratory settings, including the home. PM devices have traditionally been divided into 3 different categories [8] which are compared to a full, in-laboratory, attended PSG (a Type 1 device). A Type 2 device can record the same signals as a Type 1 device but can be used unattended outside the sleep laboratory. A Type 3 device typically measures respiratory and cardiac variables, as well as pulse oximetry, but not fast frequency signals required for recording of sleep and sleep disturbance, such as EEG, EMG and EOG. A Type 4 device typically measures a smaller number of variables such as pulse oximetry and airflow.

In general, PM has some theoretical advantages over in-laboratory PSG including convenience and reduced cost [8]. It may be more convenient in that it can be performed in the patient’s usual sleeping environment or on a hospital ward. Sleeping in the usual sleeping environment may be more comfortable for some individuals compared to sleeping in a hospital or laboratory environment, potentially leading to better quality sleep. Most PM devices are less costly than in-laboratory devices. There are also likely to be reduced costs as specialised facilities are not required. Additionally, PM is less resource-intensive, with reduced staffing requirements. However, there are also limitations of PM that must be considered [8]. When the test is conducted in an uncontrolled environment there is little or no knowledge about concurrent events that may disturb sleep, and as the test is unmonitored there are increased chances of sensor detachment and device malfunction, which can reduce the quality of recordings. Additionally, the reduced recording capabilities of PM devices could have consequences for analysis and interpretation of the recorded data.

In the past PM was generally not considered a valid alternative to in-laboratory PSG for OSA diagnosis [17-19]. However, more recently, PM has been
recognised as an acceptable method for OSA diagnosis [8, 20, 21], with
acknowledgement and understanding of its accompanying limitations. In 2007
the AASM published the clinical guidelines for the use of PM in the diagnosis of
OSA in adult patients [8]. The authors noted that PM devices have only been
shown to have good specificity and sensitivity in populations evaluated by sleep
specialists, considered to be at high risk for OSA, and without significant co-
morbid medical or sleep disorders. As such, the guidelines recommend that PM
may be used to rule-in OSA in adults who have a high pre-test probability of
moderate to severe disease. The guidelines recommended against the use of
PM for the diagnosis of OSA in patients with significant co-morbid medical
conditions or co-morbid sleep disorders. They also noted that PM is not
appropriate for general screening of asymptomatic populations that have not
been evaluated by a sleep specialist, in view of the higher false-negative liability
[8, 22, 23].

However these recommendations were largely based on Type 3 devices that
are essentially a cardiopulmonary recorder and unable to record fast frequency
channels. As previously mentioned, fast frequency channels allow the recording
of signals such as EEG, EMG and EOG, and therefore, the measurement and
quantification of sleep and cortical arousals. Type 3 devices are inherently less
sensitive than in-laboratory PSG as: (i) subtle respiratory events such as
respiratory effort related arousals and hypopnoeas requiring accompanying
cortical arousal cannot be scored due to the lack of ability to detect cortical
arousals; (ii) the denominator for respiratory indices is total recording time
rather than total sleep time (TST), usually resulting in a lower AHI, to the extent
that lack of respiratory disturbance may reflect lack of sleep rather than
absence of OSA; (iii) many Type 3 devices do not record position, so that
position dependent OSA may be missed; and (iv) the lack of sleep recordings
means that rapid eye movement (REM) sleep dependent OSA may be missed.

Despite the potential advantages of Type 2 devices that are capable of
recording sleep and cortical arousals, they were not specifically considered in
the most recent guidelines [8]. This was because there were no new data
available comparing such devices to PSG since previous guidelines [24], which stated that evidence was lacking to recommend their clinical use.

Chapter 2 of this thesis partly addresses the lack of evidence regarding Type 2 PM devices. It is an methodological chapter that considers how the use of a restricted Type 2 device, with the ability to measure sleep and arousals, but with a smaller number of channels than in-laboratory PSG, would impact on the analysis of the recording. This information is important to help determine if such a device is suitable as a screening tool for OSA.

1.3. Prevalence
OSA is considered common, with 4% of men and 2% of women estimated to have OSA in a North American population, where OSA was defined as an AHI greater than 5·h\(^{-1}\) plus symptoms of EDS [5]. These figures are from the Wisconsin Sleep Cohort Study (WSCS) and date from the early 1990’s. More recent data from the same population published in 2013 has estimated that 14% of men and 5% of women have an AHI greater than 5·h\(^{-1}\) plus EDS [25]. This increase in prevalence is expected given the increasing prevalence of obesity [26] which is a key risk factor for OSA [27]. It has also been suggested that because future increases in obesity prevalence are expected, increases in OSA prevalence are also likely to occur [28].

With regard to the Australian context, the Sleep Health Foundation (www.sleephealthfoundation.org.au) commissioned a survey of a large representative sample of Australians in 2010 (n=1512), which contained questions relating to sleep habits, sleep disorders (including snoring and OSA), as well as daytime impairments associated with sleep disturbance. From this survey, OSA prevalence was estimated at approximately 5% of the population [29], which is broadly consistent with the earliest North American prevalence estimates. In the Australian survey, the prevalence of OSA was derived by determining the proportion of respondents who snored loudly at least a few times a week, plus had breathing pauses observed during sleep at least a few
times a month. The prevalence was greater in males (6.4%) than females (3.6%), which is also in line with epidemiological studies [5, 25].

1.4. Risk factors
The main risk factors for OSA include obesity, male gender, age, craniofacial or upper airway structure, and genetics. These risk factors will be outlined in further detail below.

1.4.1. Obesity
Obesity is recognised as a key risk factor for OSA [5, 27, 30-32]. Using data from the WSCS, Young and colleagues [5] reported that a one standard deviation (SD) increase in body mass index (BMI) was associated with an approximate four-fold increased risk of having sleep disordered breathing (SDB), defined as having an AHI > 5·h⁻¹. In that study nasal airflow was estimated using a thermocouple and oral airflow using an end-tidal carbon dioxide ($P_{ET}CO_2$) gauge; an apnoea was defined as complete cessation of airflow lasting ≥ 10 seconds and a hypopnoea was defined as a discernible reduction in airflow accompanied by a ≥ 4% oxyhaemoglobin desaturation. In a longitudinal analysis of the same cohort a 10% increase in body weight predicted a 32% (95% confidence intervals [CI], 20-45%) increase in the severity of OSA, a 10% weight loss predicted a 26% (95% CI, 18-34%) reduction in OSA severity, and a 10% weight gain in those initially free of OSA, predicted a 6-fold (95% CI, 2.2-17.0) increase in the odds of developing moderate-to-severe SDB. In particular, central obesity with fat deposition in the neck and abdomen is considered a more important risk factor for OSA than peripheral obesity with fat deposition in the hips and legs [4, 32, 33].

Although the specific physiological mechanisms are uncertain, a number of mechanisms have been suggested by which obesity may contribute to OSA pathogenesis [30, 33] including: increased pharyngeal airway collapsibility due to fat deposition around the neck, reduced upper airway tethering effects caused by reduced lung volumes related to abdominal fat deposition, and functional impairment of upper airway muscles.
Since OSA and obesity share a number of co-morbidities and clinical associations, it is generally acknowledged that when studying OSA it is important to consider and account for obesity. If obesity is not accounted for, clinical associations seen in obese OSA subjects could be related to increased BMI rather than OSA [4].

1.4.2. Male gender
Male gender is considered a strong risk factor for OSA [4, 27, 30, 33]. In the earliest population-based epidemiological studies men were approximately three times as likely as women to have SDB [5]. It has been suggested that the preponderance of central obesity in males compared to peripheral obesity in females may account for the male predominance of OSA [4, 33]. Other factors suggested to play a role [30] include: differences in fat deposition around the pharyngeal airway, differences in pharyngeal length, and differences in ventilatory response to arousal from sleep. However, it has also been noted that a reporting bias may exist, with men more likely to report snoring, snorting, gasping and sleepiness, and women more likely to report unrefreshing sleep, fatigue, insomnia, and depression [34]. Suggested reasons for these differences include disparity in astuteness of bed partners as well as disparity in perceived social acceptance of symptoms.

1.4.3. Craniofacial structure
It has been recognised that craniofacial and upper airway structure may be important risk factors in OSA occurrence [2, 4, 27, 35-37]. Craniofacial structures are discussed in more detail in section 1.7 relating to pathophysiology of OSA, however, features more commonly associated with OSA include: shorter maxillary (upper jaw) length, maxillary constriction (narrower and more tapered maxillary arch), smaller mandibular area (area enclosed by the mandible ramus), greater mandibular retroposition, inferior displacement of the hyoid bone (increased mandibular plane to hyoid distance) and smaller cranial base [37].
1.4.4. Genetics
A positive family history of OSA has been shown to increase the risk of SDB [38-40]. Although the genetic predisposition for obesity is recognised as a factor that may be important in the inheritance of OSA [4], it is thought that other factors are also involved. Redline et al. [38] reported that SDB was more prevalent in the relatives of those diagnosed with SDB (21%) compared to control subjects (12%). After adjusting for obesity as well as age, gender and race, they reported odds of SDB that were 1.3 times greater in individuals with one family member with SDB, and 2.3 times greater in subjects with three family members with SDB, when compared with subjects without SDB family members. In another study Mathur et al. [39] reported that relatives of OSA patients, that were restricted to a BMI of <30 kg·m⁻², had a median AHI that was approximately four times greater than a control group matched for age, gender, height and weight. In that study differences were related to anatomical and craniofacial features; relatives of those with SDB having narrower upper airways with greater maxillae and mandible retroposition, as well as longer soft palates with wider uvulae. There is also evidence of a genetic basis in OSA for ventilatory control abnormalities [41]; in particular blunting of the hypoxic ventilatory response and impairments in respiratory responses to resistive loading during sleep [42].

1.4.5. Age
One of the earliest epidemiological studies [5], reported increasing prevalence of SDB in males from the 30-39 age group to the 40-49 age group, without further increase in the 50-59 age group. As the study failed to show a continuous increase in the prevalence of SDB with older age it was suggested that age is not a strong risk factor for SDB for middle-aged adults. Similarly, in a population sample of 741 men Bixler et al. [43] reported a maximum prevalence of OSA in those aged 45-60. The increase into middle-age is thought to relate to both anatomical and physiological changes that occur with age; in particular due to preferential pharyngeal region fat deposition, age-related lengthening of the upper airway, increased arousal from sleep leading to respiratory control instability, deterioration of the upper airway neuromuscular response, and
increased prevalence of co-morbidities; for example the apolipoprotein E gene is a common risk factor for Alzheimer’s disease and is associated with an increased risk of SDB. [30, 44]. The lack of increase into older age may be related to a larger calibre pharyngeal airway observed [45]. Using magnetic resonance imaging (MRI) and acoustic reflection Carlisle et al. [45] reported greater airway calibre in a group aged over 60 years compared to a group aged less than 40 years, despite other observations that would be expected to decreased airway calibre such as soft tissue enlargement. In that study groups were well matched for BMI, neck circumference, and daytime sleepiness, there were no differences in craniofacial features that would explain the results, and both groups were screened to exclude OSA. The authors speculated that the increased calibre may have been due to neuromuscular compensation. It has also been suggested that the lack of increase into older age may due to survivor effects [30].

1.4.6. Other
In addition to the major factors already discussed, other risk factors that have been described include alcohol use, sedative use, sleep deprivation, supine posture, nasal congestion/obstruction, mouth breathing, REM sleep, ethnicity, and disorders with craniofacial abnormalities such as Down syndrome [4, 30]. In terms of ethnicity, it has been noted that prevalence of OSA across different ethnicities is similar despite differences in craniofacial features and obesity across populations. This suggests that the different underlying risk factors prevalent in each ethnic group, result in similar overall risk of OSA [37]. By way of example, when comparing Chinese to Caucasian OSA patients who were matched for OSA severity and height, Lee et al. [46] reported that Chinese OSA patients have a smaller bony enclosure surrounding the airway, whereas Caucasian OSA patients showed increased obesity.

1.5. Co-morbidities
OSA is associated with a number of adverse outcomes including daytime somnolence [5], impaired cognition [47], reduced quality of life [48], increased motor vehicle accident risk [49], metabolic impairment [50, 51], cardiovascular
disease [52, 53] and all-cause mortality [54-56]. In terms of cardiovascular disease, the evidence is particularly strong for an independent association with hypertension [57-59]. Co-morbidities associated with OSA are outlined in further detail below.

1.5.1. Cardiovascular disease

1.5.1.1. Hypertension

Two large population based cross-sectional studies have shown strong associations with OSA severity and hypertension, after adjustment for known confounders such as BMI [60, 61]. Additionally, a longitudinal analysis from the WSCS, reported an odds ratio for prevalent hypertension of 2.03 (95% CI: 1.29–3.17) for mild OSA and 2.89 (1.46–5.64) for moderate or worse OSA, after adjustment for known confounders [53]. A meta-analysis of continuous positive airway pressure (CPAP) usage, which is considered the principal treatment for OSA [2], demonstrated a small, but significant, decrease in mean 24-h blood pressure, with larger decreases observed in those with more severe OSA and in those with increased CPAP usage [62]. That analysis estimated that CPAP use would decrease 24-hour ambulatory mean blood pressure by 0.89 mmHg for each increase in pre-treatment apnoea-hypopnoea index of 10·h⁻¹, and by 1.39 mmHg for each 1-hour increase in CPAP usage. A more recent meta-analysis of randomised controlled trials examining the impact of CPAP on blood pressure in patients with OSA and resistant hypertension, reported that CPAP resulted in an overall reduction in 24-h ambulatory systolic blood pressure of 4.78 mmHg (95% CI: 1.61, 7.95) and a reduction in diastolic pressure of 2.95 (95% CI: 0.53, 5.37) [63]. Another meta-analysis also reported a small but significant reduction in pulmonary hypertension [64].

1.5.1.2. Stroke

Cross-sectional studies have also found an independent association between OSA and stroke. Shahar and colleagues [65] reported findings from the Sleep Heart Health Study (SHHS), a large population based study, revealing an odds ratio for self-reported stroke of 1.58 (95% CI: 1.02, 2.46) for an AHI in the upper quartile compared to the lower quartile, after adjustment for confounders.
Cross-sectional analysis from the WSCS also revealed an increased risk of stroke in those with OSA, with an adjusted odds ratio of 4.33 (95% CI: 1.32, 14.24) for an AHI of > 20·h⁻¹, compared to an AHI < 5·h⁻¹ [66]. In the same cohort, longitudinal analysis revealed an elevated risk of suffering a first-ever stroke over a four year period in those with OSA, although the risk was not significant after adjustment for age, gender and BMI. Subsequent longitudinal analysis of the SHHS data revealed OSA significantly increased the incidence of ischaemic stroke in men but not women; men in the highest quartile of obstructive AHI had an adjusted hazard ratio of 2.86 (95% CI: 1.1, 7.4), compared to the lowest quartile [67]. Additionally, for men with moderate OSA (obstructive AHI 5-25·h⁻¹), each one-unit increase in obstructive AHI was associated with a 6% (95% CI: 2–10%) increase in stroke risk. The non-significant finding in the WSCS was thought to relate to lack of statistical power [67], due to the low number of incident strokes. In a meta-analysis, OSA was significantly associated with incident stroke with an odds ratio of 2.24 (95% CI, 1.57–3.19). That analysis also identified a significant dose response relationship; a 10-unit increase in AHI was associated with a relative increase of 36% (95% CI, 26–43%) in the odds of stroke or cardiac death [58]. Similarly, another meta-analysis incorporating 12 prospective cohort studies including a total of 25,760 subjects, also reported a relative risk of incident fatal and non-fatal stroke for those with severe OSA compared with no sleep apnoea of 2.15 (95% CI, 1.42-3.24) [68]. Despite these findings there is conflicting evidence as to whether treatment of OSA with CPAP can reduce the incidence of stroke [69, 70]. A systematic review and meta-analysis reported a lower incidence of stroke after OSA treatment with CPAP of 0.27 (95% CI, 0.14–0.53) based on meta-analysis of cohort studies, which was not reproduced with a randomised controlled trial or studies using administrative data [70]. Additionally, a recent international, multicenter, randomised controlled trial conducted on over 2000 OSA patients with established cardiovascular disease, found that CPAP did not reduce the risk of cardiovascular events, including stroke [71]. However, there was lower risk of stroke when a subset of the CPAP group that were adherent to CPAP, were compared to a matched control group.
1.5.1.3. **Coronary artery disease**

Evidence for an association between OSA and coronary artery disease (CAD), including angina and myocardial infarction, is not as strong as the association with hypertension or stroke. Cross-sectional analysis of the SHHS reported a non-significant odds ratio for self-reported coronary artery disease of 1.27 (95% CI: 0.99–1.62) when comparing the upper to the lower quartile of AHI, after adjustment for confounders [65]. Similarly, using longitudinal analysis, significant associations between AHI with CAD in men after adjustments for age, race, BMI, and smoking status, became non-significant after further adjustments for baseline diabetes mellitus and lipid measures, and were further diminished by adjustment for blood pressure and antihypertensive medication use [72]. After all adjustments the odds for CAD in men were 1.33 (95% CI: 0.91, 1.95) when comparing those with an AHI $\geq 30\cdot h^{-1}$ to those with an AHI $< 5\cdot h^{-1}$. After initial adjustments for age, race, BMI, and smoking status there was no significant association in women. These results are consistent with a meta-analysis that showed that when considering studies that predominately recruited men there was a significant association between OSA and CAD, but when all studies were included, and therefore both genders, the association was non-significant [58].

1.5.1.4. **Congestive heart failure**

In the SHHS cross sectional analysis [65], associations between OSA and self-reported congestive heart failure (CHF) were stronger than associations between OSA and self-reported CAD. Compared to subjects in the lowest quartile of AHI, subjects in the highest quartile of AHI had an adjusted odds ratio of 2.38 (95%CI: 1.22, 4.62) for the self-reported diagnosis of CHF. Longitudinal analysis in the same cohort showed that of those without cardiovascular disease at baseline, there was a strong and significant association of AHI with incident heart failure in men (but not in women) after adjustments for age, race, smoking, and BMI. This association was still significant after accounting for other confounders such as diabetes mellitus and blood pressure, with an adjusted odds ratio of 1.13 (95% CI: 1.02 to 1.26) per 10-unit increase in AHI in men [72].
1.5.1.5. Arrhythmia

A number of cross-sectional and longitudinal observational studies have shown increased prevalence of cardiac arrhythmias in patients with OSA (see reviews by Rossi et al. [73] and Lavergne et al. [74]).

As an example, a SHHS study compared the prevalence of arrhythmias in 228 subjects with SDB (defined as having a respiratory disturbance index (RDI) ≥30) with that of 338 patients without SDB (RDI <5) [75]. After adjustment for age, gender, BMI and prevalent coronary heart disease, compared to those without SDB, those with SDB had approximately 4-times the odds for atrial fibrillation (odds ratio 4.02; 95% CI, 1.03-15.74), 3-times the odds for non-sustained ventricular tachycardia (3.40; 95% CI: 1.03-11.20), and almost twice the odds for complex ventricular ectopy (1.74; 95% CI: 1.11-2.74) [75].

More recently, cross-sectional analysis of 697 veterans with suspected SDB found that subjects with moderate-to-severe SDB (AHI > 15) had approximately twice the odds of having any nocturnal cardiac arrhythmias (2.24; 1.48-3.39; p = 0.004) compared to those without SDB (AHI < 5), after adjusting for age, BMI, gender, and cardiovascular diseases. Also, increased rates of obstructive respiratory events and hypoxia were associated with increased arrhythmia risk [76].

In a recent Australian longitudinal study [77] the relationship between OSA and atrial fibrillation (AF) was examined in nearly 7000 middle-aged adults with suspected OSA, over a median follow-up period of almost 12 years. That study reported that an AHI >5 was an independent predictor of incident AF after multivariate adjustment (hazard ratio 1.55; 95% CI: 1.21-2.00).

There is some suggestion that OSA treatment with CPAP may reduce cardiac arrhythmias [73, 74], however it has been noted that more randomised controlled trials are required in this area [73, 74, 77, 78].
Although the exact mechanisms are not certain, there are a number of possible mechanisms by which OSA can result in development of cardiac arrhythmias [73]. These include; (i) intrathoracic pressure swings and associated increased transmural gradients, which may lead to structural remodelling of cardiac walls, (ii) sympathetic activation related to repeated cortical arousals, intermittent hypoxia, and/or intrathoracic pressure swings which is thought to induce electrical and structural remodelling, and, (iii) intermittent hypoxia may also lead to oxidative stress and subsequent electrical remodelling.

1.5.1.6. Importance of oxygen desaturation
While there a many possible mechanisms that may lead to increased risk of cardiovascular disease in OSA [79], there is some suggestion that oxygen desaturation during respiratory events may be important. Cross-sectional analysis from the SHHS noted that hypopnoeas accompanied by oxyhaemoglobin desaturation of ≥4% were associated with cardiovascular disease after accounting for confounding variables, however no association was observed with hypopnoeas associated with lesser desaturation or arousals [52]. In that study cardiovascular disease was determined via questionnaire and included angina, history of heart failure, a previous myocardial infarction, or stroke, as well as a history of bypass surgery or coronary angioplasty. As mentioned above, intermittent hypoxia has also been proposed as a possible mechanism leading to cardiac arrhythmias [73]. Hypoxia is thought to result in arrhythmia by two possible pathways: (i) by inducing sympathetic activation which is thought to induce electrical remodelling and electrical instability in the myocardium, and (ii) by increasing oxidative stress which would also induce electrical remodelling [73]. A recent longitudinal study [77] also reported that time with oxygen saturation <90% was a stronger predictor of AF than was continuous AHI, after a median follow-up of approximately 12 years, suggesting that oxygen desaturation may be an important intermediary in the relationship between OSA and AF.
1.5.2. Cancer
A recent meta-analysis investigated the links between OSA and cancer incidence [80]. From 5 studies rated as good quality, and incorporating almost 35,000 patients with OSA/SDB, that meta-analysis, using unadjusted analysis, reported that OSA/SDB patients had almost 50% greater overall cancer risk compared to those without SDB. While attenuated, the risk remained significant after adjustments were made for risk factors, such as age, gender, obesity, smoking and alcohol use (relative risk: 1.40; 95% CI: 1.01-1.95).

That study outlined possible mechanisms leading to an association between OSA/SDB, focussing on intermittent hypoxia as a possible mediator. They highlighted that intermittent hypoxia increases tumour growth, tumour necrosis and predisposition to metastasis, that reactive oxygen species, generated during re-oxygenation are important in key tumour genesis pathways, and that patients with OSA have decreases antioxidant capacity [80].

In the Australian context, one of the studies included in the above meta-analysis used 20 –year follow-up data from a Western Australian cohort, fully adjusted for possible confounders. That study reported that moderate-severe OSA was significantly associated with cancer mortality (hazard ratio: 3.4; 95% CI: 1.1, 10.2) and incident cancer (2.5; 1.2, 5.0) [54],

1.5.3. Metabolic impairment
An independent association between SDB and metabolic impairment has been reported. Punjabi and colleagues [81], reporting data from the SHHS, identified adjusted odds ratios for glucose intolerance of 1.27 (95%CI: 0.98-1.64) and 1.46 (1.09, 1.97) for subjects with mild and moderate-to-severe OSA respectively, relative to subjects without OSA. In that analysis adjustments were made for several confounders, including age, gender, smoking status, BMI, waist circumference, and self-reported sleep duration. Further analysis determined that respiratory events with desaturation between 2-4% were associated with increased risk of hyperglycaemia, after adjustment for confounders, but not respiratory events with <2% desaturation [51].
Nevertheless, it has been noted that in order to support a causal link between OSA and glucose metabolism there is a need for further longitudinal data with PSG defined OSA from large cohorts [82].

1.5.4. Motor-vehicle accidents, neurocognitive impairment & other consequences

A systematic review of the literature [83] reported that patients with OSA have a two- to three-fold increased risk of motor vehicle accidents compared to the general population, although consistent associations between daytime sleepiness and crash risk, and the severity of OSA and crash risk, were not observed.

In terms of neurocognitive impairment, a review of the literature noted that the majority of studies have reported spared global cognitive and language functioning. However, impairments have been more commonly noted in attention/vigilance and executive functioning [84]. Additionally, OSA has been associated with increase rates of mental illness, including depression [85], increased risk of occupational injuries [86] and poorer quality of life [48]. More generally it has been noted that patients with sleep problems have higher levels of absenteeism from work and reduced productivity due to fatigue, compared to the general population [86].

1.6. Costs

In a report prepared for Sleep Health Australia in 2004 [87] and later published in a scientific journal [88], the total cost of sleep disorders in Australia in that year was estimated at $A 10.3 billion (including direct health costs of managing sleep disorders and associated medical conditions, the indirect financial costs of associated work-related injuries and motor vehicle crashes, and lost productivity, and the nonfinancial costs derived from loss of quality of life and premature death). Extrapolated to the United States population it was estimated that the total cost of sleep disorders was greater than the total cost of asthma and chronic obstructive pulmonary disease, while being similar to that of diabetes [89]. Australian health costs of sleep disorders (as opposed to total
costs) were estimated at $A 628 million (nearly one percent of the total Australian health costs), a similar order of magnitude to asthma. Although the specific costs of OSA were not outlined separately, it was also estimated in the report that OSA accounted for approximately two-thirds of all sleep disorders.

More recently, the Sleep Health Foundation (www.sleephealthfoundation.org.au) commissioned a report that analysed the direct and indirect costs associated with sleep disorders in Australia for 2010 [90]. This report estimated the health costs of the three most common sleep disorders at $A 818 million per year. Of these costs, $A 657 million per year related to OSA: $A 248 million for OSA itself and $A 409 million for the health costs of conditions attributable to OSA, including hypertension, vascular disease, depression, and motor vehicle and workplace accidents. Indirect financial costs associated with OSA, including lost productivity and other indirect costs associated with accidents, amounted to $A 2.6 billion. Importantly, as has been pointed out [29], these figures are likely an under estimation of the total costs associated with OSA as they did not include the effects on quality of life in terms of disability-adjusted life-years, conservative estimates of prevalence were used, and potential co-morbidities such as metabolic disorders were excluded.

In a review study, Tarasiuk et al. [91] reported that the estimated health costs of those with untreated OSA, of about two-fold greater than controls, was largely attributable to cardiovascular disease morbidity.

1.7. Pathogenesis

As has been described above, OSA is common, it is associated with a number of adverse outcomes and it is costly, and thus the pathophysiology of OSA has been a topic of intense research.

In humans the upper airway, from the posterior end of the hard palate to the epiglottis, has relatively little rigid support and is therefore prone to collapse [30, 35]. Forces that act to collapse the airway include the negative pressure
generated during inspiration as well as the pressure exerted by tissues surrounding the airway. OSA patients tend to have an anatomically small pharyngeal airway [92, 93] which is more prone to collapse [94]. Importantly, this is also observed under passive conditions of general anaesthesia and muscle paralysis, showing that the narrowing in OSA is not due solely to differences in upper airway dilator muscle activity [95]. The narrowed upper airway in OSA patients is thought to relate to increased soft tissue surrounding the airway [92, 96], or the airway and soft tissue being enclosed by a small bony compartment, such as in individuals with a small maxilla and mandible [35]. Thus, upper airway anatomy is considered a key factor in the pathogenesis of OSA [30, 35, 97].

Although upper airway anatomy is important in the pathogenesis of OSA, it has been recognised that anatomy alone does not fully explain the variability in incidence or severity observed [30, 35, 98-100]. The forces leading to collapse of the pharyngeal airway are offset by dilating forces, a key one being pharyngeal dilator muscle activity [35]. Thus, pharyngeal dilator muscle responsiveness and efficacy, or the ability of the upper airway dilator muscles to respond to challenge, has also been considered as a possible contributor in the pathogenesis of OSA [30, 35], and is one target of the current research. Other factors considered important [30, 35, 97] include arousability or arousal threshold (the propensity to wake in response to increased respiratory drive) and loop gain (the stability of the respiratory control system). Another aspect that has been considered, and which is another target of the current research, is the possibility of a deficit in the sensory detection of respiratory challenge in OSA patients. Such a deficit may also ultimately result in impaired upper airway muscle responsiveness.

This section outlines and discusses some of the key factors considered important in the pathogenesis of OSA and how these factors may contribute to disease pathogenesis. This section pays particular attention to pharyngeal dilator muscle responsiveness to negative airway pressure and the sensory
detection of negative airway pressure, which are targets of the research conducted for this thesis.

1.7.1. Anatomy
Anatomy of the pharyngeal airway and upper airway dilator muscles are shown in figure 1-2 below.
Numerous studies using varied imaging techniques have shown the airway lumen size to be smaller in OSA subjects compared to healthy controls during wakefulness [92, 95, 96, 102, 103]. Differences are seen in both retroglossal and retropalatal regions although the differences are greater in the retropalatal
region where the airway is at its narrowest [92]. Over the respiratory cycle the retropalatal airway is narrowest at end expiration [103]. Perhaps the most important study examining airway lumen size differences between OSA and control subjects is that of Isono et al. [95]. That study separated out the influence of neuromuscular and anatomical factors by using endoscopy to visualise the upper airway during complete muscle paralysis under general anaesthesia. They compared a group with severe SDB, a group with mild SDB, and a group without SDB, classified by overnight pulse oximetry. They examined the static properties of the pharynx by determining its pressure-area relationship. A pressure delivery device was used to experimentally reduce the upper airway pressure from 20 cmH₂O to the pressure at which the velopharynx (retropalatal oropharynx) and the oropharynx (retroglossal space) closed completely, while the subject remained apnoeic due to the muscle paralysis (i.e. without mechanical ventilation). Using this procedure the site of primary closure was the velopharynx in 49 subjects and the oropharynx in 8 subjects. Maximal cross sectional area at the retropalatal region at the highest values of delivered upper airway pressure were greater in those without SDB compared to either group with SDB, suggesting a narrower airway in those with SDB. Additionally the closing pressure was significantly higher in SDB groups compared to the non SDB group suggestive of a more collapsible airway. However it has been noted that there is considerable overlap in the closing pressure between those with and without SDB [2] suggesting other factors are at play in OSA pathogenesis. In addition, the slope of the pressure area curve at the velopharynx was steeper near the closing pressure for SDB patients compared to controls, independent of pharyngeal size, showing that the pharynx was more vulnerable to pressure change [95].

The increased airway collapsibility is consistent with studies examining passive critical closing pressure ($P_{\text{crit}}$). $P_{\text{crit}}$ is established using a procedure where a subject is placed on a device with ability to control airway pressure delivery. The sleeping subject is initially placed at an optimal pressure, sufficient to eliminate airflow limitation, and then the pressure is reduced in steps until the airway is completely collapsed. The pressure at this point is referred to as the $P_{\text{crit}}$, with
the passive $P_{\text{crit}}$ determined during the first few breaths after pressure reduction when there is minimal dilator muscle activity [104-106]. Kirkness et al. reported an increase in prevalence and severity of OSA with increasing passive $P_{\text{crit}}$ above pressures of -5 cmH$_2$O [106], with a correlation coefficient of 0.46 ($r^2 = 0.28$). In that study a passive $P_{\text{crit}}$ threshold of -5 cmH$_2$O correctly classified sleep apnoea (AHI >10·h$^{-1}$) in 76.7% of subjects, with a sensitivity of 96.5% and specificity of 30.6%. Thus while OSA was essentially absent in those whose passive $P_{\text{crit}}$ was less than -5 cmH$_2$O, OSA was not necessarily present in those with a passive $P_{\text{crit}}$ above -5 cmH$_2$O. These findings are consistent with the concept that while the passive upper airway anatomy predisposes to OSA, the structural loads may be overcome with active neuromuscular mechanisms that protect individuals from sleep apnoea [106].

In addition to airway size in terms of cross-sectional area, shape and length of the upper airway have also been considered in terms of their contribution to OSA pathogenesis. A number of studies have suggested that the upper airway is larger in the lateral direction in healthy individuals, compared to a more elliptical shape, oriented in the anterior-posterior direction, in individuals with OSA [92, 107]. This orientation possibly puts the upper airway dilator muscles of those with OSA at a functional disadvantage [2, 108], limiting the ability of muscles that also have an anterior-posterior orientation, such as the genioglossus, to improve airway patency. It must be noted however, that not all studies have shown differences in airway shape between OSA and control subjects [109]. It has also been suggested that a longer airway would put the upper airway at a mechanical disadvantage and more prone to collapse [2, 110].

A number of soft tissue structures have been shown to have increased dimensions in OSA patients, possibly contributing to the differences in airway size and shape. Using MRI, Schwab et al. [92] demonstrated that OSA patients had increased lateral pharyngeal muscular wall, soft palate, tongue, and pharyngeal fat pads dimensions, however the dimensions of the lateral pharyngeal walls explained the largest part of the variance in airway calibre.
The increased soft palate and tongue dimensions were thought to be of lesser importance in their contribution to airway narrowing as their increased dimensions were not in an orientation expected to impinge on airway dimensions (i.e. anterior-posterior width). While the pharyngeal fat pads were increased in volume, they were not increased in area or width at the level of greatest airway narrowing. Using a more sophisticated volumetric MRI analysis, Schwab and colleagues [96] later showed that the greatest odds ratios for sleep apnoea risk were observed with increased lateral pharyngeal walls, tongue, and total soft tissue volume. In multivariate analysis, tongue and lateral pharyngeal wall volume independently increased the risk of sleep apnoea [96].

Craniofacial features have also been suggested as being important in predisposing individuals to OSA [2, 35-37, 97]. The upper airway is surrounded by a bony enclosure, which includes mandible (lower jaw), maxillae (upper jaw and palate) the spine, and the base of the skull [36]. The importance of craniofacial features in OSA was demonstrated by Shelton and colleagues [36]. They studied 30 subjects with varying degrees of OSA using MRI and found a smaller area within the mandible was correlated with greater OSA severity. Mandible area (area enclosed by the mandible ramus) and length (distance from the teeth to the posterior mandible ramus) both correlated with OSA severity, however using stepwise regression most variance in OSA severity was explained with a model that included weight, mandible area and height, in order of strength of association. In an invited review Sutherland and colleagues [37] summarised the literature relating to the importance of craniofacial structure in combination with obesity in OSA. From that review, some of the craniofacial features more commonly associated with OSA include: shorter maxillary length, maxillary constriction (narrower and more tapered maxillary arch), smaller mandibular area (area enclosed by the mandible ramus), greater mandibular retroposition, inferior displacement of the hyoid bone (increased mandibular plane to hyoid distance), smaller cranial base, increased anterior facial height, and increased craniocervical angle (extended natural head position). However the authors highlighted that the latter two are likely to be compensatory mechanisms to maintain airway patency rather than risk factors. As discussed
above, obesity is a well-recognised risk factor for OSA [27]. OSA prevalence increases with increasing BMI and there is evidence that excess weight is a causal factor in OSA. A longitudinal analysis from the WSCS showed that change in weight and change in AHI were related to each other in a dose-response manner [31]. Using conditional logistic regression the authors estimated that adults without OSA experiencing a 10% weight gain were 6 times more likely to develop moderate to severe SDB (\(\text{AHI} \geq 15 \cdot \text{h}^{-1}\)) on follow-up compared to those with a stable body weight. As outlined in the Sutherland et al. review [37] an interaction between craniofacial features and obesity has been demonstrated. While craniofacial abnormalities are more commonly seen in non-obese patients with OSA and increased soft tissue volumes are seen in obese patients, there is overlap between the occurrences of each resulting in intermediate phenotypes [111, 112]. Additionally, the ratio of tongue to oral cavity volume is greater in OSA [113], and larger tongues are seen in OSA compared to controls, matched for maxillomandibular dimensions and obesity [114]. Thus it is thought that a small bony enclosure relative to the volume of soft tissue would predispose individuals to OSA [2, 35-37, 114].

**1.7.2. Arousability**

The current standard definition of a cortical arousal from sleep recommended by the AASM is, “an abrupt shift of EEG frequency including alpha, theta and/or frequencies greater than 16 Hz (but not spindles) that lasts at least 3 seconds, with at least 10 seconds of stable sleep preceding the change” [9, 12]. Cortical arousal in REM sleep, but not NREM sleep, also requires “concurrent increase in submental EMG lasting at least 1 second”. While there is some argument that this definition of arousal is too restrictive (see White and Younes [2] for a commentary) this arousal definition is almost unchanged from the original standardised definition proposed by the American Sleep Disorders Association [15]. Cyclical arousals associated with termination of respiratory events (hypopnoeas and apnoeas), leading to sleep fragmentation, is a typical feature of OSA. Sleep fragmentation has been associated with objective daytime sleepiness, as well as decrements in mood, cognitive functioning and
psychomotor performance [115-117], independent from sleep duration and respiratory event associated oxygen desaturation [117].

The predominant view is that the key respiratory stimulus that leads to arousal in OSA is negative intrathoracic pressure [2, 118-121] as opposed to chemoreceptor stimuli such as hypoxia or hypercapnia. Gleeson et al. [121] presented three different stimuli to induce arousal from non-REM sleep in 8 healthy subjects. They presented a 30 cmH$_2$O·L$^{-1}$·s resistive load, progressive hypoxia and progressive hyperoxic hypercapnia and found that arousal occurred at varying levels of ventilation and estimated blood partial pressure of carbon dioxide ($P_{CO_2}$) and oxygen ($P_{O_2}$) for the three conditions, but that the intrathoracic pressure at the point of arousal was similar. Berry et al. [120] measured the effect of hyperoxia on the arousal response to an occlusion stimulus with similar findings. While the time to arousal was significantly longer during hyperoxia, there was no difference in the intrathoracic pressure prior to arousal. Similarly, in OSA subjects Kimoff et al. [119] reported that experimental O$_2$ and CO$_2$ administration resulted in increases and decreases in the apnoea duration, respectively. However, while the estimated end apnoea $P_{O_2}$ and $P_{CO_2}$ were different under these conditions, the level of inspiratory effort associated with arousal was not. While the peak negative intrathoracic pressures prior to arousal (arousal threshold) are similar within subjects between conditions, there is variation between subjects [119-121].

White and Younes [2] provide an interesting historical perspective into the role of arousals and arousal threshold in the pathogenesis of OSA. While it was initially thought that the arousal was protective, and necessary to re-establish airway patency, it is now recognised that respiratory events can be terminated without arousal and that many patients with periods of cyclical OSA may have periods of stable ventilation, without sleep state or body position change. From this arose the concept that arousals may be deleterious and contribute to OSA in some individuals; the arousal threshold may occur at levels of chemical stimuli that are nonthreatening and prior to mechanisms (such as
neuromuscular compensation) that would assist opening of the airway and stabilising ventilation.

1.7.3. Respiratory control instability
Respiratory control instability has been suggested as an important factor in OSA pathogenesis [2, 30, 35, 97, 122]. In brief, the concept proposes that the arousal from sleep may increase instability of basic chemistry respiratory control, thereby perpetuating the cycle of respiratory events and arousals. Respiratory control instability is often described in terms of loop gain, which is an engineering term used to describe instability in a system controlled by feedback loops [30, 122]. To summarise, important aspects of respiratory control instability are:

1. There is tight regulation of ventilation to maintain carbon dioxide and to a lesser extent oxygen levels within narrow limits. Skatrud and Dempsey [123] demonstrated that apnoea occurred during sleep when $P_{ET}CO_2$ was experimentally reduced by 1-2 mmHg below the waking level, with apnoea duration significantly related to the degree of hypocapnia. This reduction in respiratory drive is associated with a reduction in the size of the pharyngeal airway, which more commonly results in complete airway occlusion in those with OSA compared to normal controls [124].

2. This regulation of ventilation occurs by multiple feedback loops, primarily involving chemoreceptors as sensors and respiratory muscles as effectors, with lung characteristics influencing the effectiveness of respiratory muscles in altering blood gas levels.

3. Any system with feedback loops has the potential to become unstable. This is particularly so if:
   a. The ‘gain’ of the feedback loop is high. The gain of a feedback loop is high if the system responds quickly and vigorously to a disturbance. A high gain respiratory system would be one with a brisk ventilatory response to hypercapnia (referred to as controller gain), with a small change in ventilation producing a large $PCO_2$ change (referred to as plant gain).
b. There is a phase delay between detection of the disturbance and the response. This is the case in the respiratory system due to the delay between blood $P_{CO_2}$ changes at the lung and detection at the carotid body and brainstem.

Thus respiratory control instability during sleep may result in perpetuation of respiratory events as follows: with sleep onset, there is reduced output from brainstem central pattern generator; ventilation decreases and blood $P_{CO_2}$ increases. The reduced output of the central pattern generator may result in decreased output to upper airway dilator muscles, resulting in airway narrowing and collapse, increased resistance to airflow, and leading to obstructive apnoea in a compromised airway. During the apnoea respiratory effort and $P_{CO_2}$ increase, while $P_{O_2}$ decreases. Arousal from sleep will occur, generally as a result of increased intra-thoracic pressure. After the arousal there is increased upper airway muscle activity and responsiveness, and hyperventilation resulting in a reduction in $P_{CO_2}$ and a rapid increase in $P_{O_2}$. Upon return to sleep the $P_{CO_2}$ may be below the apnoea threshold resulting in reduction of output from the central pattern generator and continuation of the cycle.

1.7.4. Lung volume

The relationship between lung volume and airway patency is thought to be another possible contributor to OSA pathogeneses [30, 35, 97]. Increasing lung volume is thought to stiffen the airway and decrease airway collapsibility due to increased caudal traction on the airway. Increased lung volume may also increase respiratory control stability by increasing oxygen and carbon dioxide stores, thus buffering the blood gases from changes in ventilation [97].

Studies have shown that increasing lung volumes, by manipulating extra-thoracic pressure, reduces airway collapsibility in healthy control subjects [125] and OSA patients [126], reduces the severity of SDB in OSA patients [127], and decreases CPAP pressure required to treat OSA patients [128]. Reduced lung
volume in obesity is thought to be one of the factors that contribute to obesity being a key risk factor for OSA [30, 97].

1.7.5. Surface tension
Surface tension of the mucosal lining liquid of the upper airway has been shown to have an influence on upper airway patency. Reducing the surface tension using a surfactant stabilises the airway and improves airway patency in both anaesthetised subjects without known OSA [129] and sleeping OSA patients [130]. Compared to healthy subjects, OSA patients have been shown to have greater surface tension [131] and reducing the surface tension in OSA patients reduces the severity of SDB [130]. Of interest to the influence of route of breathing on OSA, an overnight reduction in surface tension observed in those with predominantly nasal breathing is not observed with oral breathing [130]. Additionally, a lesser AHI is seen for those OSA patients with predominately nasal breathing compared to oral breathing [130].

1.7.6. Fluid retention and shift
Fluid retention and shift in fluid from lower extremities to the upper body and neck, may lead to upper airway narrowing, particularly in conditions leading to increased extracellular fluid, such as heart failure, end stage renal disease and hypertension [97], but also in general OSA populations [132]. Redistribution of fluid has shown to lead to improvement in OSA: a recent randomised controlled study in an unselected OSA population showed that wearing lower leg compression stockings during the day resulted in a modest reduction in AHI, a reduction in night time fluid shift from the legs, and an increase in the morning upper airway cross sectional area [132]. This followed an earlier randomised, controlled, open-label double-crossover trial examining the use of thigh length compression stockings in non-obese subjects with OSA and chronic venous insufficiency, a condition resulting in fluid accumulation in the legs [133]. In that study there was also a modest reduction in AHI of approximately 35%, in association with a reduction in the overnight leg fluid volume change of 62% and a reduction in the overnight neck circumference increase of 60% [133].
1.7.7. Neuromuscular impairment

The forces leading to collapse of the pharyngeal airway are offset by dilating forces, the key one being pharyngeal dilator muscle activity [35, 104]. Thus, pharyngeal dilator muscle responsiveness, or the ability of the upper airway dilator muscles to respond to challenge, has also been considered as a possible contributor in the pathogenesis of OSA [30, 35].

Due to its importance as an upper airway dilator, and because of its accessibility, the genioglossus is the best studied upper airway muscle. Its contraction protrudes the tongue and stiffens and enlarges the pharyngeal airway [134-139]. It has a pattern of activity which is inspiratory phasic (greater activity during inspiration) which is thought to prevent collapse due to inspiratory negative airway pressure [140]. This activity is greater during wakefulness in OSA patients compared to healthy controls during basal breathing [141-143], which is thought to compensate for an anatomically compromised upper airway [30, 141], and is diminished upon transition from wakefulness to sleep, more so in OSA patients than healthy controls [142, 143].

There is substantial evidence that the genioglossus muscle is responsive to upper airway negative pressure. This has been termed the ‘negative pressure reflex’ having been demonstrated to occur in response to negative pressure pulses [144-153] as well as stimuli presented over multiple breaths such as supra-threshold resistive loads and negative pressure ventilation [141, 149, 151, 154-156]. This response has been extensively studied and it is attenuated but not abolished in sleep [147, 155, 157, 158], although later studies suggest this may be posture dependent [145, 151]. Genioglossus muscle responses to sudden onset negative pressure pulses have not been found to be impaired in OSA during wakefulness [148, 149]. Similarly, while previous multi-breath studies have reported a tight relationship between the collapsing forces of negative epiglottic pressure ($P_{epi}$) and genioglossus EMG activity (EMGgg) [154, 156], no differences have been observed in EMGgg sensitivity between OSA subjects and controls during wakefulness [141, 143], as expressed by the slope of the relationship between EMGgg and negative pressure. Recent
studies during sleep also suggest little difference in genioglossus muscle responsiveness between OSA patients and healthy control subjects [159, 160]. From these observations it has been suggested that OSA may result from an anatomically compromised airway in combination with normal reduction in EMGgg activity and responsiveness during sleep [30, 143]. One aspect that has not been assessed experimentally however, is the threshold negative pressure stimulus required to elicit an EMGgg response, nor have studies specifically targeted responses to small negative pressure stimuli close to the conscious detection threshold. Thus the genioglossus EMG response to small negative pressure stimuli is a key target of the current research and is discussed in more detail in chapters 5 and 8.

1.7.8. Sensory impairment
As mentioned previously, it is possible that a deficit in neuromuscular response to compensate for negative pressure challenge of the pharyngeal airway may result from a deficit in the sensory detection of negative pressure.

Traditionally detection of respiratory challenge in the upper airway has been studied by examining conscious perception of inspiratory resistive loads, using threshold detection [161-170] or magnitude estimation [165, 171-173]. In this situation resistive loads are generally applied by the patient inspiring through a circuit containing some type of porous material, such as a metal screen [161-163, 165, 173] or filter paper [164, 166, 171]. The material is generally organised in series with ports between successive resistive screens and opening and closing of various port combinations causes inspiratory flow to be shunted through the screens and for inspiration to occur against varied resistive loads (Figure 1-3).
The resistive load impedes airflow into the airway and results in more negative airway and pleural pressures, and increases in the pressure difference across the diaphragm [170].

The detection threshold for resistive loads is commonly defined as the added resistance ($\Delta R$) that can be detected for 50% of its presentations ($\Delta R_{50}$) (e.g. Davenport and Chan [174]). It has been reported that resistive load detection is related to the background resistance ($R_0$), which is made up of the resistance of the extrinsic breathing circuit and the subject’s intrinsic airways resistance [167]. Wiley and Zechman [167] demonstrated detection thresholds with a constant ratio ($\Delta R_{50}/R_0$ or Weber fraction) of approximately 0.3. As such, the same added resistive load may be detected at a relatively lower but not a higher background resistance. This may be an important concept when examining OSA subjects due to the possibility that they will have increased intrinsic resistance. Increased intrinsic resistance in OSA has been suggested in a number of studies using various methods [175-179]. Other studies have found no differences [180-185], although differences appear to be more likely with subjects in a supine position [179, 183, 185].

Only two studies have examined conscious detection threshold in OSA [178, 186]. Clerk et al. [178] found no difference between OSA subjects (BMI < 35
kg·m$^{-2}$), snorers and controls, whereas an earlier study found an increased detection threshold for OSA subjects compared to controls [186]. Another study demonstrated reduced magnitude estimation of large increases in respiratory resistance in OSA subjects [172].

Conscious detection of resistive loads is subjective, entails the possibility of false positive responses and is dependent on subject attention. This may be a particular problem when examining OSA patients due to the prevalence of EDS [5] and impaired attention [84] observed in this patient group. The respiratory related evoked potential (RREP) is considered a more objective method to examine sensory function and respiratory load detection, largely due the characteristics of its early components [187]. The RREP is the averaged cortical response (EEG) to a respiratory load and, as with event related potentials from other sensory modalities, the RREP is derived by averaging the EEG response to many presentations of the same stimulus [188]. Multiple presentations are required to allow the signal to stand out from the background noise.

The RREP has been recorded in response to airway occlusion [189-191], inspiratory resistive loads [173, 174, 192, 193], and to negative pressure pulses [194, 195], and is made up of a number of negative and positive components (Figure 1-4). Although the components may differ depending on the EEG recording configuration, the key components during wakefulness are: Nf recorded maximally over frontal regions of the cortex, P1 recorded maximally over centro-parietal regions, N1 and P2 recorded maximally over central regions and P3 recorded maximally over parietal regions. The early components, P1 in particular, are thought to represent arrival of the primary afferent information at the cortex and are not augmented by attention [174, 191]; the later components, P3 in particular, are thought to reflect cognitive processing of the respiratory signal and are augmented by attention [174, 187, 189, 191, 196, 197].
In healthy subjects, it has been reported that a resistive load threshold for elicitation of RREPs exists [174] and that it relates to the conscious detection threshold of resistive loads. Loads below the threshold for conscious detection do not elicit the RREP, whereas for loads above the threshold, many RREP component amplitudes increase with increasing resistive load magnitude [173, 174, 192, 193]. Also, as with the conscious detection threshold, the RREP is influenced by background resistance. Chou and Davenport [198] demonstrated that an RREP could be abolished by increasing the background resistance so that the Weber fraction ($\Delta R/R_0$) of the particular resistive load decreased below 0.3. The relationship between conscious detection and the RREP is discussed further in chapter 4, followed by an experimental assessment of this relationship in chapter 6.
In OSA, studies have examined the RREP during wakefulness in response to large negative pressure stimuli such as airway occlusion [190, 199, 200] or negative pressure pulses [201] with some inconsistent results. However, of these the majority have suggested that there is preservation of the amplitude of the early P1 component [149, 190, 199, 200], and only two studies have suggested an amplitude reduction of early components in OSA [201, 202]. Grippo et al. [202] also reported that all control, but less than half of OSA participants showed a cortical response to a relatively small negative pressure pulse (-1 cmH₂O), suggestive of a raised threshold for eliciting the RREP in OSA.

The majority of OSA studies to date, however, have not examined RREP responses to small negative pressure stimuli, and none have attempted to quantitate thresholds for eliciting RREP components. This is another key target of the current research, with more detail provided in chapters 4 and 7.

1.8. Summary and thesis aims

OSA is a common disorder, characterised by repetitive collapse of the pharyngeal airway during sleep, with key risk factors including obesity, male gender, craniofacial structure, genetics and age. There are a number of adverse consequences associated with OSA including excessive daytime sleepiness, cognitive impairment, cardiovascular disease, metabolic impairment, and increased motor-vehicle crash and work-place accident risk; however, perhaps the strongest association observed to date is with hypertension. As a result there is a significant burden on individuals with the condition, as well as on society due to increased health and other associated costs and there is intense interest in factors contributing to OSA pathogenesis. While anatomy is considered a key factor in OSA pathogenesis it has been recognised that other factors must be involved; other factors considered include arousability, respiratory control instability, lung volumes, neuromuscular impairment and sensory impairment.
Negative airway pressure is a key force or load contributing to airway collapse and this force is opposed by pharyngeal dilator muscle activity. While no consistent evidence for impairment of neuromuscular response to negative pressure, or sensory detection of negative pressure, has been observed in OSA patients, past studies have generally utilised large negative pressure stimuli. Thresholds for sensory detection of negative pressure, and thresholds for neuromuscular response to negative pressure, have not been examined in OSA during wakefulness or during sleep. This may be an important factor in the pathogenesis of OSA, as failure to detect and respond to minor threats to airway patency may lead to worsening collapse which is difficult to remedy by later muscle recruitment. Alternatively it may mean that dilator muscle recruitment occurs too late to prevent arousal, with repetition of the cycle of upper airway collapse on resumption of sleep.

The key aims of this thesis were to explore if there were impairments in OSA patients during wakefulness in the detection of, and neuromuscular compensation for, negative pressure respiratory load stimuli close to the conscious detection threshold.

It was hypothesised that OSA patients would have impaired detection of, and neuromuscular compensation for, threshold inspiratory resistive loads during wakefulness, when compared to healthy control subjects.

If impairment is observed it is possible that it may either be a contributor to OSA pathogenesis or may be a result of OSA; either due to the repeated mechanical trauma of obstructive events, or to sleep deprivation. Treatment of OSA can elucidate the likelihood of one or the other. If impairments are corrected following treatment it is likely that impairments are a result of OSA; if impairments are not corrected it may be that damage caused by OSA is irreversible, or that the impairment may play a role in the pathogeneses of OSA.

For the current research it was hypothesised that any abnormalities observed would not be corrected after treatment of OSA for 6 months.
2. Experiment 1: Polysomnography using abbreviated signal montages: Impact on sleep and cortical arousal scoring

2.1. Preface

The key experimental chapters of this thesis (Chapters 7 and 8) involve the comparison of individuals with severe OSA to healthy individuals without OSA. The standard method for OSA diagnosis, or for exclusion of OSA, is in-laboratory attended PSG [7]. PSG is the simultaneous monitoring of many physiological signals and standards for in-laboratory PSG in use at the time of commencing the investigations required at least one EEG channel, one submental EMG channel and two EOG channels for measurement of sleep and arousals [14]. The vast majority of OSA patients for this thesis were recruited following diagnosis with in-laboratory PSG.

An alternative to in-laboratory PSG is to use PM devices [8]. As stated in chapter 1, these devices have the advantage of being able to be used in the participant's usual sleeping environment and have cost advantages given that the investigation can be unattended; however a significant disadvantage is that they may provide a lesser number of signals than that used in in-laboratory PSG, with unknown impact on PSG analysis.

For screening of control subjects, while it was decided to investigate participants in a laboratory environment, matching the environment for diagnosis of OSA participants, staffing and equipment availability logistics dictated that the investigations be conducted with a PM device. The device available at the time matched the recording capabilities of in-laboratory equipment except that only one EEG channel and one EOG channel were available for recording of sleep and cortical arousals; omitting one of the EOG channels and the EMG channel typically
included in in-laboratory PSG. The following experiment was designed to assess whether PSG analysis using such a PM device, with an abbreviated signal montage, would be comparable to PSG analysis using an in-laboratory signal montage. The results were used to help assess the suitability of the PM device for screening control subjects, ultimately guiding the use of a more sophisticated PM device that matched the recording capabilities of in-laboratory equipment.

While the study was primarily used to assess the screening device for control subjects, the following experiment was conducted on subjects being investigated for suspected OSA, which ultimately included a range of subjects from those without OSA (Chicago AHI less than 15·h\(^{-1}\)) to those with severe OSA (maximum ~80·h\(^{-1}\)). It was thought that the use of such a sample would increase the generalisability of results to clinical practice and increase chances of successful publication. To further increase the utility of the study the use of a signal montage that included only 1 combined EEG/EOG signal for sleep and arousal scoring was also assessed. It was reasoned this montage would be the minimum that could possibly be used for sleep and cortical arousal scoring of PSG, and the investigators were aware of its use in Australian clinical sleep laboratories.

This study was published in a peer reviewed scientific journal, Sleep Medicine, in January 2015 [1], and a copy of this publication is presented in its entirety below.

The specific contribution of the publication authors are as follows, however as mentioned in the thesis preface, all authors agreed that the primary author (PhD candidate) contributed greater than 50% of the work involved in the publication:

Warren Ruehland (PhD candidate) designed the study, scored PSGs, collected and analysed the data, interpreted the results, and prepared, revised, edited and approved the manuscript.
Thomas Churchward and Tristia Lakey contributed to study design, scored PSGs, and reviewed and approved the manuscript.

Linda Schachter and Peter Rochford contributed to study design, and reviewed and approved the manuscript.

Natalie Tarquinio scored PSGs, and reviewed and approved the manuscript.

Fergal O'Donoghue and Maree Barnes reviewed and approved the manuscript.

2.2. Published manuscript
The published manuscript is included below.
Polysomnography using abbreviated signal montages: impact on sleep and cortical arousal scoring

Warren R. Ruehlman 1,*, Thomas J. Churchward 2, Linda M. Schacter 5, Trista Lakey 6, Natalie Tarquino 5, Fergus J. O'Donoghue 4, Maree Barnes 4, Peter D. Rochford 4

1 Institute for Breathing and Sleep, Austin Health, Heidelberg, Vic., Australia
2 Department of Medicine (Asthma and Sleep), Faculty of Medicine, University of Melbourne, Heidelberg, Vic., Australia
3 Sleep Support Services, Melbourne, Vic., Australia
4 Advanced Pulmonary and Sleep Diagnostics, Melbourne, Vic., Australia
5 Sleep Research, Austin Health, Melbourne, Vic., Australia

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ABSTRACT

Objectives: This study examined the impact of using two abbreviated signal montages on the accuracy, precision and intra-rater reliability of polysomnography (PSG) sleep and arousal scoring, compared to a standard reference montage, in a cohort of patients investigated for obstructive sleep apnoea (OSA). One abbreviated montage incorporated two signals dedicated to sleep and arousal scoring, and the other incorporated a single signal.

Methods: Four scorers from two laboratories each scored 15 PSGs four times in random order, once using each abbreviated montage and twice using the reference montage.

Results: Use of the two-montage montage resulted in small changes in the distribution of sleep stages, a reduction in the arousal index and resultant reductions in sleep and arousal scoring agreement. For the one-montage montage, although similar magnitude sleep stage distribution changes were observed, there were large reductions in the arousal index, and sleep and arousal scoring accuracy. Additionally, using the one-montage montage, there were statistically significant reductions in the precision of summary statistics including total sleep time (TST) and the amount of rapid eye movement (REM) sleep scored, and reductions in the intra-rater reliability of REM sleep and arousal scoring.

Conclusions: These findings demonstrate that abbreviated signal montages may result in underestimation of the arousal index and, depending on the montage, poorer precision in TST and REM sleep scoring, with potential consequences for apnoea–hypopnoea index (AHI) measures and OSA diagnosis. The results highlight the importance of careful evaluation of PSG results when using portable devices that have restricted signals, and they offer guidance for future PSG and portable monitoring standards.

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1. Introduction

It is standard clinical practice to confirm obstructive sleep apnoea (OSA) diagnosis using in-laboratory polysomnography (PSG). However, it is increasingly recognised that portable monitoring (PM) may be an acceptable alternative, with acknowledgement and understanding of accompanying limitations [1].

Although many PM devices have abbreviated signal recording capabilities compared to full PSG, there is an advantage in quantifying sleep and cortical arousals, requiring the recording of fast sampling rate signals such as electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG). Recording of these signals allows: (i) assessment of the impact of any respiratory disturbance on brain architecture; (ii) the use of total sleep time (TST) rather than total recording time (TRT) as the denominator in calculating indices of respiratory or sleep disturbances; (iii) verification of rapid eye movement (REM) sleep sampling, important due to the incidence of left-related OSA, estimated to have a prevalence of approximately 1% in clinical OSA populations [2]; and (iv) the use of respiratory event scoring criteria requiring airflow reduction accompanied by cortical arousal.

Despite these theoretical advantages, the most recent clinical guidelines for use of PM to diagnose OSA [1] did not consider devices that were able to measure sleep. This was because there were no new data available comparing such devices to PSG since previous guidelines [3], stated that evidence was lacking to recommend their clinical use.
PM devices with fast sampling capability may still be restricted to the number of signals that can be dedicated to the recording and scoring of sleep and cortical arousals. Current guidelines [4] recommend the use of six primary signals (three EEG, two EOG, and one EMG) for recording and scoring of sleep and cortical arousals in PSG. We have previously shown that the use of four primary signals, incorporating one EEG, results in only small changes in the distribution of sleep stages [5] and no statistically significant differences in sleep or cortical arousal scoring inter-scorer or intra-scorer reliability. However, there are no data to guide clinical practice on how further signal restrictions may impact the scoring of sleep and arousals. Such information is crucial for those using PM devices with limited signal recording capabilities.

This study aimed to examine the impact of using two abbreviated signal montages on the accuracy, precision, and inter-scorer reliability of PSG sleep and arousal scoring, compared to a standard reference montage, in a cohort of patients presenting for the investigation of OSA. One abbreviated montage incorporated two signals dedicated to recording and scoring of sleep and cortical arousals, whereas the other utilized a single signal.

2. Methods

2.1. Design

This study was a prospective, non-blinded, randomized comparison of sleep and arousal scoring using two abbreviated montages compared to a standard reference montage; it was approved by the institutional human research ethics committee.

2.2. Patient selection

The study utilized 13 single-night PSGs sourced during July and August 2006 from the Austin Health Sleep Laboratory in Melbourne, Australia, from consecutive patients investigated for OSA. PSGs were not considered if they were primarily conducted for non-OSA sleep disorders, research or treatment implementation.

2.3. PSG recordings

PSGs were recorded using CompuMedics S-series or E-series monitoring equipment (CompuMedics, VIC, Australia). The recording configuration consisted of: one EEG signal (C3/A1), two EOG signals (left and right eye movements [CZF/PSG], or combined EEG/EOG signal [Fp1/OC]), submental EMG, electrocardiogram (ECG), nasal pressure, body position, thoracic and abdominal excursion (inductance plethysmography), oxygen saturation via finger pulse oximetry (Nellcor M-95S; Nellcor Inc., Bedfont, LD, UK), left and right leg movements and sound.

2.4. PSG scoring

Sleep and arousal scoring were performed manually, in a single pass, using 'Procion PSG 2 software (CompuMedics, Abbotsford, VIC, Australia), based on published standards available at the time of the study [6]. Apnoea-hypopnea indices (AHI) determined using "Chicago Criteria" [8] during the original clinical investigation characterized the patient sample.

During scoring, PSGs were configured to display one of three montages: (i) a reference montage (Mref) incorporating one EEG signal (C3/A1), two EOG signals and one EMG signal, selected as it was in the minimum configuration recommended for use in PSG [6]; (ii) an abbreviated two-signal montage (M2) incorporating one EEG signal (C3/A1) and one EOG signal (LOC/ROC); or (iii) an abbreviated one-signal montage (M1) incorporating the single combined EEG/EOG signal (Fp1/ROC). Care was taken to ensure that the display size of all signals was identical regardless of the number of signals displayed.

During abbreviated montage scoring, the start and end of REM sleep were not defined by EMG changes, but they were defined by the presence/absence of REMs and stage 2 sleep features; arousals in REM did not require concurrent EMG elevation with EEG frequency shift.

2.5. Scoring

Four scorers from two separate Australian clinical sleep investigation services participated: two from Sleep Services Australia, Melbourne, and two from the Austin Hospital, Melbourne. All scorers participated in scoring concordance programs and they were experienced in abbreviated montage scoring.

2.6. Protocol

For each scorer, all PSGs and versions were de-identified and presented in random order with the exception that no 2 versions of the same PSG were ever presented consecutively. A second copy of Mref (M2ref) was later scored to allow comparison of abbreviated montage accuracy and precision against intra-montage scoring repeatability. Thus, each scorer analyzed all 13 PSGs four times each, twice using Mref and once each using M2 and M1.

2.7. Analysis

The analysis involved assessment of: (i) PSG summary statistic accuracy; (ii) PSG summary statistic precision; (iii) event-by-event/epoch-by-epoch accuracy; and (iv) event-by-event/epoch-by-epoch inter-scorer reliability. For all assessments of accuracy and precision, the mean value of all four scorers was used for statistical analysis. Distributions of the differences between numerous parameter pairs were skewed and so non-parametric Friedman tests were undertaken for all comparisons, with post-hoc analysis conducted using Wilcoxon signed-rank tests.

2.7.1. Summary statistics accuracy

Statistical analysis compared repeated measure differences in PSG sleep and arousal summary statistics between Mref, M2, M2ref, and M1. The distribution of epoch-by-epoch sleep stage-specific discrepancies was examined to elucidate the cause of any observed differences.

2.7.2. Summary statistics precision

Precision of PSG sleep and arousal summary statistics for Mref, M2, and M2ref each was assessed using the mean absolute deviation (MAD) about the mean difference from Mref. Statistical analysis compared repeated measure differences in precision between Mref, M2, and M2ref.

2.7.3. Epoch-by-epoch/epoch-by-event accuracy

Epoch-by-epoch accuracy of sleep and arousal scoring for Mref, M2, and M2ref each versus Mref was assessed using Cohen's pair-wise kappa [9], modified for continuous measurements for arousal scoring [10]. Statistical analysis compared repeated measure differences in accuracy between Mref, M2, and M2ref. Raw agreement, expressed as percentage agreement [11] for sleep and in proportion of specific agreement (PSA) for positive ratings [12] for arousals, was also presented for comparison.

2.7.4. Epoch-by-epoch/epoch-by-event inter-scorer reliability

Epoch-by-epoch inter-scorer reliability of sleep and arousal scoring for Mref, M2, and M2ref each was assessed using Fleiss' multi-scorer kappa [11,12], modified for continuous measurements for
Table 1

<table>
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<td>–</td>
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</table>

Values are median where appropriate.
Abbreviations: IQR = Interquartile range; BMI = Body mass index; ESS = Epworth Sleepiness Scale; AHI = apnea-hypopnea index.

3. Results

3.1. Patient characteristics

The characteristics of the 15 patients studied are presented in Table 1.

3.2. Summary statistics: accuracy

<table>
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<th>Difference Relative to M00</th>
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</thead>
<tbody>
<tr>
<td>Total sleep time (min)</td>
<td>M00</td>
<td>292.9</td>
<td>252.5, 340.9</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>289.8</td>
<td>254.8, 334.5</td>
<td>–</td>
<td>3.1 (−1.4, 5.5)</td>
</tr>
<tr>
<td>M02</td>
<td>288.2</td>
<td>254.5, 341.1</td>
<td>–</td>
<td>3.1 (−1.8, 5.8)</td>
</tr>
<tr>
<td>M03</td>
<td>3.3</td>
<td>236.4, 348.5</td>
<td>–</td>
<td>–3.3 (−5.5, −1.1)</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>M00</td>
<td>7.1</td>
<td>6.3, 7.7</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>7.2</td>
<td>6.5, 7.9</td>
<td>–</td>
<td>6.8 (0.5, 12.2)</td>
</tr>
<tr>
<td>M02</td>
<td>7.3</td>
<td>6.5, 8.3</td>
<td>–</td>
<td>6.4 (0.7, 12.0)</td>
</tr>
<tr>
<td>M03</td>
<td>7.6</td>
<td>6.5, 9.0</td>
<td>–</td>
<td>−0.6 (−2.3, 1.0)</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>M00</td>
<td>9.2</td>
<td>8.0, 27.8</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>12.0</td>
<td>10.3, 26.0</td>
<td>–</td>
<td>12.0 (−0.0, 26.0)</td>
</tr>
<tr>
<td>M02</td>
<td>12.3</td>
<td>10.1, 26.0</td>
<td>–</td>
<td>12.3 (0.0, 26.0)</td>
</tr>
<tr>
<td>M03</td>
<td>12.1</td>
<td>11.2, 28.0</td>
<td>–</td>
<td>11.2 (−0.1, 21.0)</td>
</tr>
<tr>
<td>REM latency (min)</td>
<td>M00</td>
<td>95.6</td>
<td>58.8, 145.7</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>94.1</td>
<td>59.9, 141.0</td>
<td>–</td>
<td>69.7 (−1.4, 126.3)</td>
</tr>
<tr>
<td>M02</td>
<td>92.6</td>
<td>56.0, 131.7</td>
<td>–</td>
<td>72.6 (−1.6, 129.7)</td>
</tr>
<tr>
<td>M03</td>
<td>92.6</td>
<td>56.0, 131.0</td>
<td>–</td>
<td>72.6 (−1.6, 129.7)</td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>M00</td>
<td>476.2</td>
<td>400.0, 580.0</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>482.2</td>
<td>418.0, 582.0</td>
<td>–</td>
<td>24.0 (−15.5, 37.1)</td>
</tr>
<tr>
<td>M02</td>
<td>482.2</td>
<td>418.0, 582.0</td>
<td>–</td>
<td>24.0 (−15.5, 37.1)</td>
</tr>
<tr>
<td>M03</td>
<td>482.2</td>
<td>418.0, 582.0</td>
<td>–</td>
<td>24.0 (−15.5, 37.1)</td>
</tr>
<tr>
<td>Time in each sleep stage (min) Stage 1</td>
<td>M00</td>
<td>23.6</td>
<td>20.0, 33.5</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>23.6</td>
<td>20.0, 33.5</td>
<td>–</td>
<td>18.5 (−14.5, 33.5)</td>
</tr>
<tr>
<td>M02</td>
<td>23.6</td>
<td>20.0, 33.5</td>
<td>–</td>
<td>18.5 (−14.5, 33.5)</td>
</tr>
<tr>
<td>M03</td>
<td>23.6</td>
<td>20.0, 33.5</td>
<td>–</td>
<td>18.5 (−14.5, 33.5)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>M00</td>
<td>127.1</td>
<td>117.2, 137.3</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>127.1</td>
<td>117.2, 137.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M02</td>
<td>127.1</td>
<td>117.2, 137.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M03</td>
<td>127.1</td>
<td>117.2, 137.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Slow wave sleep (%)</td>
<td>M00</td>
<td>43.5</td>
<td>29.5, 55.7</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>47.1</td>
<td>27.0, 60.0</td>
<td>–</td>
<td>17.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>M02</td>
<td>47.1</td>
<td>27.0, 60.0</td>
<td>–</td>
<td>17.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>M03</td>
<td>47.1</td>
<td>27.0, 60.0</td>
<td>–</td>
<td>17.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>REM</td>
<td>M00</td>
<td>50.0</td>
<td>35.8, 64.5</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>50.0</td>
<td>35.8, 64.5</td>
<td>–</td>
<td>13.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>M02</td>
<td>50.0</td>
<td>35.8, 64.5</td>
<td>–</td>
<td>13.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>M03</td>
<td>50.0</td>
<td>35.8, 64.5</td>
<td>–</td>
<td>13.1 (−14.0, 31.0)</td>
</tr>
<tr>
<td>Neural Index (%)</td>
<td>M00</td>
<td>5.0</td>
<td>4.0, 5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M01</td>
<td>5.0</td>
<td>4.0, 5.0</td>
<td>0.0</td>
<td>2.0 (−0.5, 4.5)</td>
</tr>
<tr>
<td>M02</td>
<td>5.0</td>
<td>4.0, 5.0</td>
<td>0.0</td>
<td>2.0 (−0.5, 4.5)</td>
</tr>
<tr>
<td>M03</td>
<td>5.0</td>
<td>4.0, 5.0</td>
<td>0.0</td>
<td>2.0 (−0.5, 4.5)</td>
</tr>
<tr>
<td>Neural Count</td>
<td>M00</td>
<td>772.0</td>
<td>543.0, 910.0</td>
<td>–</td>
</tr>
<tr>
<td>M01</td>
<td>772.0</td>
<td>543.0, 910.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M02</td>
<td>772.0</td>
<td>543.0, 910.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M03</td>
<td>772.0</td>
<td>543.0, 910.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data reported as median (interquartile range); abbreviations: M00 = reference montage; M01 = one central EEG, one EOG montage; M02 = combined EEG/EOG montage; M03 = repeated scoring of M00 NS; P < 0.05 using Friedman test.
2.2. M1 versus M2
There was a statistically significant reduction of 35 (21, 50) in stage 1 sleep and a statistically significant increase of 8 (5, 15) in stage 2 sleep; there was also a statistically significant arousal index reduction of 41 (20, 51)% (Table 2; Fig. 1). No statistically significant difference was found for either sleep summary statistics. For the arousal index, the median reductions were greater for M1 compared to M2 versus M0 (P = 0.001). A net shift from stage 1 to stage 2 of 11.3 (3.1, 16.9) min and a net shift from stage 1 to stage 2 of 7.5 (-2.9, 10.8) min contributed to the reduction in stage 1. The shift from stage 1 to stage 2 as well as a net shift from SWS to stage 2 sleep of 14.8 (-1.9, 16.6) min contributed to the increase in stage 2.

3.2.3. M10 versus M0
There was a statistically significant increase of 14 (-1, 34.8) in SWS, largely due to a net shift from stage 2 to SWS of 7.6 (-20.3, 17.4) min.

3.3. Summary statistics precision
3.3.1. M2 versus M0
There were no statistically significant differences in the amount of stage REM sleep, compared to the precision of M0 (Table 3). The reduced precision of M2 for those parameters is also reflected in the wider interquartile ranges of the differences from M0 (Table 2; Fig. 2).

3.4. Epoch-by-epoch/event-by-event accuracy
There was a statistically significant reduction in the accuracy of M2 versus M0 (P = 0.01), compared to M10 versus M0 for overall sleep scoring, and for individual sleep stages; stage 1, stage 2, and REM sleep (Table 4). There was a statistically significant reduction in the accuracy of M1 versus M0, compared to M0 versus M0 for overall sleep scoring, and for all sleep stages individually. For overall sleep scoring, the reduction in accuracy was greater for M0 compared to M1 (P = 0.001), and the equivalent raw percentage agreements were 86.0% (82.1, 88.9) for M0 versus M0, and 95.6% (85.4, 98.0) for M0 versus M0 respectively. The arousal scoring accuracy was reduced for both M1 and M2 versus M0 (Table 5), and the reduction was greater for M1 compared to M0 (P = 0.001). For arousal scoring raw agreement, the equivalent PSAs were 0.67 (0.64, 0.75), 0.55 (0.47, 0.61) and 0.73 (0.70, 0.78) when using M0, M1, and M2 respectively.

3.5. Inter-scorer reliability
There were no statistically significant reductions in multi-rater inter-scorer reliability of sleep or arousal scoring when scoring with either M2 or M10 versus M0 (Table 5). With M1 versus M0, there was a statistically significant inter-scorer reliability reduction for scoring REM sleep and stage 1 sleep. For overall sleep scoring, the equivalent raw percentage agreements were 79.85 (77.0, 82.3, 78.3, 82.5) for M1 versus M0, and 80.28 (77.3, 82.1) for M0 versus M0 respectively. For arousal scoring raw agreement, the equivalent PSAs were 0.58 (0.56, 0.60), 0.60 (0.58, 0.60) and 0.64 (0.62, 0.67) when using M1, M2, M10, and M0 respectively.

4. Discussion
This study examined the impact of using a single abbreviated sleep montage on the accuracy, precision, and inter-scorer reliability of sleep and arousal scoring, compared to a standard reference montage. Using an abbreviated montage incorporating one central EEG signal and one EOG signal for sleep and arousal scoring, we found that there was an increase in the amount of SWS and a reduction in stage 1 sleep, as well as a reduction in the cortical arousal index. Despite the small but statistically significant reduction in epoch-by-epoch sleep scoring event-by-event accuracy, we found that there was a statistically significant difference in the precision of PSG summary statistics compared to repeated scoring using the reference montage. Additionally, there were no statistically significant differences in multi-rater inter-scorer reliability of sleep or arousal scoring compared to the reference montage.
**Table 3**

Precision of sleep and arousal scoring summary statistics derived using two abbreviated signal montages (M1 and M2 vs. M4) compared to repeated scoring using a full montage (M4 vs. M4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Montage</th>
<th>Value</th>
<th>Difference Relative to M4</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time (min)</td>
<td>M4</td>
<td>4.41 (4.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>4.41 (4.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>4.41 (4.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>M4</td>
<td>0.00 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>0.00 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>0.00 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>M4</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage REM latency (min)</td>
<td>M4</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>2.41 (4.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>M4</td>
<td>1.01 (1.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>1.01 (1.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>1.01 (1.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to reach sleep stage (min)</td>
<td>Stage 1</td>
<td>M4</td>
<td>2.31 (1.42)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>2.31 (1.42)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M1</td>
<td>2.31 (1.42)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage 2</td>
<td>M4</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Stage 3</td>
<td>M4</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>0.31 (0.36)</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

All data reported as median (inter-quartile range); absolute deviation around the median; difference from M4; abbreviations: M4: reference montage; M2: one critical EEG and EOG montage; M1: one combined EEG/EOG montage; NS: P > 0.05 using Friedman tests.

**Table 4**

Epoch-by-epoch and event-by-event accuracy ( kappa) of polytomography sleep and arousal scoring using two abbreviated signal montages relative to a full montage (M2 and M1 vs. M4) in comparison to repeated scoring of a full montage (M4 vs. M4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Montage Comparison</th>
<th>Value</th>
<th>Difference Relative to M4</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep – Overall</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Arousal (REM/REM)</td>
<td>M4 vs. M2</td>
<td>0.40 (0.37, 0.43)</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Sleep – Specific Sleep Stages</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Stage 1</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Stage 2</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Stage 3</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>REM</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Arousal</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Arousal – Overall</td>
<td>M4 vs. M2</td>
<td>0.86 (0.79, 0.92)</td>
<td></td>
<td>0.004</td>
</tr>
</tbody>
</table>

All data reported as median (inter-quartile range); abbreviations: M4: reference montage; M2: one critical EEG, one EOG montage; M1: one combined EEG/EOG montage; M4: repeated scoring of M4; NS: P > 0.05 using Friedman tests.
## Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Montage 1 (SWS/REM)</th>
<th>Montage 2</th>
<th>Difference Relative to Montage 1</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep - Overall</td>
<td>M_d</td>
<td>M_d</td>
<td>0.71 (0.88, 0.34)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_{log}</td>
<td>M_{log}</td>
<td>0.72 (0.88, 0.37)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.70 (0.88, 0.37)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.71 (0.88, 0.34)</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep - specific sleep stages</td>
<td>Stage 1</td>
<td>M_d</td>
<td>0.20 (0.25, 0.36)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_{log}</td>
<td>M_{log}</td>
<td>0.25 (0.25, 0.35)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.25 (0.25, 0.32)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.25 (0.25, 0.34)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>M_d</td>
<td>0.06 (0.3, 0.72)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_{log}</td>
<td>M_{log}</td>
<td>0.06 (0.3, 0.72)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.06 (0.3, 0.72)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.06 (0.3, 0.72)</td>
<td>NS</td>
</tr>
<tr>
<td>Slow-wave sleep</td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.64 (0.66, 0.71)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.64 (0.66, 0.71)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.64 (0.66, 0.71)</td>
<td>NS</td>
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<tr>
<td>REM</td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.57 (0.35, 0.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.57 (0.35, 0.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.57 (0.35, 0.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Arousal</td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.06 (0.06, 0.07)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.06 (0.06, 0.07)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.06 (0.06, 0.07)</td>
<td>NS</td>
</tr>
<tr>
<td>Arousal - Overall</td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.75 (0.45, 0.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_{log}</td>
<td>0.75 (0.45, 0.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>M_d</td>
<td>M_d</td>
<td>-0.75 (0.45, 0.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

All data as median [inter-quartile range]; abbreviations: M_d: reference montage; M_{log}: one channel EEG, one EOG montage; M_{log}d: one combined EEG/EOG montage; M_{log}d: repeated scoring of M_d, M_{log}; NS: P > 0.05 using Friedman test.

Similar to the two-signal montage, using the one-signal montage resulted in a change in the distribution of sleep stages, in particular, a reduction in stage 1 sleep and an increase in stage 2 sleep. However, compared to the two-signal montage, there was a greater reduction in arousals scored and the reductions in epoch-by-epoch and event-by-event accuracy were larger and involved more sleep stages. Additionally, using the one-signal montage, there were reductions in the precision of sleep scoring summary statistics (TST, SE, REM latency, and total REM), and there were reductions in inter-scorer reliability of REM sleep and arousal scoring.

### 4.1 Sleep summary statistics

It is of interest that the two-signal montage resulted in an increase in SWS sleep whereas the single-montage did not. One may expect that, given that electrodes are placed more frontally in the single-signal montage and that slow waves used to score SWS are more prominent in frontal regions of the cortex, there would be an increase in scored SWS. Our finding is possibly contributed by the electrode being placed too far forward to pick up frontal lobe predominant slow waves, or it is due to the smaller inter-electrode distance, which is known to result in EEG amplitude reduction [14]. For the two-signal montage increase in SWS, given that reference montage scoring also resulted in a small but significant increase in SWS, one must also consider the possibility of a false positive finding. While it seems plausible that eye movement-related EEG fluctuations being mistaken for K-complexes may contribute to the reductions in stage 1 sleep for both abbreviated montages, this was not the case. The reduction in precision for both abbreviated montages in the amount of REM sleep scored, significantly so for the one-signal montage, suggests confusion between eye movement deflections and K-complexes but not one being consistently mistaken for the other.

A bias or shift in the distribution of sleep stages may not be a great concern but a decrease in precision is more worrisome as the impact of using an abbreviated montage becomes less preferable or systematic. Precision was only reduced using the one-signal montage, significantly so for TST, sleep efficiency, REM latency, and the amount of REM sleep scored. The reduced precision for TST is of particular concern, given its use as the denominator in the AHI and Act.

### 4.2 Sleep epoch-by-epoch accuracy

A reduction in epoch-by-epoch accuracy using abbreviated montages is expected given the shifts in sleep stages observed for both abbreviated montages and the reduced precision observed for the one-signal montage. The median reduction in overall epoch-by-epoch sleep staging accuracy for the two-signal montage was similar in magnitude to the small average difference in inter-scorer reliability seen when scoring with one versus three EEGs [5] or when using Kales and Kales (R&K) [6] versus American Academy of Sleep Medicine (AASM) [4] sleep scoring recommendations [15]. The median reduction for the one-signal montage was three to four times greater than the two-signal montage. Additionally, individual sleep stage epoch-by-epoch accuracy reductions were greater when using the one-signal montage compared to the two signal montage.

### 4.3 Sleep inter-scorer reliability

The only statistically significant reduction in sleep inter-scorer reliability was for REM sleep using the one-signal montage.
Together with the lack of precision in the amount of REM scored and the reduced REM epoch-by-epoch accuracy, this finding when using the one-signal montage offers any advantage gained in being able to identify REM sleep.

4.4. Cortical arousal summary statistics

It is worth noting that both abbreviated montages resulted in reductions in the number of scored arousals. Given that the two-signal montage used the same EEG electrode placement as the reference montage, the reduction observed likely relates to the lack of EMG. Even though EMG activation is only a requirement for cortical scoring in REM sleep, as previously suggested [15], EMG activation in NREM sleep may act to cue for closer inspection of EEG. The further reduction in scored arousals using the one-signal montage likely then relates to electrode placement. This further reduction is inconsistent with our previous finding suggesting an increase in arousals with frontal leads [17], indicating that the combined frontal EEG/EOG is too far forward to effectively detect all frontal lobe arousals. Although not examined in this study, due to the reduction in arousals scored, a reduction in AHI is likely when hypopnoea scoring is dependent on cortical arousal association. The magnitude of this AHI reduction requires further investigation; however, we were able to examine data from a previous study [18] where we determined that a median of 35% of hypopnoeas were scored based on the presence of an associated arousal alone when using the 2007 AASM alternative hypopnoea definition [4]. Applying the median reduction of 12.5% in the arousal count observed in the present study using the two-signal montage resulted in a reduction in AHI of 3.8 (0.2, 16.0) or 4.7 (1.1, 2.2); applying the median arousal count reduction of 42.6% observed in the present study using the one-signal montage resulted in an AHI reduction of 12.2 (0.4, 4.1) to 12.5 (1.1, 0.0) in the present study. These observations also highlight the need for care when extrapolating the results of the present study to more severe OSA populations.

4.5. Cortical arousal event-by-event accuracy

The present finding of a reduction in event-by-event arousal scoring accuracy for both abbreviated montages is expected given the lower number of arousals scored.

4.6. Cortical arousal inter-scorer reliability

Both abbreviated montages resulted in lower inter-scorer reliability, but only significantly so for the one-signal montage. This reduction in inter-scorer reliability is a concern given that inter-scorer reliability of arousal scoring is already generally poorer than sleep or respiratory event scoring [11]. Again, this finding may at least in part be explained by the lack of EMG cue in the abbreviated montages; however, this does not explain the larger reliability decline using the one-signal abbreviated montage. Reduced inter-scorer reliability also has implications for the reliability of scoring cortical arousal-associated respiratory events.

4.7. Study Limitations

One potential limitation of the current study is that it was commenced prior to the publication of current standards [4], and therefore the study used recording and scoring techniques based on previous guidelines [6,7]. However, as noted by the Italian Association of Sleep Medicine [19], the AASM manual’s sleep and arousal scoring specifications remain much of the framework of previous guidelines. Thus, only small changes in sleep stage distributions have been observed when comparing AASM to R & K scoring [20], or multiple to single EEG derivations [5], suggesting that the results obtained in the present study are applicable to current guidelines.

A limitation that must also be recognised in the present study is that scorers could not be blinded to scoring method and therefore could be subject to bias. We tried to limit this by using multiple scorers from different sleep centres, which also had the advantage of increasing the generalisability of the study findings.

One advantage of the one-signal montage used in the present study is that the electrodes sit outside the headband, which allows the use of pre-gelled adhesive electrodes, simplifying electrode application and removal. This advantage comes at the cost of reduced accuracy, precision and reliability of sleep scoring, and reduced accuracy and reliability of cortical arousal scoring; however, it is important to recognise that this does not necessarily preclude the use of single-electrode montages with different electrode placement. For example, Dyson et al. [21] compared sleep scoring with EEG electrode placement outside the headband (+/−2 cm) to standard R & K placement (+2 cm), and reported comparable sleep scoring. Thus, further studies are required to investigate the utility of different electrode placements to those used in the present study.

Additionally, it remains unknown whether non-epoch-based scoring approaches such as cyclic alternating pattern (CAP) [22], which better account for sleep microstructure such as K-complexes, sleep spindles and delta bursts, would also be impacted by abbreviated montages. One may speculate that information would be lost by limiting the channels available for analysis.

It is also important to recognise that non-statistically significant findings in the present study do not necessarily imply the equivalence of methods; it is possible that with a larger sample significant differences would be found. It is clear, however, that more statistically significant differences were observed for the one-signal montage compared to the two-signal montage, showing that differences were larger and for more consistent.

5. Conclusion

The main findings of this study were that using an abbreviated montage of one central EEG and one EEG during PSG in a subset of patients being investigated for OSA resulted in a change in the distribution in sleep stages and a reduction in the arousal index and thus a small reduction in epoch-by-epoch and event-by-event accuracy. Similar to the two-signal montage, the one-signal montage resulted in a change in the distribution of sleep stages; however, compared to the two-signal montage, using the one-signal montage, the reduction in the arousal index was greater, and the reduction in epoch-by-epoch and event-by-event accuracy were larger and involved more sleep stages. Additionally, using the one-signal montage, there were reductions in the precision of sleep scoring summary statistics and inter-scorer reliability of sleep and arousal scoring, not observed using the two-signal montage. The reduction in precision of ISI is of particular concern given its use as the denominator of AHI and AI.

These findings are valuable for those using portable devices that have restricted signal options, and they offer guidance for future standards for recording and scoring sleep and related events. They reflect greater uncertainty in scoring sleep and arousals using one compared to two signals during PSG and bring into question the utility in scoring sleep and arousals using a single signal, although it should be recognised that alternative electrode placement or subject populations may alter these findings.
Disclosure statement

Mr Ruchland has received research support from Resmed, Fisher and Paykel Healthcare and Philips Respironics. Mr Ruchland and Mr Ruchland are directors of Respiratory QA Pty Ltd (trading as OSleep), which provides quality control services for respiratory and sleep laboratories. Dr ODonoghue is a partner in a private sleep laboratory service as well as a partner in a business that provides home polysomnography. The remaining authors have declared no conflict of interest.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.11.005.

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References


2.3. Summary of results and implications

The main findings of this study were that using an abbreviated montage of one EEG and one EOG during PSG, in a cohort of patients being investigated for OSA, resulted in a change in the distribution in sleep stages and a reduction in the arousal index, compared to a full in-laboratory montage. Similar to the 2-signal montage, a 1-signal montage resulted in a change in the distribution of sleep stages, however compared to the 2-signal montage, using the 1-signal montage the reduction in the arousal index was greater, the sleep stage changes were greater, and there were reductions in the precision of sleep scoring summary statistics and inter-scorer reliability of sleep and arousal scoring, not observed using the 2-signal montage.

As has been mentioned above, the use of a 2-signal montage was being considered for use for when conducting PSG on control subjects for the current PhD project. As there were no differences in TST between the 2-signal montage and the reference montage, the change in the distribution of sleep stages would have minimal implication for the detection of OSA. This is because TST is the denominator for AHI and because AHI is the key index used for OSA quantification. The reduction in the number of scored arousals however has the potential to impact the AHI. This is because an associated scored arousal is a requirement for scoring of some respiratory events, both using the recommended standards used in this thesis [13] and more recent recommended standards [9, 11, 12]. For this reason, for the current research it was decided to use a more sophisticated recording device for detection of OSA, which matched the recording capabilities of full in-laboratory PSG.
3. Conscious detection and sensation of respiratory loading

3.1. Introduction

The rhythm of breathing is controlled automatically by neurons in the medulla oblongata and pons of the brainstem (central pattern generator), with efferent projections to inspiratory pump muscles such as the diaphragm and intercostal muscles, as well as muscles modulating airway resistance such as the laryngeal skeletal muscles and bronchial smooth muscles [203, 204].

The central pattern generator’s activity is influenced by central and peripheral chemoreceptors that detect $P_{O_2}$, $P_{CO_2}$ and/or pH, providing feedback designed to maintain blood gas or acid/base homeostasis, as well as mechanoreceptors in the airway, lungs and chest wall that respond to non-chemical stimuli, such as volume and pressure changes [203, 204]. Additionally control of breathing can be influenced by emotional state, via input from the limbic system, sleep state or be overridden or moderated by voluntary activity, controlled by the forebrain; and related to this is the conscious perception or detection of respiratory sensations.

In general, there is no conscious perception or awareness of basal breathing. However, sensory perception or detection of mechanical respiratory loads is important, particularly in respiratory pathology such as asthma as well as OSA, and is therefore the focus of this chapter. In asthma, conscious detection of increased respiratory load caused by airway narrowing is necessary if patients are to initiate treatment early during exacerbations, and there is evidence that ‘poor perceivers’ are at greater risk of serious exacerbations and hospitalisations [205, 206]. In OSA, sensory detection of the increased load imposed by a narrow airway is important in initiating compensatory upper airway dilator muscle responses, thus impaired sensory detection may be an etiological factor in OSA.
This chapter outlines the methodologies used to examine sensory detection of respiratory loads, with a focus on threshold detection, and examines the literature using these methods to study sensory detection in OSA compared to healthy control groups. Additionally this chapter examines the literature concerning sensation of non-respiratory stimuli in the upper airway, as this has been an area of interest related to sensory detection in OSA.

### 3.2. Mechanical load stimulus

Traditionally detection of respiratory loads has been studied by applying inspiratory resistive load stimuli [161-173]. Resistive loads most closely mimic the internal flow limitations experienced by patients with a narrowed airway in asthma, chronic bronchitis and OSA [162, 167, 186]. The methods used to apply resistive loads have been described in chapter 1. Alternatively, other studies have imposed elastic loads, which are produced when subjects breathe from varying sizes of rigid airtight containers [169, 207-210]. However elastic loads are more applicable to those patients with restrictive lung disease, such as those with interstitial fibrosis [210].

A resistive load generally results in a reduction in airflow into the lung and more negative upper airway and pleural pressures (as estimated by oesophageal balloon) and increases the transdiaphragmatic pressure (the pressure difference across the diaphragm measured with gastric and oesophageal balloons) [170]. While the resistive load is mediated by an external device the pressure changes are driven by the subject’s own inspiratory activity. This is opposed to negative pressure pulses, which have been used in studies examining cortical [149, 194, 195, 201, 202] and neuromuscular responses [144-153] to negative pressure, where the stimulus is largely independent of the subject.

### 3.3. Respiratory detection methodologies

Studies utilising resistive load stimuli have generally utilised conscious detection threshold [161-170] or magnitude scaling techniques [165, 171-173]. The
techniques have been described in a review article by Zechman and Wiley, originally published in 1986 and republished more recently [211]. Some of these techniques are briefly outlined below, however the current research was interested in the detection and response to small stimuli close to the conscious detection threshold, and therefore threshold detection techniques are discussed in more detail than other techniques.

3.3.1. Magnitude estimation
For this method subjects provide a numerical estimate of the magnitude of a series of stimuli above the threshold of detection. Numerical estimates can be based on a closed scale, where stimuli are compared to a reference stimulus, or an open scale, where there is no reference.

3.3.2. Magnitude production
For this method the subject manipulates the stimulus intensity to match the subjective magnitude of a numerical value supplied by the experimenter.

3.3.3. Cross modality matching
For cross modality matching the subject matches respiratory sensation intensity with a sensation from a different sensory modality, such as tension production when squeezing a handgrip.

3.3.4. Threshold measures
In threshold detection studies a number of resistive loads are added to the breathing circuit, for single breaths or for multiple consecutive breaths, with a varying number of unloaded breaths between the various loads. Loads are generally presented during inspiration, in a randomised order, and with intensities ranging from those that would never be detected to those that would almost always be detected, with the likelihood of detection increasing with increasing intensity. Subjects are usually screened from the apparatus and are instructed to indicate detection with a button press. To maintain attention and minimise response
variability subjects can be cued prior to stimulus delivery and a zero load control can be presented, which serves to provide an index of false positive responses.

Subsequently, the proportion of yes and no responses for each stimulus intensity are quantified and plotted against load intensity. The threshold is estimated from the resulting curve and is defined as the resistive load intensity at which the proportion of yes responses is 50%.

Bennet et al. [212] were the first to report on the threshold detection of added respiratory resistance and reported a threshold for detection of approximately 0.6 cmH$_2$O·L$^{-1}$·s. However these findings were later extended by Wiley and Zechman [167] in an important study that showed the significance of background conditions on detection of resistive loads.

3.3.4.1. The effect of background resistance on threshold detection

Wiley and Zechman [167] not only reported that resistive load detection is related to the background resistance of the extrinsic breathing circuit, they also demonstrated that the subject’s intrinsic airways resistance was important. For example, when subjects were placed in a supine posture detection threshold increased from 0.6 to 1.0 cmH$_2$O·L$^{-1}$·s with an associated increase in the internal resistance from 1.89 to 2.56 cmH$_2$O·L$^{-1}$·s, such that the detection threshold occurred at a common ratio of added resistance to background resistance of approximately 0.25-0.30. Consistent with this, subjects with increased intrinsic resistance due to chronic bronchitis had increased detection thresholds, which were similar to normal controls after the background conditions were taken into account.

The authors suggested that these results indicated that Weber’s law applied to perception of respiratory stimuli, in a similar fashion to perception of other modality
differences such as light intensity or added weights to the hand. Weber’s law states that the smallest difference between two stimuli that can be consciously perceived increases with background stimulus intensity [213]. This relationship means that same absolute added resistive load may be detected at a relatively lower but not a higher background resistance. This is an important concept when comparing OSA to control subjects because of the possibility that subject groups will have differing airway resistance.

Increased intrinsic resistance in OSA has been demonstrated in a number of previous studies using various methodologies to assess intrinsic resistance, such as rhinomanometry to measure nasal resistance [175, 176], catheters to measure upper airway resistance [177], plethysmography to measure total airway resistance [178] and forced oscillation technique to measure total respiratory resistance [179]. However other studies have reported no difference [180-182, 184] or increased resistance in a supine but not a seated position [183, 185, 214]. Increased resistance may be due to a smaller upper airway observed in OSA; related to increased soft tissue volume [92, 96] or craniofacial differences [215]. Inflammation related oedema may also be a factor in decreasing airway patency and increasing resistance [216]. Increased nasal resistance in particular has been recognised as a potential etiological factor in OSA [180] as flow limitation in the nasopharynx would lead to more negative pressure in the pharyngeal airway, favouring collapse. Current thinking however is that nasal resistance only plays a minor role in OSA pathogenesis [175, 217, 218] or that it may be important in association with certain pharyngeal conditions such as high pharyngeal resistance [217, 219].

### 3.3.4.2. The effect of stimulus timing on threshold detection

Conscious threshold detection studies have often applied the stimulus over the entire inspiration or multiple inspirations [161-165, 167-170] however for the current research a key interest was in the sensory detection of respiratory resistance using RREPs, which are best elicited by stimuli applied in mid inspiration [220].
An early study reported that mid-inspiratory stimulus delivery increases the threshold of detection [166] compared to an inspiratory onset stimulus, suggesting information generated early in the breath is important. However, a more recent study with methodology similar to the current research [174], examining the relationship between conscious detection thresholds and the RREP, reported that stimulus timing had no influence on the conscious detection threshold.

### 3.4. Conscious detection of respiratory and non-respiratory stimuli in OSA

It has been postulated that impaired sensory function may contribute to OSA pathogenesis as it may mean that compensatory responses to airway narrowing are inadequate to prevent further collapse. Previous investigators have studied sensory function in the upper airway in awake OSA subjects, using various non-respiratory methods including temperature sensitivity [221], two-point discrimination (2PD) [222, 223], as well as thresholds for vibration detection [222] and endoscopic air pulses [224]. Other studies have compared OSA subjects and controls in their ability to consciously detect respiratory resistive loads [172, 178, 186]. These studies will be discussed in further detail below.

#### 3.4.1. Non-respiratory stimuli

##### 3.4.1.1. Temperature sensitivity

Larsson et al. [221] examined temperature sensitivity in the oropharynx in 15 OSA subjects compared to 15 age-matched controls. They found that OSA patients had higher thresholds for warm temperatures that were more prominent at the anterior tonsillar pillar than the tip of the tongue, while the threshold for cold temperatures did not differ significantly. The authors postulated that this reduced sensitivity to warm temperature may be due to oedema caused by vibration and suction during snoring, or neuropathy in the pharyngeal mucosa, which may be primary, or secondary to vibration from snoring or mechanical stretching of pharyngeal structures during apnoeas; although no treatment was applied to help differentiate
between primary and secondary mechanisms. They suggested that the reduced sensitivity may be reflective of general defective function of local receptors, including those that subserve the local reflex motor regulation of the dilator muscles of the upper airway.

### 3.4.1.2. Two-point discrimination

2PD has been examined in OSA in a number of studies [222-224], where 2PD threshold is defined as the smallest inter-probe distance at which the subject correctly identified one versus two points.

Kimoff et al. [222] measured 2PD to assess sensory function in 37 OSA patients, 12 non-apnoeic snorers and 15 controls. 2PD in the upper airway was compared to two control sites on the lower lip and hand. 2PD was selectively reduced in OSA subjects and snorers in the upper airway compared to controls, however snorers and OSA subjects were not significantly different from each other. In 23 OSA patients 2PD did not improve after 6 months of treatment with CPAP. In that study subject groups were not matched for BMI, however the authors argued this was not a factor given that there was no differences in 2PD at control sites. The findings of a deficit in 2PD in OSA were also replicated in a later study by the same group [224].

Similar testing has also been performed in lean OSA subjects, compared to those with a more subtle respiratory disturbance: upper airway resistance syndrome (UARS), and controls, that were matched for BMI [223]. That study reported deficits in palatal 2PD in OSA subjects but not UARS subjects or controls. They postulated that these results could be explained by: (i) differences in the amount or longevity of snoring, (ii) differences in the type of snoring (e.g. retropalatal vs. retroglossal), or (iii) differences in features such as reflux and thoracic pressures, that may alter the time course of destruction of palatal mucosa.
3.4.1.3. Vibration detection threshold

In the study that examined 2PD, Kimoff et al. [222] also examined vibration detection thresholds (VT). In that component of the study a controller delivered a vibrating stimulus at 120 Hz, using a 1.5 cm plastic post, to fingertip, lower lip, and oropharynx, and the amplitude of vibration was determined by varying the voltage at the controller unit. Similar to 2PD results they found that VTs were impaired in OSA patients and snorers in the oropharynx, compared to controls. However in contrast to 2PD, there were significant improvements in VT after treatment with CPAP, although the mean values were still greater than control subjects. Taken together with the 2PD results the authors suggested these findings indicate the presence of a selective and partially reversible impairment in the detection of mechanical stimuli in the upper airway of patients with OSA and snoring. The authors postulated that deficits were most likely secondary to mechanical trauma in OSA, however as only partial reversibility was observed, the authors could not rule out sensory impairment as an etiological factor in OSA development. The findings of a deficit in VT in OSA were also replicated in a later study by the same group [224].

3.4.1.4. Endoscopic air pulses

The above findings suggesting impairment of upper airway mucosal sensory function in OSA were extended by a study using an endoscopic air pulse as a stimulus [224]. Such a stimulus allowed testing of sensory function in other regions of the upper airway including the velopharynx, hypopharynx and larynx, in addition to the oropharynx. They tested 39 untreated OSA patients and 17 controls and found significant impairment in the detection threshold of the air pulses in the oropharynx, velopharynx and larynx but not the hypopharynx. Impairment at the larynx was also demonstrated more objectively by an increased threshold stimulus intensity required to elicit the protective laryngeal adductor reflex, which is a protective reflex characterised by brief closure of the true vocal chords.
3.4.1.5. Summary

Numerous investigators have shown impaired upper airway mucosal sensory function in awake OSA subjects and snorers by examining conscious detection of various non-respiratory stimuli. This impaired sensory function appears to be selective in that it is seen in the upper airway but not at control sites such as the lip and hand. The predominant view is that this deficit is a secondary phenomenon with sensory impairment developing as a consequence of the mechanical trauma associated with OSA [30, 222, 224]. In support of this view there is evidence that OSA can lead to changes in upper airway structures (reviewed by Saboisky et al. [225]). For example, an increase in type IIA muscle fibres has been observed in OSA, which is thought to be as a result of a training effect due to repetitive loading. Also, OSA has been associated with inflammatory changes to upper airway soft tissue structures, with localised swelling possibly leading to airway narrowing and exacerbation of airway collapse. Nevertheless, a sensory deficit has not been ruled out as an etiological factor in OSA, and it remains possible that impaired upper airway mucosal sensory function may be a result of a combination of primary and secondary mechanisms.

Authors have suggested that a general attenuation of upper airway mucosal sensory function may denote a defect in the afferent limb of the negative pressure reflex, which may result in reduction of upper airway dilator muscle activity and thereby contributing to upper airway collapse [222, 224]. This idea is supported by studies showing that topical anaesthesia reduces upper airway dilator muscle activity [152, 226, 227]. Although studies suggest impairment of upper airway mucosal sensory function in OSA, it must be noted that the stimuli are not respiratory in nature and therefore do not take into account the many potential sites for receptors subserving respiratory load perception. For example Gandevia et al. [228] examined the threshold for detection of negative pressure changes applied at the mouth during inspiration. They found that the threshold for detection was elevated by closing the glottis and confining the pressure to the upper airway and from this and other observations concluded that information from active inspiratory
muscle contraction played an important role in sensory detection of negative pressure.

### 3.4.2. Respiratory stimuli

Studies examining conscious detection of increased respiratory resistance, arguably a more physiologically relevant stimulus, have shown mixed results. Clerk et al. [178] found no difference between OSA subjects, snorers and controls, whereas an earlier study found an increased detection threshold for OSA subjects compared to controls [186]. Another study demonstrated reduced magnitude estimation of large increases in respiratory resistance in OSA subjects [172]. These studies will be discussed in more detail below.

McNicholas et al. [186] examined threshold detection of flow-resistive loads during wakefulness in 5 OSA patients compared to 9 healthy controls. They reported that the threshold level of load detection was higher in the OSA patients than in the healthy subjects and that this difference persisted when the threshold load was corrected for background resistance (mean ± SD.: 1.05 ± 0.11 vs. 0.45 ± 0.07; \( P < 0.001 \)). The authors argued against obesity being a contributing factor, in particular arguing that the increased elastic load in obesity would only have a small influence on threshold detection; however while they reported that 3 of 5 OSA patients were overweight they did not report the BMI differences between groups. Additionally they acknowledged that inattention could have been a contributing factor given the EDS observed in the OSA patients. However they also noted that, contrary to the importance of EDS, the patients were cued prior to stimulus presentation, there were minimal times when participants failed to respond with a button press, and there were no differences in the precision with which added loads were detected, in that correlation coefficients relating stimulus magnitude to percent detection were no different between OSA and normal participants.

In contrast to McNicholas et al. [186], Clerk and colleagues [178] reported no difference in threshold detection of resistive loads when comparing eleven OSA
patients, 7 male snorers and 10 normal controls, after corrections were made for background resistance. However in that study severely obese patients were excluded, such that the authors suggested that abdominal obesity may be the key reason for the difference between the two studies. Contrary to the McNicholas study the authors speculated that the differences may have been due to the added elastic load caused by obesity, as constant background elastic loads have been shown to increase the conscious detection threshold of resistive loads [161]. Given there were no differences in conscious detection despite many of their OSA subjects reporting excessive daytime sleepiness, Clerk and colleagues [178] argued against sleepiness and attention having an important influence on conscious detection of respiratory loads. From their results the authors also argued against a defect in sensory detection contributing to the development of OSA.

Reduced magnitude estimation of large resistive loads (10, 20 and 30 cmH₂O·L⁻¹·s⁻¹) was reported in OSA patients (seated and awake) compared to controls in a Japanese laboratory [172]. In that study there were no differences in BMI and age between groups and so these factors could not account for group differences in magnitude estimation. Also, although background resistance of the patient was not taken into consideration, any differences in background resistance would be less likely to influence the results given that they would only be a small proportion of the large resistive loads presented. Additionally, this difference was abolished after two weeks of CPAP treatment which is not consistent with background resistance contributing to results. The correction after CPAP is also suggestive that the sensory deficit is secondary in nature and not a primary factor contributing to the development of OSA. Overall, the authors suggested that their results were consistent with reversible injury caused by snoring vibration and repeated forceful suction collapse of the pharynx. Alternatively they suggested that sensory detection of resistive loads is influenced by reversible excessive daytime sleepiness.
In sum, studies examining conscious detection of increased respiratory resistance in OSA have shown mixed results. One study found no difference between lean OSA subjects and normal controls in their detection threshold of inspiratory resistance [178]. Of the studies showing significant differences between OSA subjects and controls, it has been suggested for 1 in particular [186] that obesity was a contributing factor [178]. Additionally, while there is some suggestion that a secondary sensory deficit exists, studies to date have not been able to rule out EDS and inattention contributing to their findings.

3.5. Summary and conclusions

The study of conscious detection of respiratory challenge and upper airway sensory function in OSA has been an area of interest because of the possibility that impairment in sensory function may lead to inadequate neuromuscular compensation in the face of a respiratory challenge. A number of studies have suggested a sensory deficit in OSA in response to non-respiratory stimuli that is specific to the upper airway and is partially reversible by treatment with CPAP. The predominant view is that this sensory impairment is a secondary consequence of the mechanical trauma of snoring and repetitive airway collapse, however a sensory deficit has not been ruled out as an etiological factor in OSA. It must be noted however that because experimental stimuli are not respiratory in nature they do not reflect the flow and pressure dynamics that occur in the collapsing airway. Additionally they fail to allow for the possibility that other non-upper airway respiratory structures may be involved in sensory detection of respiratory stimuli. Studies that have examined sensory detection of respiratory stimuli have reported impaired threshold detection and impaired magnitude estimation in OSA, although in general it is not possible to rule out confounding factors contributing to these results. In particular, EDS and associated impaired attention is a prominent feature of OSA [84] which may confound results of studies examining conscious detection of respiratory stimuli. For this reason more recent studies have investigated the cortical response to respiratory stimuli using the RREP.
One of the key aims of this thesis was to determine if there were impairments in OSA patients during wakefulness in the detection of small negative pressure respiratory load stimuli, close to the conscious detection threshold. Due to the limitations and confounding factors associated with conscious detection studies, the key experimental chapter (Chapter 7), examining sensory detection in OSA, did not use conscious detection measures as a primary outcome. Similar to the more recent sensory detection studies chapter 7 utilised the RREP as a primary outcome, and this technique is discussed in detail in the following chapter.
4. Respiratory Related Evoked Potentials

4.1. Introduction and definition

The RREP represents the cortical response (EEG) to a sudden onset respiratory stimulus and, as with other sensory modalities, the RREP is derived by averaging the EEG response to multiple stimulus presentations [188]. Multiple presentations are required to allow the stimulus response to stand out from background noise, or non-event related cortical currents [187]. It represents the synchronous activity of populations of neurons [229], generating a dipole, likely related to the simultaneous stimulation of a group of sensory afferents that project to the cerebral cortex [187]. The strength of the dipole is related to the number of neurons activated and the current amplitude relates to the dipole strength; and hence the current amplitude relates to the stimulus magnitude [187]. It has been suggested that the pattern of RREP peaks represent the neural basis of the temporal sequence of respiratory sensation [230]. Thus the RREP is used to study respiratory sensation and is thought of as a more objective measure of sensory function than methods involving conscious detection of a stimulus. An RREP example has been shown previously in Figure 1-4.

As synchronised activity is sometimes seen in preparation for discrete events, the non-respiratory literature has moved away from the term ‘evoked potential’ in favour of the broader term ‘event related potential’ [231]. However, this is not the case in the respiratory literature where the term ‘respiratory related evoked potential’ is used exclusively to describe the averaged cortical (EEG) response to multiple presentation of the same respiratory stimulus. As such, this term and its abbreviation, RREP, are used throughout this thesis.

This chapter outlines what is known about the RREP and its components. It outlines and discusses some of the methodological issues associated with production of the RREP in terms of the stimulus, recording and analysis. In addition this chapter discusses what is known about the relationship between conscious
detection and the RREP, and lastly, examines the literature using RREPs to study sensory detection in OSA patients compared to control groups.

4.2. RREP Components

RREPs are made up of positive and negative components. Its early components are considered exogenous as they are largely influenced by characteristics of the stimulus, whereas the later components are considered endogenous in that they are influenced by the subject’s conscious state and psychological condition [187].

Components are typically labelled in terms of their polarity (N for negative or P for positive) and either in terms of their order from the stimulus presentation (e.g. the first negative component is labelled N1 and the first positive component is labelled P1) or in terms of their typical latency from stimulus onset (e.g. A positive component typically at 300ms would be labelled P300). Both the order and latency approaches have pitfalls. For example, using the order approach Webster and Colrain [232] described an earlier component than those previously described and labelled it P1a. Labelling by latency creates difficulties if latencies differ according to stimulus characteristics or when there are individual subject differences.

Component labelling is also confounded by the fact that the RREP morphology may be influenced by the electrode placement or nature of the stimulus, or by whether or not attention is paid to the stimulus, and these issues will be discussed later in this chapter. In terms of EEG electrode placement, recent studies have generally used a linked ear or linked mastoid reference, reporting Nf, P1, N1, P2 and P3 (P300) components. The current research also used a linked mastoid reference and therefore the key components using this reference are described below.
4.2.1. Early Components

4.2.1.1. Nf

The Nf peak is the first negative peak of the RREP in response to sudden onset negative pressure stimuli, and is unique to the RREP as, for example, there is no equivalent peak in the somatosensory evoked potential in response to a tactile stimulus [197]. It was first described in children in response to occlusion, and was noted to be maximal in amplitude in frontal regions with an electrode array referenced to linked ears, but not with a cephalic reference [196]. In that study the Nf peak occurred later than the P1 peak, however subsequent studies in adults consistently show the Nf peak occurring prior to P1 [174, 197, 198, 232], with latencies from stimulus onset in the range of 25-80ms observed [171, 173, 174, 190, 198, 232]. Due to the different patterns of waveform recorded anterior and posterior to the central sulcus, as well as the temporal overlap of the Nf and P1 components, it was suggested that P1 and Nf are generated from separate cortical generators [196]. This was later supported in a dipole modelling study which suggested that Nf and P1 were likely generated from separate radial dipoles; for the Nf component the location of the dipole was consistent with a supplementary motor cortex source [233]. While it has been reported that the Nf component is not augmented by attention [191], it is unclear if Nf amplitude increases with increasing load magnitude; increasing Nf amplitude with increasing stimulus magnitude has been reported [174] but not consistently [173]. The role of Nf in the sensory processing of respiratory stimuli is not known but because of its early latency and source localisation it has been suggested that it may reflect a predictive process in cognitive functioning [187] or some form of preparatory motor response [173].

4.2.1.2. P1

The P1 component is the first positive component of the RREP (although as mentioned one study presenting occlusions reported an earlier positive component that they labelled P1a [232]), and is said to correspond to the somatosensory evoked potential P50 produced by mechanical stimulation of the leg [189]. The P1 component has been noted to be maximal in amplitude in parietal [173, 196, 233]...
and centro-parietal [191, 232, 234, 235] regions of the cortex. Using 29 scalp electrode sites Logie et al. [233] modelled the electrical dipoles produced by inspiratory occlusion, and found the P1 component was likely generated by radial cortical generators posterior to the central sulcus, consistent with a possible location in the trunk region of the primary somatosensory cortex.

P1 is typically recorded with latencies from stimulus onset in the range of 45-75ms [173, 174, 198] although latencies greater than 100ms have been reported [171, 190, 236]. This range of latencies likely relates to the fact that the stimulus in RREP studies is often generated by the subject inspiring against a resistive load or occluded breathing circuit, and is less direct compared to mechanical or electrical stimulation of a foot or hand. In fact, the mouth pressure change at 0.1 s following stimulus onset delivered at initiation of inspiration ($P_{0.1}$) has been found to be inversely correlated with the latency of the RREP P1 peak [189]; the $P_{0.1}$ is said to be a measure of respiratory drive [189, 237] but will also be influenced by the experimental breathing circuit. A number of studies have shown that the P1 peak amplitude increases with stimulus magnitude [173, 174, 192, 193], and additionally P1 amplitude is correlated with magnitude estimation [173, 193], however the P1 peak is not influenced by attention [174, 191].

From these observations, and from what is known about afferent pathways from animal studies, it is suggested that P1 peak reflects the arrival of respiratory load related sensory information at the somatosensory cortex [189], and more generally that the RREP is a neural indicator of cortical sensory information processing related to conscious detection of respiratory loads [174, 198].

4.2.2. Late Components

4.2.2.1. N1

The second negative peak of the RREP is the N1 peak, with topographical studies showing the N1 component to be maximal in amplitude in central regions of the cortex [173, 191, 232] with a wide band of maximal activity over the somatosensory
cortex [232, 234]. Source localisation using a conservative source estimation algorithm known as minimum-norm estimate (MNE) suggested an N1 origin in the lateral sensorimotor cortex plus an additional frontal cortex source not evident in topographical studies [234]. The N1 has typically been reported to have latencies from stimulus onset in the range of 85-130ms [173, 174, 198] although latencies up to 170ms have been reported in adults [190]. The evidence as to whether N1 amplitude increases with resistive load magnitude is conflicting; Webster and Colrain [173] found that N1 amplitude did not increase with increasing magnitude of resistive loads. In that study 500ms mid-inspiratory resistive loads were presented (3, 6, 12, and 24 cmH$_2$O·L$^{-1}·$s) as well as a control condition and subjects were required to attend to the load by categorising the intensity. In contrast Davenport et al. [174] reported that N1 amplitude was greater with larger resistive loads, but only when the stimulus was attended to [174]. In that study two 500 ms supra-threshold resistive loads (~7 and 11.5 cmH$_2$O·L$^{-1}·$s) were presented after the onset of inspiration, in addition to a sub-threshold resistive load and a control condition. A comparison was made between an ‘ignore’ and an ‘attend’ condition, where subjects were required to press a button if they detected the stimulus. They reported that N1 amplitude was greater for the larger supra-threshold load in the ‘attend’ but not the ‘ignore’ condition. Increased amplitude of N1 in response to occlusion has also been reported when attention is directed to the stimulus by categorising the occlusion length [191]. Thus it is possible that N1 may be influenced by the stimulus characteristics as well as subject attention.

While it is unclear what the N1 wave represents in terms of cognitive processing, due to its dependence on attention and its temporal location it has been suggested that this peak might represent a neural triggering or gating process that links the primary sensory neural information and the attention related cognitive processes [174, 187, 197, 230].

4.2.2.2. P2

The P2 component has received considerably less attention than other components and is sometimes not reported [174, 197], possibly because the
functional significance of P2 in response to respiratory and non-respiratory stimuli is not well understood [238]. The P2 has been found to be maximal in central regions of the cortex [173, 191, 232, 234], regardless of the position of the reference electrode. Using the linked ear reference its latency is later than the N1 component and earlier than the P3 component at a stimulus latency of approximately 170-220ms [171, 173, 191, 232]. While the P2 component amplitude is increased when attention is directed to the stimulus [191], it is still present without directed attention and therefore it is thought to have both endogenous and exogenous influences [171, 238], however P2 does not increase in amplitude with increasing stimulus magnitude [173].

4.2.2.3. P3 or P300

The P3 peak is part of a large positivity that in topographical studies has been observed to be maximal in parietal regions of the cortex [173, 191, 232, 234]. Consistent with this, the source localisation study of von Leupoldt et al. [234], using MNE, reported maximal activation in the lateral parietal cortices with left hemisphere dominance.

The P3 has been reported to have latencies from stimulus onset in the range of 250-350ms [171, 173, 174, 198]. The P3 component amplitude increases with increasing load magnitude [173, 174] and is associated with magnitude estimation of the stimulus [173]. Additionally P3 amplitude has consistently shown to be related to attention; it is diminished or absent if attention is not directed to the stimulus [174, 191, 239]. Its amplitude is also influenced by stimulus context; for example larger amplitudes are observed with smaller stimulus probability, where stimulus probability is manipulated by varying the number of unloaded breaths between stimulus delivery [240]. From these observations the P3 peak is considered to be related to the cognitive perception of respiratory stimuli [173, 174, 191].
4.3. RREP Methodology

As has been mentioned there are number of methodological issues when producing the RREP in terms of the stimulus, recording and analysis. This section discusses these issues and in particular highlights how decisions were made regarding methodology used in the current research.

4.3.1. Stimulus delivery

4.3.1.1. Stimulus type

The RREP has mostly been studied in response to negative airway pressure stimuli. Negative pressure is a force promoting collapse of the airway, and therefore detection of negative pressure theoretically provides a survival benefit, allowing some form of response to maintain airway patency and maintain ventilation.

The RREP has been recorded in response to various negative pressure stimuli including airway occlusion [189-191], negative pressure pulses [194, 195] and inspiratory resistive loads [173, 174, 192, 193, 241]. For the current research inspiratory resistive loads were used as the respiratory stimulus. This is because the key research interest was the detection and response to small negative pressure stimuli close to the conscious detection threshold, and inspiratory resistive loads can provide a more subtle stimulus compared to occlusion and negative pressure pulse stimuli. The method for delivering a resistive load has been described previously (Chapter 1). Briefly, the resistive load impedes airflow into the lung and results in more negative airway and pleural pressures and increases in transdiaphragmatic pressures at a given flow rate [170].

4.3.1.2. Stimulus magnitude

One of the key aims of the current research was to examine whether threshold respiratory load detection, measured using the RREP, was impaired in OSA patients. To do this it was planned to present multiple resistive load stimuli with at least some close to, and if possible, spanning the conscious detection threshold.
The conscious detection threshold will vary depending on the equipment and population sampled [167]. The pioneering study of Bennet et al. [212] reported a threshold for detection of approximately 0.6 cmH₂O·L⁻¹·s, however more recently in normal subjects the mean ± SD has been reported to be 2.66 ± 1.22 cmH₂O·L⁻¹·s (range 0.60–5.10 cmH₂O·L⁻¹·s) [174]. Similarly, pilot testing of the equipment used in the current studies in normal subjects revealed a mean conscious detection threshold of 2.5 ± 1.2 cmH₂O·L⁻¹·s (range 0.5 – 4.7 cmH₂O·L⁻¹·s). Based on this information four resistive loads were presented to the participants: ≈1.2, 2.2, 3.0, and 6.2 cmH₂O·L⁻¹·s. Pilot data predicted that for the majority of participants the lowest intensity load would be below the conscious detection threshold, the largest intensity resistive load would be above the conscious detection threshold, and the two intermediate resistive loads would be close to the conscious detection threshold. The number of different intensities presented was constrained by the need to minimise the duration of the protocol for participant comfort and compliance. Other considerations impacting the duration of the protocol included the number of presentations of each load required to produce the RREP, the frequency of stimulus presentations which in previous literature is generally in the order of every 2-6 breaths, the desire to present an occlusion stimulus to guide RREP component detection and classification, and the desire to present a zero load control to account for predictive or other non-stimulus related influences. Some of these factors are discussed in more detail below.

In addition to the 4 resistive loads an occlusion stimulus was presented. The occlusion stimulus was primarily used to aid RREP component detection and allow for individual subject differences in the latency of components. Based on the RREP component latencies produced by occlusion, component detection windows were created for each individual subject. These windows were set around the component latencies in response to occlusion, on the EEG channel where the component was maximal. The windows were then used to examine the ensemble averaged responses to the other various stimulus intensities to judge whether
RREP components were present or absent. This technique was possible as latency generally does not change with stimulus magnitude [173].

A zero load control stimulus was also presented in the current studies. This stimulus is important for conscious detection studies as it provides an index of false positive responses. In terms of the RREP it has been recognised that anticipation, artefacts or other non-stimulus related experimental factors may lead to deviation in the ensemble average trace; the zero load control load accounts for these deviations by subtracting the waveform from the loaded condition averages [192, 193]. Only some studies have used a zero load presentation [174, 192, 193, 220] and on even fewer occasions has the zero load control been accounted for as described above [192, 193].

In total then, in the current RREP studies there were 6 different stimulus conditions presented to the participants, 4 resistive loads of varying intensity, an occlusion stimulus and a zero load control. These stimuli were presented in a random order to minimise temporal, order, and sequence effects.

### 4.3.1.3. Stimulus delivery route

The majority of RREP studies have delivered the stimulus via the oral route [174, 189, 191, 196-198, 201, 202, 242] while a small number have presented the stimulus via the nasal route [149, 171, 199]. This is despite the nose being the normal breathing route during basal breathing [243]. Using the nasal route has the potential to influence the RREP due to increased resistance compared to the oral route [244, 245] and because increased background resistance is likely to reduce RREP amplitude [198]. Despite this one study has reported no significant differences in RREPs elicited by occlusion during nose breathing compared to mouth breathing [246]. Pilot testing for the current research suggested increased comfort via the nasal route over the oral route, which was a significant consideration given the lengthy 3-4 hour protocols.
4.3.1.4. Stimulus timing

The majority of RREP studies have delivered the negative pressure stimulus during inspiration [171, 174, 189, 191, 197, 198, 220, 232, 240]. This likely relates to the fact that collapsing force of negative pressure is greatest during this part of the respiratory cycle. However studies have varied as to whether the stimulus is presented at onset of inspiration or as an interruption to inspiration when the negative pressure is greater. One of the first studies to record RREPs presented the stimulus immediately prior to onset of inspiration [189], however Revelette et al. [220] later examined the impact of stimulus timing on RREP amplitudes and latencies. That study compared inspiratory onset occlusions to mid-inspiratory occlusions. Occlusions from the onset of inspiration were performed by the experimenter pressing a footswitch to inflate a balloon occluder during the subject’s expiratory phase. The subsequent inspiratory effort began against the occluded airway. Mid-inspiratory occlusions were performed by computer activation of a solenoid that initiated the balloon inflation with a preset delay after the beginning of inspiration. The delay was adjusted such that interruption of inspiration occurred 150 ms before peak flow and was maintained constant for each individual. They found that the peak amplitude of RREPs was greater and the peak latency shorter for the evoked potentials produced by mid-inspiratory occlusions. Many subsequent studies have thus also initiated stimuli after commencement of inspiration [171, 174, 191, 193, 197, 198, 232, 235, 240, 247, 248], as is the case for the current research.

4.3.1.5. Stimulus duration

Stimulus duration has been shown not to influence the RREP component amplitudes or latencies [191], indicating that the beginning of the pressure change is the important aspect in producing the RREP. However it is important that the stimulus presentation is longer in duration that the latest component of interest; pilot testing revealed that pressure changes at stimulus offset may also produce deflections in the ensemble average EEG traces. Prior research has indicated that
the latest component of interest for the current study, the P3, occurs at average latencies from stimulus onset in the range of 250-350ms [171, 173, 174, 198], although pilot testing for the current study revealed latencies over 400ms. In the current study, to mitigate the influence of pressure changes at the offset of the stimulus on the RREP it was decided to deliver the resistive load stimuli until the end of inspiration. For reasons of participant comfort the occlusion stimulus was delivered for a maximum of 800ms.

4.3.1.6. Stimulus frequency

Previous RREP studies have used various frequencies of stimulus presentation, generally without explanation of rationale, including every 4-7 breaths [174, 192], 3-7 breaths [189, 193, 198], 3-6 breaths [220], 3-5 breaths [233], 2-6 breaths [171, 197], and 3-10 breaths [241]. As there is no stimulus presented during the intervening unloaded breaths, presentation of the stimulus in this manner is referred to as a single stimulus paradigm, as opposed to an “oddball” setting where there are two stimuli; a target and a standard [240].

Although not explicitly outlined, the rationale for frequency of stimulus presentation in previous research likely relates to prevention of habituation or refractoriness. A study examining the impact of stimulus frequency (probability) on RREP demonstrated increased P3 amplitude with decreased probability of presentation (ranging from every second to every 20th breath) [240] but no impact on earlier components such as Nf, P1 or N1. However the relevance of this finding is questionable for the current research, as subjects are cued on the breath prior to stimulus presentation with an auditory message. Another study examined the RREP response to paired 150ms occlusions in a single breath, separated by a 500ms interval, and reported reduced peak Nf, P1 and N1 amplitudes for the second presentation compared to the first presentation. Thus it is clear that early component amplitudes may be impacted if a second stimulus is presented within a single breath, but not if consecutive stimuli are presented with an intervening
unloaded breath. This data also suggests chemosensory feedback has little impact on RREPs in response to subsequent loads.

While no studies have examined the impact of presenting stimuli on consecutive breaths, for the current research it was decided to present the stimuli every 2-4 breaths. This frequency was chosen as it minimised the influence of habituation or refractoriness on RREP amplitudes, it allowed comparison to similar previous research, and it maximised the comfort of participants by minimising the length of the protocol. Importantly, the key focus of the research was on the early components of the RREP, which are minimally influenced by stimulus frequency.

4.3.1.7. Number of stimulus presentations
Examination of the threshold stimuli for elicitation of the RREP requires multiple presentations of multiple different stimulus intensities, with a separate ensemble average generated for each stimulus magnitude. The RREP benefits by increasing the number of stimulus presentations by increasing the signal-to-noise ratio, however the signal-to-noise ratio only increases as a function of the square root of the number of trials [249]. Thus increasing the numbers of stimulus presentations from 16 to 64 will double the signal-to-noise ratio, however to double it again would require 256 presentations. The number of presentations thus becomes a trade off between maximising signal-to-noise and comfort and tolerance of participants regarding the length of the protocol. This is a particular consideration for RREPs as opposed to evoked responses to non-respiratory stimuli, as respiratory stimulus presentations are generally limited by the respiratory cycle (e.g. respiratory stimuli are commonly presented at a particular point in the respiratory cycle, every 2-6 breaths, and basal breath rates are generally in the range of 13-15 breaths per minute). The early study of Davenport et al. presented 256 occlusion stimuli in a protocol lasting 60-90 minutes [189]. However, more recent studies with multiple load intensities have used as few as 50-75 stimulus presentations for each intensity [173, 174] in producing the RREP. Additionally the number of load
presentations included in the RREP is likely to be smaller than the number presented, as individual presentations are generally only included if they are correctly timed to produce the expected pattern of negative pressure change, and if the EEG is stable and free from blink or movement artefact [174, 242]. In the current study it was particularly important to maximise the signal-to-noise ratio, given that it examined responses to loads close to the conscious detection threshold, however it was not feasible to present 256 presentations of each stimulus magnitude because of the time it would take to complete the protocol. It was decided to present 90-100 presentations of each load magnitude, allowing for up to 1/3 of them to be excluded from the RREP due to problems with the stimulus presentation or EEG artefact described above. Pilot testing revealed that this number of presentations would produce discernible RREP components.

4.3.1.8. Cueing prior to stimulus delivery
To maintain subject attention and minimise response variability subjects can be cued prior to stimulus delivery. Cueing the subject by drawing attention to the stimulus, may also serve to maximise the amplitude of later components of the RREP; N1, P2 and P3 have all been shown to increase in amplitude when attention is paid to the stimulus [174, 191, 239]. In the current study the participants were cued via headphones at end inspiration on the breath prior to stimulus presentation with an automatically generated message “next breath”. Maintenance of attention was a particular consideration in the current study due to the lengthy and monotonous protocol participants were subjected to.

4.3.1.9. Masking of equipment noises
A number of actions were taken in the current experiments to minimise the influence the experimental noises, which may influence the RREP. Firstly, the resistive loading device (including the balloon valves) was located in a room adjacent and separated by a brick wall from the patient. Secondly, the participants wore headphones which also served to deliver a prompt to the participant prior to stimulus delivery. Thirdly, music of the participant’s choice was played inside the
same room as the participant. Fourthly, although not necessary to do so, balloon valves were fired when presenting the zero load control.

**4.3.1.10. Description of stimulus delivery equipment**
The equipment used in the current research is shown below in Figure 4-1. The key element for the delivery of resistive loads to the study participant is a loading manifold. The loading manifold used in the current studies was custom made from clear acrylic (10 mm, Plasticut, Campbellfield, Victoria, Australia) with dimensions of 210 x 210 x 470 mm. The manifold was subdivided by clear acrylic partitions with cut-outs of various diameters. The cut-outs were covered with perforated stainless steel sheet (thickness: 0.9 mm; perforation diameter: 0.1 mm; open area: 3%; ActionLaser, Hornsby, New South Wales, Australia). Each subdivision of the manifold contained a port to the manifold exterior containing a fast actuating balloon valve (9340 series, Hans Rudolph, Shawnee Mission, KS, USA). Another port at one end of manifold was connected to the study participant located in an adjacent room via a t-piece balloon valve (8250 series, Hans Rudolph), reinforced tubing, a non-rebreathing valve (series 2600, Hans Rudolph, Kansas City, MO, USA) and a modified non-vented nasal CPAP mask (Profile Lite, Philips Respironics, Murraysville, PA, USA).

The balloon valves controlling manifold airflow were regulated by a balloon valve controller (2430 series, Hans Rudolph), which maintained balloon inflation/deflation times of less than 120ms. Application of stimuli was achieved by activation of a t-piece balloon valve, directing inspiratory flow through the manifold, and simultaneous activation of various combinations of the manifold port balloon valves. This allowed presentation of a zero load control condition, as well as various resistive loads, with good linearity characteristics, designed to span the detection threshold (≈1.2, 2.2, 3.0, and 6.2 cmH₂O·L⁻¹·s). During bypass of the manifold (between stimulus breaths), which was achieved by de-activation of the t-piece balloon valve at the manifold entrance, as well as during the zero load control, the circuit resistance was approximately 2 cmH₂O·L⁻¹·s. The manifold could
also be completely occluded. Stimuli were delivered during mid-inspiration and continued until end inspiration for all stimuli except for the occlusion stimulus which, for participant comfort, was presented for 800 ms.
Figure 4-1: Equipment diagram. 
Notes: See text for detailed explanation. Abbreviations: EEG: electroencephalogram; EOG: Electrooculogram; EMG: Electromyogram.
4.3.11. Automation of stimulus delivery

Regulation of the balloon valve controller and therefore load presentations was performed using a custom script developed using Spike 2 software (Cambridge Electronic Design, Cambridge, England). The automated script was responsible for:

1. Detection of the start and end of inspiration using the flow signal,
2. The delay in stimulus delivery so that it occurred at mid-inspiration,
3. The timing of stimulus delivery so that they occurred every 2-4 breaths,
4. The firing of the various combinations of balloon valves (using a transistor-transistor-logic (TTL) pulse), via the balloon valve controller, to deliver the various stimulus intensities,
5. The order of presentation and randomisation of the various stimulus intensities (block design with one presentation of each stimulus intensity in each block),
6. Cueing the subject prior to stimulus delivery,
7. Marking the recording traces at the point of firing of the TTL pulse (used later for averaging traces), and
8. Counting the number of stimulus presentations.

4.3.2. Recording Considerations

4.3.2.1. Electrode location

Vertex vs. Linked earlobe reference

One of the earliest studies examining the RREP in humans used a small number of electrodes with a cephalic (vertex) reference (Cz according to the 10-20 electrode placement convention) [189]. In that study occlusions at the onset of inspiration produced RREPs in electrode pairs thought to overlay the left somatosensory cortex (e.g. C3-Cz), producing a consistent pattern of peaks, identified as P1, N1, P2, and N2. RREPs were not apparent with other electrode pairs (Fz-Cz, Fz-Fh), or during unoccluded breaths. Many subsequent studies also used a similar vertex
reference [192, 193, 195, 220, 234, 246, 250] although others have used a single mastoid reference [241] or a reference on the torso [209], which in the context of somatosensory evoked potentials is said to be useful if subcortical components are of interest [251]. The majority of the most recent studies however have used a joined ear lobe (or mastoid) reference in recording the RREP [149, 173, 174, 190, 191, 197, 198, 232, 235, 242, 248] and as such so did the current studies. The ear or mastoid reference largely eliminates subcortical components, as they are also picked up at the reference electrode, and has the theoretical advantage of being relatively electrically quiescent compared to a vertex reference [251-253]. In fact it has been argued that the presence of RREP components with a vertex reference and closely spaced electrodes in the landmark RREP study by Davenport [189] was fortuitous and likely related to the lateralization of the somatosensory cortical generators [254]. Linking of electrodes allows ‘common mode rejection’ of non-physiological electrical activity common to both electrodes.

The use of differing reference electrodes results in different waveforms [187]. In particular, with a linked-earlobe but not the vertex reference, a short latency negative peak is seen most prominently with frontal leads (Nf), and a late positive peak is observed when attention is directed to the stimulus (P3 or P300). In contrast an N2 component is observed with a vertex but not linked-earlobe reference. Thus the components typically observed with a vertex reference are P1-N1-P2-N2, whereas components typically observed with a joined earlobe reference include Nf-P1-N1-P2-P3.

One RREP study that has utilised both a vertex and joined ear-lobe reference in a single study is that of Davenport and colleagues [196], in paediatric subjects. They reported that a particular positive peak was observed most prominently at the C3, C4, P3, and P4 electrode sites with a joined-earlobe reference, which the authors suggested corresponded to the P1 peaks with the vertex reference. Whether this observation can be extrapolated to adults is questionable however, as there are a number of points of difference with that study in children [196], compared to
subsequent studies in adults. Namely, the first negative peak (Nf) occurred later than P1 in children, whereas studies in adults consistently show the peak occurring prior to P1 [174, 197, 198, 232]. Additionally, RREP components were not observed in midline electrode locations with a linked-ear reference in children [196], however in adults components are routinely observed using midline electrodes [173, 197, 232, 233]. Ultimately therefore it is unclear how the RREP peaks measured with a vertex reference relate to the peaks measured with a linked-ear reference in adults, complicating comparison between studies.

The early study of Davenport and colleagues [189] related the waveforms observed with a vertex reference to those observed with somatosensory event related potentials, recorded in response to stimulation of the upper and lower limbs. The prominent pattern of cortical components produced by somatosensory stimuli have been described as a ‘W’ complex [253, 255]; components produced by lower-limb tibial nerve stimulation include P39-N50-P60-N75, and for upper limb median nerve stimulation include P22-N30-P45-N60 [253], suggested by Davenport [189 ] to be comparable to P1-N1-P2-N2 of the RREP with a cephalic reference. However it is unknown how the peaks of the RREP produced with a linked ear or mastoid reference relate to somatosensory event related potentials.

**Midline vs. Lateral electrode locations**

Likely based on studies suggesting source localisation of early P1 in the trunk region of the primary somatosensory cortex [233] many of the more recent studies have used lateral electrode locations to assess component magnitude and latencies; in particular they have used F3 and F4 to assess Nf component amplitudes and latencies while using C3’ and C4’ to assess P1 components, where C3’ and C4’ are positioned 2 cm caudal to C3 and C4 respectively [174, 197, 198, 248].
Using the multiple electrode linked-ear configuration in children, RREP components were observed with lateral derivations (e.g. P₃, P₄, C₃, C₄) but not midline electrode derivations (e.g. Pz, Cz) [196]. In contrast, topographical studies in adults using multiple electrodes show little differentiation in amplitude between components measured from midline and lateral locations [232-234]. In fact Webster and Colrain [173], examining RREP responses to inspiratory resistive loads from 29 scalp sites, reported that many components were maximal at the midline. Examples from a number of studies have also shown successful recordings from midline electrodes referenced to linked earlobes or mastoid (Figure 4-2).

Based on these observations for the current research EEG was recorded from Fz, Cz, and Pz referenced to linked mastoid. Fz was included to optimise Nf measurement, whereas Cz and Pz were recorded to optimise measurement of P1 and P3 components.
Figure 4-2: Example of RREPs from lateral and mid-line electrodes. Top panel: The averaged respiratory related evoked potential elicited with paired occlusions in a single breath. Note the P1 component was elicited with midline as well as lateral electrode placements. From Chan et al. [197]. Bottom panel: Grand average evoked potentials elicited in response to occlusion. Note that P1 and Nf components were elicited with midline placement. From Webster and Colrain [191].
4.3.3. Analysis of RREP components

As has been previously mentioned the RREP represents the averaged cortical response to multiple presentations of a respiratory stimulus. The techniques for averaging the multiple stimulus presentations require a number of procedural decisions and these will be discussed in the following section. Additionally the naming of RREP peaks based on morphology and latency was a significant issue during the conduct of the current research and is also discussed in detail in this section.

4.3.3.1. Time-lock for averaging

In order to generate the average cortical response to a stimulus, a point of the stimulus must be chosen as the time-lock for averaging the multiple presentations. While a number of the past studies have used mouth pressure change as the time-lock for stimulus averaging [189, 220], including those of the Webster group [191, 232, 233, 240] (via personal communication with K. Webster, 2010) or balloon valve pressure [192, 193], many, including the most recent Davenport group studies have used the electrical pulse that initiated the stimulus [174, 196-198, 241].

This electrical pulse has the advantage of being highly reproducible and easily detectible compared to pressure changes. Additionally, it has an advantage over using mouth/mask pressure changes, as the electrical pulse can be fired during a zero load control, allowing for comparable average techniques when no pressure change occurs with the stimulus presentation. For these reasons the electrical pulse generating the stimulus was used as the time-lock in the current research. While there is no direct comparison of different time-lock methods in the RREP literature, it is important to recognise that there is a delay between equipment activation and the resultant pressure and flow change [191, 241], which are typically used to define stimulus onset, as described in the following section.
4.3.3.2. Stimulus onset for measurement of peak latencies

In RREP research it is important to clearly define stimulus onset, as component latencies are measured relative to this point. The stimulus onset should not be confused with the time-lock for averaging, defined above. As opposed to the time-lock for averaging, it is not desirable for the stimulus onset to be defined by the point of equipment activation, due to the delay between that point and the start of any pressure or flow changes. There are various ways stimulus onset has been described in the literature, although most define stimulus onset in terms of the start of the pressure change, and as such so does the current research. The various ways of describing stimulus onset include: “the last flow point preceding the sudden decrement in inspiratory flow following solenoid activation” [171], “onset of mouth pressure change” [197], “start of the Pm [mask pressure] change” [191, 232, 240], “onset of the occlusion (indicated by the change in Pm)” [174], and, “the point at which Pm departed from control levels” [220]. In situations where loads were applied during the expiratory phase descriptions include: “the onset of inspiration measured by mouth pressure” [189, 220], and, “the point of intersection of two tangent lines on the mouth pressure trace” [192]. Others have not specified methodology [193, 198].

Although the start of mask pressure change is the most commonly described stimulus onset for RREP latency evaluation, studies generally do not specifically outline how the pressure change was detected, or in particular if manual or automated methods were utilised. The following describes the automated method used in the current research as to how the pressure change was detected using custom scripts developed with Spike 2 software.

1. A calculated channel was added to the recording indicating the slope of the mask pressure channel with a time constant $p$ of 0.02 seconds (20ms). The slope at time $t$ is calculated using an equal weighting of the points from time $t - p$ to $t + p$. 

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2. The channel marking the TTL electrical pulse that resulted in balloon valve activation and stimulus presentation was scanned.

3. When a stimulus presentation was located (except for the zero load control):
   a. Steepest slope was identified (i.e. the fastest rate of change in mask pressure) and set as a start point.
   b. A backward search was then undertaken from that point to find where the slope reached 15% of its maximal value. This percentage is recommended by the software and hardware manufactures of the equipment used for the current research in finding the start and end of a fast up or down stroke in a waveform, and on visual inspection accurately detected the start of the pressure change.

For the zero load control condition, in the absence of a decrement in mask pressure, it was necessary to calculate a stimulus onset. For the zero load control the stimulus onset was set to the average stimulus onset of all other stimulus conditions for that individual.

4.3.3.3. Peak identification and measurement
For the current research there was a range of stimulus intensities presented, ranging from the zero load control to occlusion of the resistive loading manifold. Therefore it was expected that RREP components may be absent, small or large depending on the stimulus magnitude. As has been previously mentioned, to assist in picking RREP component peaks component detection windows were created for each individual subject, based on a large stimulus, occlusion, to guide where components would be expected to occur. This was possible as component latencies generally do not change with stimulus magnitude [173]. This methodology also allowed for factors that have been shown to influence component latencies, such as individual differences in respiratory drive and equipment factors [189, 190]. When determining the presence of peaks subjectivity was minimised by blinding the assessor to stimulus intensity.
In RREP studies identified component peaks are typically measured from zero-to-peak [174] or baseline-to-peak [173, 198, 233]. Baseline-to-peak measurements have the advantage of taking into account any DC drift in individual channels. The baseline is usually the mean of the waveform during a pre-stimulus window and to minimise the influence of noise the use of a longer window (e.g. 200+ ms) has been recommended [257, 258]. An alternative is to take peak measurements relative to an adjacent peak (or trough) in the waveform (peak-to-peak). However, when taking peak-to-peak measurements it must be noted that successive peaks may reflect different physiological and/or functional processes [258].

4.3.3.4. Ocular artefact
Eye movements and blinks change the electrical fields around the eyes, which effects the electrical fields over the scalp, resulting in ocular artefact recorded in EEG signals and potential to impact RREP waveforms.

Three mechanisms have been proposed as to the source of the eye movement and blinking voltage [259] (the first two requiring eye movement, the third requiring eyelid movement only): (i) the potential difference between the positively charged cornea and the negatively charged retina, (ii) the potential difference across the retina, and (iii) movement of the eyelids over the eyeball (blinking) where it is thought that the eyelid acts as a sliding potential source. However, regardless of the voltage source, blinks and eye movements have the potential to influence RREP waveforms as they distort the electrical fields over the scalp.

The approaches to dealing with ocular artefact [259] include: (i) recording with eyes closed, (ii) requesting the subject fixate on a point and not blink during stimulus presentation, (iii) rejecting trials where large eye movements or blinks are noted during stimulus presentation, and, (iv) EEG correction algorithms based on EOG recordings. Some of these may be used in combination and will be discussed further below.
Recording with eyes closed was rejected for the current RREP studies, due to the risk that the participant would fall asleep during the experimental protocol. Therefore, as the subjects had their eyes open during stimulus presentation, they were requested to fixate gaze and not blink during stimulus presentations. An examination of the effect of applying a post-hoc ocular artefact correction, compared to rejecting trials with ocular artefact, was undertaken in two individuals with OSA, chosen due to having a relatively low number of ocular artefact free stimulus presentations. There are a number of methods described using EEG algorithms to correct for ocular artefact in the EEG (see Croft and Barry [259]) and one of the simplest methods is that described by Semlitsch [260]. This is a regression technique based on a single vertical EOG channel and involves the following steps: (i) identification of blinks throughout the study in a single vertical EOG channel, (ii) creation of an ensemble average of the EOG channel and each of the EEG channels, time locked to blinks, (iii) generation of regression based correction factors for each individual and separately for each EEG channel, and, (iv) application of the correction factors to each of the EEG channels for the entire recording. In summary, ocular artefact correction using this method increased the number of stimulus presentations included in the EEG ensemble average. However, there was no observed benefit in using the ocular artefact correction, with no substantive alteration in the shape of the waveform or improvement in ability to pick out waveforms from background. This is likely a result of the fact the signal-to-noise ratio only increases as a square root of the number of trials [249]. Ocular artefact correction was therefore not utilised for analysis during this study.

In summary, for the current research the approach to dealing with ocular artefact was to instruct participants to fixate gaze and not to blink during the stimulus presentation, and to reject stimulus presentations where a blink or eye movement occurred.
4.3.3.5. Issues relating to naming of RREP components

When analysing RREP components for this thesis an issue arose concerning component naming. This was because the first discernible positive component had a mean latency (~160 ms) that was outside the mean latency range seen for P1 in previous RREP studies and approaching the latency of the P2 component. In adults P1 is typically recorded with latencies from stimulus onset in the range of 45-75 ms [173, 174, 198] although latencies greater than 100ms have been reported [171, 190, 236]; the longest P1 latency reported in the adult literature is in the order of 105-115 ms [190], however P1 latencies in the order of 130-140 ms have recently been reported in children [236]. P2 has generally been reported with a latency in the range of 170-220 ms [171, 173, 191, 232].

Implications

Whether the first positive peak observed in the current research is considered to be P1 or P2 has implications for the interpretation of the data. While P1 is considered to represent arrival of somatosensory information at the cortex [189], the functional significance of P2 is not well understood [238]. Also, while P1 is considered an exogenous component in that its attributes are determined by the physical properties of the externally elicited stimulus, P2 is considered to have both exogenous and endogenous influences [171, 238]. That is, its attributes are influenced by the stimulus properties and the interaction between the stimulus and the subject [229]. In particular the amplitude of P2 component of the RREP, but not P1, is diminished if attention is not directed to the stimulus [191]. This is important for studies investigating differences between OSA and controls, as is the case in this thesis, as OSA patients are known to have deficits in attention [84]. Therefore, if the first positive component observed in the current study were to be considered to be P2, any deficits observed in OSA subjects could be attributed to an attentional rather than a sensory deficit. However, a finding of no observed deficits would still imply that there is no sensory deficit, regardless of whether the first positive component was considered to be P1 or P2.
There are a number of reasons why for the current research, the first positive component was considered to be a delayed P1 rather than P2. These reasons will be detailed below.

**Possible mechanism leading to delay**

Delays in latency of RREP components and their possible explanation have been discussed in previous literature. The pioneering RREP study of Davenport *et al.* [189], recorded with a cephalic EEG reference, highlighted the similarity of the RREP morphology to the somatosensory event related potential elicited by direct mechanical stimulation of the hand or ankle, and noted that the latency of the RREP was approximately 30 ms longer than finger and 15 ms longer than ankle stimulation. They explained this delay by emphasising the fact that event related potentials resulting from respiratory stimuli are produced by a less direct stimulus (slower onset) than say those resulting from mechanical touch or electrical stimulation, and for the RREP there is likely a delay between the start of the pressure change and mechanoreceptor activation and subsequent afferent transmission. This concept was supported by data showing that a lesser $P_{0.1}$, which is considered a marker of respiratory drive, was associated with a longer P1 latency. Similarly Gora *et al.* [190] attributed the delayed component latencies observed in their study to the slow occlusion stimulus negative pressure change, which was generated manually using a hand-held stopcock. Other authors have also recognised that the RREP is generated by a less direct stimulus than those used to elicit somatosensory evoked responses, and therefore that RREP component latencies may be influenced by subject’s breathing pattern or equipment factors [187, 233]. It is thus possible that delays observed in the current RREP studies were caused by an equipment-related slower stimulus rise-time than previous studies, leading to a delay in mechanoreceptor activation.

**Evidence and observations supporting a delay**

In support of the concept that the equipment used in the current studies resulted in a delayed RREP, the rate of pressure change in the current studies of (15-
30 cmH$_2$O·s$^{-1}$) was estimated to be 2-10 times slower than prior studies with the most comparable methodologies [171, 174, 190, 198] (although this was estimated from publication figures as stimulus rate of pressure change is generally not reported in RREP studies).

Also supporting the concept that equipment factors lead to a delay in RREP components, three of the control participants attended on a separate occasion to the main experimental testing and RREP responses to occlusions with and without the loading manifold in circuit were compared. Removing the loading manifold reduced the volume and therefore the elastic component of the breathing circuit and resulted in maximal rate of pressure change increase from approximately 15 cmH$_2$O·s$^{-1}$ to 85 cmH$_2$O·s$^{-1}$. This resulted in a reduction in the latency of the first positive component in the order of 60 ms on average to an average latency of 97 ms. Negative pressure pulses were delivered to a further three subjects (in early rather than mid inspiration) resulting in a maximal rate of pressure change of approximately 300 cmH$_2$O·s$^{-1}$ and a reduction in the first positive component latency to approximately 80ms. The faster rate of pressure change only reduced component latency and did not change the pattern of components observed; importantly no additional components were observed with the faster rate of pressure change so that there was no evidence of missing components at the lower rate of pressure change stimuli (Figure 4-3). This data suggests an estimated delay from start of pressure change to receptor activation in the order of 80ms when the resistive loading manifold is included in the circuit. This delay raises the possibility of applying a correction factor to the latency of components observed in the current research. However there are a number of reasons why a correction factor would be undesirable: (i) this value is estimated from a small number of subjects, (ii) patient factors, in particular respiratory drive, are likely to influence any correction, and therefore it is not possible to isolate equipment effects alone, and, (iii) applying a correction factor would not provide a true representation of the response to the stimulus.
Figure 4-3: A. RREP examples at the C2 electrode site from a single participant in response to stimuli with varied rate of pressure change. B. Corresponding mask pressure traces. Notes: Dotted line in Figure A indicates latency of first positive component. Vertical dashed line represents stimulus onset. Occlusions were delivered during mid-inspiration for 800ms in an ‘attend’ condition, whereas the pressure pulse was delivered in early inspiration for 250ms in an ‘ignore’ condition. Abbreviations: \( P_{\text{mask}} \): mask pressure.
Another point in favour of the concept that the equipment used in the current studies resulted in a delayed RREP, and therefore that the first component peak was P1 in the current research, comes from a recent study in children [236]. Importantly that study utilised an identical electrode configuration (Fz, Cz and Pz referenced to linked mastoid) and balloon valve controllers as that used in the current research. That study reported P1 latencies in the order of 130-145ms (Figure 4-4), which is outside the P1 latencies previously reported in adults.

Additionally, it has been suggested that RREP components occur earlier in children compared to adults due to the shorter nerve path length and thus a reduced transmission distance [196].

Figure 4-4: Respiratory related evoked potential at Cz elicited by occlusion in children with OSA before treatment compared with after treatment and controls.

Note: The black vertical line symbolises initiation of pressure deflection and represents stimulus onset. From Tapia [236].

The main argument in favour of the first positive component observed in the current research being P2, is that its latency is approaching that previously reported for the P2 component latency [171, 173, 191, 232]. However further arguments in favour of the first positive component being P1 include: (i) All
components from the current research were delayed; for example the first negative peak occurred at an average latency of approximately 90 ms, compared to past studies in the range of 25-80 ms [171, 173, 174, 190, 198, 232, 236], (ii) the RREP morphology was preserved in comparison to past studies. In particular, consistent with P1 but not P2 the first positive component was followed by a negative component (Figure 4-3), and, (iii) the first positive component increased in amplitude with increasing stimulus magnitude, which is also consistent with a P1 component but not P2. P2 amplitude has been shown to be independent of stimulus magnitude [173].

Summary
The latency of the first positive RREP component recorded in the current studies was later than P1 reported in prior studies, raising some doubt as to the labelling of this peak. However, there are number of observations, including: (i) stimulus rise-time characteristics, (ii) RREP morphology, (iii) RREP component response characteristics, and, (iv) experimental observations, that suggest that this peak is in fact a delayed P1 and not the later P2 component.

4.4. RREP afferent source
There are many potential sources of the RREP and indeed it is possible that there are multiple sources. Daubenspeck et al. [261], established the importance of the upper airway contribution to the RREP. That study showed that bypassing much of the upper airway with a laryngeal mask substantially reduced (but did not eliminate) the cortical response to negative pressure pulses. It has been suggested that RREP persistence may be due either to contribution of RREP sources lower than the upper airway or to incomplete bypass of the upper airway by the laryngeal mask [261, 262]. The finding that upper airway anaesthesia does not alter the RREP [246, 261] suggests either involvement of deeper joint and intramuscular receptors, or that the contribution of the upper airway is redundant, although this second suggestion is not consistent with the laryngeal mask findings.
Perhaps the best evidence for contribution of lower structures comes from a study involving trachostomised double lung transplant patients, albeit involving only 2 patients [248]. In that study the RREP was observed when the stimulus was delivered via tracheostomy, bypassing the upper airway, although the RREP was reduced in amplitude. As the upper airway was bypassed and double lung transplant involves denervation of lung vagal afferents, the authors argued that respiratory muscle afferents (inspiratory pump mechanoreceptors in particular) are the likely RREP source. Consistent with this view, stimulation of intercostals muscles in humans [263] and the phrenic nerve in cats [264] leads to cortical evoked potentials. The RREP amplitude reduction in the Davenport study [248] may have been the result of a lower intensity stimulus delivered via tracheostomy compared to the mouth, or due to the lack of contribution from upper airway or lung vagal afferents. However, the finding that double lung transplantation does not alter the early RREP components in response to mouth delivered occlusion [235, 248] suggests that pulmonary mechanoreceptors, innervated by the vagus nerve, are not a major contributor to the RREP.

Opposing the importance of inspiratory pump mechanoreceptors in RREP production is a study that induced predominant diaphragm dysfunction by respiratory loading [265]. If inspiratory pump mechanoreceptors were important in RREP production, diaphragm dysfunction would be expected to alter the RREP morphology, however early RREP components were unchanged following the intervention.

Donzel-Raynaud et al. [262] also examined RREP responses to mouth vs. tracheostomy delivered mid-inspiratory occlusions. As opposed to Davenport et al. [248] they demonstrated that an occlusion stimulus delivered via the mouth but not via tracheostomy was able to produce an RREP, however that study used 8 patients with chronic respiratory failure, 4 due to quadriplegia. While the results pointed to a contribution of upper airway afferents to the RREP, the contribution of respiratory muscle afferents could not be discounted due to the loss of, or
impaired, respiratory muscle function in the subjects studied, and because the
RREP pattern was atypical in 6 of the 8 subjects (although earlier components
were better preserved). With respect to the quadriplegic patients, as rib cage and
respiratory muscle afferents were interrupted, the lack of RREP via tracheostomy
again suggests vagal afferents are not sufficient to produce an RREP.

In summary, the afferent source of the RREP is not clearly delineated; however
evidence to date suggests a likely contribution form a submucosal upper airway
source, as well as a contribution from lower respiratory muscle afferents.
Pulmonary mechanoreceptors, innervated by the vagus nerve, are not likely to be a
major contributor to the RREP.

4.5. Relationship between conscious detection and the
RREP

In healthy subjects, a number of observations have suggested a relationship
between the conscious detection of respiratory loading and the RREP elicited by
resistive loads, and these are summarised below.

A resistive load threshold for elicitation of RREPs exists and it relates to the
conscious detection threshold of resistive loads

A number of studies have shown that when a no load control condition is presented
no RREP peaks are elicited [174, 189, 196, 220, 235]. Additionally it has also been
reported that small resistive loads clearly below the conscious detection threshold
(~30-50% of the threshold load) do not elicit any RREP components, whereas
resistive loads clearly above the conscious detection threshold do [174, 198].

RREP component amplitudes increase with load intensity

For loads above the threshold, many RREP component amplitudes increase with
increasing resistive load magnitude [173, 174, 192, 193]. Knafelc and Davenport
[192] reported that P1 amplitude of the RREP increased with increasing load
magnitude and also that the P1 amplitude was related to magnitude estimation of
the resistive loads. The same authors [193] reproduced and extended these findings by reporting that mouth pressure, oesophageal pressure and transdiaphragmatic pressure became more negative with increasing load magnitude and were also closely related to the magnitude estimation of the resistive loads. Webster et al. [173] also confirmed the relationship between P1 amplitude and stimulus intensity and also reported that the P3 peak amplitude increased with increasing load magnitude, after excluding poor perceivers where no P3 was observed. More recently, Davenport et al. [174] reported that the P1, Nf, and P3 amplitudes were related to resistive load magnitude, as was the N1 peak, but only when the stimulus was attended to.

**RREPs are impacted by background resistance in a similar way to conscious detection**

As has been mentioned previously, it has been reported that resistive load conscious detection is related to the \( R_0 \) – which is made up of the resistance of the extrinsic breathing circuit and the subject’s intrinsic airways resistance [167]. Wiley and Zechman [167] demonstrated detection thresholds with a constant ratio of \( \Delta R_{50} / R_0 \) of approximately 0.3. Thus a particular resistive load magnitude may be consciously detected at a relatively lower but not a higher background resistance.

Chou and Davenport [198] demonstrated that the RREP is influenced by background resistance in a similar fashion. That study presented three resistive loads (0.2, 3.8, and 23.3 cmH\(_2\)O·L\(^{-1}\)·s) under two conditions (no added background resistance and added background resistance of 13.3 cmH\(_2\)O·L\(^{-1}\)·s). They found that, (i) the smallest resistive load, which was below the theoretical conscious detection threshold \( \Delta R/R_0 = 0.3 \) for both conditions, never produced an RREP, (ii) the largest resistive load, which was above the conscious detection threshold for both conditions, always produced an RREP, although the amplitude of RREP components was generally greater for the condition with no added background resistance, and (iii) the middle respiratory resistance only produced an RREP for
the condition without background resistance where the load was above the conscious detection threshold \((\Delta R/R_0 > 1.5)\), but not for the condition with added background resistance, where the load was below the conscious detection threshold \((\Delta R/R_0 < 0.15)\). Thus, similar to conscious detection threshold studies, an RREP can be abolished by increasing the background resistance.

### 4.5.1. Gating of respiratory stimuli

The observed relationship between conscious detection and the RREP in response to respiratory loads has been used to support the idea of sensory gating of respiratory stimuli.

Gating of respiratory stimuli has been proposed by Davenport and colleagues \([174, 187, 198, 230, 266]\), where the “gate” is considered as a “filter” receiving and evaluating sensory stimuli. Gating has been demonstrated in other sensory modalities \([267, 268]\) and is considered important as it allows attention to be directed to essential physiological functions such as breathing \([266]\) while protecting cognitive processing from being flooded with redundant sensory stimuli.

In general there is no awareness or sensation of basal breathing; however sensation of basal breathing is possible if attention is directed to it. This implies that respiratory stimulus transduction occurs from respiratory sensory receptors during basal breathing, but that the afferent activity is ‘gated out’ from cognitive processing \([266]\). Similarly, a challenge to breathing such as an increase in resistance to airflow may be sensed by an individual, but only if the stimulus magnitude is above a certain threshold \([167]\). For a sub-threshold respiratory challenge respiratory stimulus transmission may also occur but is gated out from cognitive processing. For a supra-threshold load, respiratory mechanosensory afferent activity is ‘gated in’ resulting in conscious awareness of the stimulus.

Davenport and colleagues have proposed a *subcortical* threshold gating process \([197, 198, 230, 266]\), whereby if stimulus magnitude is sufficient the subcortical
gate allows respiratory information to be transmitted to the somatosensory regions of the cortex. This is opposed to sensory information filtering which reflects whether or not attention is directed to primary somatosensory information that reaches the somatosensory cortex resulting in further cognitive processing, and possible affective or behavioural responses [266].

The following RREP data to date is consistent with the concept of subcortical gating in that:

1. Loads well below the conscious detection threshold do not produce any components of the RREP, whereas supra-threshold loads do result in a RREP production [174]

2. An RREP in response to an added resistive load of particular magnitude can be abolished entirely by increasing background circuit resistance so that the ratio of resistive load magnitude to background resistance is less than what would be expected at the conscious detection threshold.

It should be noted however that these observations may also result if mechanoreceptors operate under Weber’s law. That is, if small stimuli, below the conscious detection threshold, do not result in mechanoreceptor activation.

The intensity based ‘threshold’ gating described above is also distinct from a ‘frequency’ gating mechanism, whereby the second stimulus of a respiratory stimulus pair, delivered during a single inspiration, produces smaller amplitude RREP components [197]. However, the fact that all components are reduced in response to the second stimulus may also suggest a subcortical process [197].

Although the anatomical site of the gating mechanism is unknown, the thalamus has been suggested as a possible candidate [187, 230]. This is related to observations that: (i) the thalamus is considered an essential relay point in sensory processing [269], (ii) the thalamus has been implicated in sensory gating of other sensory modalities [270, 271], and (iii) in animal models, direct or indirect
stimulation of respiratory muscle nerves has shown neural pathways exist via ventroposterolateral (VPL) thalamic neurons to the somatosensory cortex [264, 272, 273] and additionally, increased activity of VPL neurons has been observed with inspiratory occlusions in rats [273].

4.5.2. Summary
A number of observations suggest there is a close relationship between conscious detection of respiratory loading and the RREP. These include: (i) loads below the conscious detection threshold, including unloaded conditions, do not produce an RREP whereas RREPs are produced in response to supra-threshold loads, (ii) for supra-threshold loads many RREP components increase in amplitudes with increasing load intensity, and (iii) conscious detection and RREPs are impacted by background resistance in a similar manner. Some of these observations have also been used to support the concept of gating of respiratory stimuli. It must be noted however that the intensity of sub-threshold loads presented in these studies have been well below the conscious detection threshold, at approximately 30-50% of its value, and therefore it is unknown if larger intensity sub-threshold loads would produce RREP components. Chapter 6 of this thesis is an experimental chapter examining the relationship between conscious detection and the RREP in a more directed manner than has occurred in previous studies, by particularly targeting resistive loads at, or close to, the conscious threshold, rather than loads clearly above and below the threshold as occurred in previous RREP studies [174, 198]. This may provide some insight into sensory gating mechanisms that have been proposed.

4.6. RREP studies in OSA
In OSA patients, a number of studies have examined the RREP during wakefulness in response to large respiratory stimuli such as airway occlusion [190, 199, 200] or negative pressure pulses during inspiration [149, 201] or expiration [202], with some inconsistent results. These studies will now be discussed.
One of the earliest studies comparing OSA and control subjects in RREP morphology during wakefulness was that of Gora et al. [190]. That study examined the RREP response during wakefulness and sleep in 9 mild OSA patients and 8 age and BMI matched controls, although they only reported on 6 of each due to participant difficulty in falling asleep during the protocol. The RREP was produced by interruption of inspiration with an occlusion stimulus, generated by turning a stop cock in early inspiration. The RREP waveform was broadly similar between OSA and control participants during wakefulness, with only the N1 component showing reduced amplitude in the patients during wakefulness. As the N1 component is influenced by attention the authors attributed this finding to differences in EDS between groups. No latency differences were observed during wakefulness. While they also reported a blunted cortical response in OSA during sleep they argued it was not due to compromised mechanoreceptor function, due to the essentially normal RREP seen during wakefulness.

Another study that used an occlusion stimulus during wakefulness and sleep was that of Affifi et al. [199]. They compared 10 moderate OSA participants to 10 controls in their RREP response to a 500ms occlusion, triggered by manual activation of a solenoid during early inspiration and delivered by a nasal mask. While there was no significant difference in mask pressure generated by the stimulus between groups, there was no measurement of airway pressure. That study reported no significant difference in the amplitude of any RREP components between groups. Although they reported longer Nf and shorter P3 latency in the OSA group, similar to Gora et al. [190], they interpreted their findings as reflecting no difference in RREP between OSA and controls during wakefulness.

Donzel-Raynaud et al. [200] recorded RREPs evoked by mid-inspiratory occlusion and found no difference in component amplitudes, between a group of severe OSA patients compared to controls, but reported delays in components later than P1. They interpreted this as indicating a normal transmission to the cortex of the
sensory information related to inspiratory occlusions, but an impaired processing of this information.

In contrast Akay et al. [201] reported that the RREP was altered in treated OSA subjects in response to oral negative pressure pulses delivered at the start of inspiration. That study used a global field power technique that does not distinguish RREP components and reported a deficit in amplitude in the 55-70 ms region, consistent with a deficit in the arrival of somatosensory information. While age and BMI were not matched in this study, the authors argued they were not important factors after being accounted for in statistical analysis. They postulated that the mechanism for a reduced afferent signal may be that the airway structure in which the mechanoreceptors lie may be less compliant in OSA subjects or that the receptors may be less sensitive to distortion.

A later study that delivered -15 cmH$_2$O negative pressure pulses approximately 250 to 300 ms after onset of inspiration [149] found no differences in the RREP amplitude of components between 12 OSA and 13 control subjects, but found a delay in a component equivalent to N1 in other studies. They suggested their results reflected intact sensory pathways but a deficit in sensory processing that may be related to hypoxia or sleep fragmentation/deprivation, however it was noted that OSA subjects were older and had significantly larger BMI. Importantly, the stimulus profile and magnitude at the level of the mask and at the epiglottis were similar between controls and OSA subjects. This is opposed to other studies that have generally not reported pressure comparisons in the airway [190, 199-202]. In those studies there is always a possibility that pressure transmission, and therefore the key sensed stimulus, is different in OSA subjects to normal controls.

Grippo et al. [202] utilised a different technique to produce RREP in that the negative pressure pulse stimulus was delivered during expiration. They studied 10 untreated OSA subjects compared to 12 healthy controls, in their RREP response to negative pressure pulses of -10 cmH$_2$O, -5 cmH$_2$O and -1 cmH$_2$O, delivered via
the mouth. They found that all RREP amplitudes were reduced in OSA subjects; however, in contrast to other studies, there were no significant differences in component latencies. Grippo et al. [202] is also the only study to have suggested a raised threshold for eliciting the RREP; they reported that all control but less than half of OSA participants showed a cortical response to a relatively small negative pressure pulse (-1 cmH₂O). It must be noted that differences in intrinsic airways resistance between subject groups was not taken into account, which may be important when delivering small stimuli.

In sum, while the results are inconsistent, of the studies comparing RREPs in OSA subjects and normal controls in wakefulness, the majority have suggested that there is preservation of the amplitude of the early P1 component [149, 190, 199, 200], which is thought to reflect arrival of somatosensory information at the cortex. Only two studies have suggested an amplitude reduction of early components in OSA [201, 202]. Differences in results are possibly explained by methodological differences, both in terms of the analysis methods and stimulus delivery, with few studies reporting on the negative pressure change in the airway. In terms of latency, there is some suggestion of delayed latency of the early Nf but not P1 [199]; a result that has been replicated in children [236].

There is also some inconsistency in whether a deficit exists for later components, which are thought to reflect cognitive processing of the respiratory stimulus. The most common finding is a component delay [149, 200] or of no differences in latency [190, 202] although a shorter latency of later components has also been reported in OSA [199]. Only one study reported an amplitude reduction in the later N1 component [190]. Any deficit in later components however could be attributed to EDS and deficits in attention, which are a prominent feature of OSA.

While there is some indication of a raised threshold for eliciting the RREP in OSA [202] the vast majority of studies have utilised large magnitude stimuli, and none have examined responses to small negative pressure stimuli produced by a more
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subtle resistive load. The examination of RREP responses to small negative pressure stimuli, close to the conscious detection threshold, is a novel and key target of the current research, and is the focus of a following experimental chapter (Chapter 7).

4.7. Summary and conclusions

The RREP and its components were discussed in detail in this chapter. The RREP is the averaged cortical response, measured using EEG, to a sudden onset respiratory stimulus. It has been recorded in response to occlusion, negative pressure pulses and inspiratory resistive loads. When recording RREPs there are a number of methodological decisions to be made in terms of stimulus delivery, and recording and analysis of waveforms, and these were also outlined in this chapter. These methodological decisions include stimulus type, magnitude, timing, frequency and duration, as well as EEG electrode location. Some issues relating to identification of RREP peaks that arose during the research were also discussed.

The RREP is thought of as a more objective measure of airway sensory function than conscious detection; particularly its early P1 component which is thought to reflect arrival of somatosensory information at the cortex. It is for this reason that the RREP, and in particular the P1 component, is utilised as the primary outcome measure in the key experimental chapter examining sensory detection in OSA (Chapter 7). However, it is also recognised that a relationship exists between conscious detection and the RREP and this relationship is examined in a following experimental chapter (Chapter 6).

While RREP studies in OSA patients have shown some mixed results, the majority of studies indicate that there is preservation of the early P1 component of the RREP in OSA, indicating normal transmission of sensory information to the cortex. Despite there being some suggestion of a raised threshold for eliciting the RREP, the majority of OSA studies to date have not examined responses to small negative pressure stimuli, such as respiratory resistive loads. This may be an
important omission as failure to detect and respond to minor threats to airway
patency may lead to further collapse which is difficult to remedy. The comparison
of RREP responses to small resistive load stimuli, close to the conscious detection
threshold, in OSA compared to controls is a key target of this thesis and is
examined in a following experimental chapter (Chapter 7).
5. Responsiveness of upper airway muscles to negative pressure

As has been previously mentioned, even if sensory detection of collapsing pressure is preserved in OSA, it does not preclude the presence of a deficit in the neuromuscular response. This chapter briefly introduces the anatomy of the upper airway, which is the site of collapse in OSA. It focuses on the muscles of the upper airway, and in particular the genioglossus muscle of the tongue, which is considered to be one of the key upper airway dilator muscles [136, 154, 274, 275]. Information regarding the action of the genioglossus will be presented along with research relating to function and the response of the genioglossus muscle to negative pressure, the key force that promotes airway collapse. Finally, studies comparing genioglossus function in OSA patients compared to healthy individuals will also be discussed.

5.1. The upper airway and the site of collapse in OSA
The upper airway is made up of the extrathoracic trachea, the larynx, the pharynx, and the nose, however the pharynx is the main section that is prone to collapse as it lacks rigid support [30, 35, 276]. The pharynx is separated into three broad regions: (i) the nasopharynx, (ii) oropharynx, and, (iii) hypopharynx [276, 277], however the oropharynx has been observed to be the most common location of airway collapse. In particular, the most common location of collapse has been observed to be at the level of the soft palate (retropalatal or velopharynx region of the oropharynx), although collapse has also been observed behind the posterior surface of the tongue (the retroglossal or glossopharynx region of the oropharynx) [93, 136, 139, 278-280].

5.2. Pharyngeal muscles
While one of the key factors promoting airway collapse is the inspiratory negative airway pressure generated by the respiratory pump (the diaphragm), the primary process opposing airway collapse is the activation of pharyngeal muscles that
dilate the pharynx [35]. However, it is important to note that the pharynx is made up of a complex arrangement of muscles that may act to increase or decrease airway calibre or that may act to change compliance (or stiffness) of the airway (see Figure 1-2 for a diagram of upper airway muscles). This is related to the fact that the upper airway muscles not only have a role in respiration; they also play a role in functions such as swallowing and phonation.

The main muscles that constrict the airway are the superior, middle and inferior constrictors located on the posterior and lateral wall of the oropharynx. These muscles are mainly used in swallowing [276, 281]. The muscles that dilate the oropharynx are predominantly located laterally and anteriorly [281]. In addition to the constrictors, other muscles that make up the lateral walls of the oropharynx include the hyoglossus, styloglossus, stylohyoid, stylopharyngeas, palatopharyngeas and palatoglossus muscles [276]. Of these the hyoglossus, styloglossus, and palatoglossus extend anteriorly into the tongue which, along with the soft palate, makes up the anterior wall of the oropharynx [282]. The position of the soft palate determines the route of breathing. The tensor veli palatini tightens the soft palate to allow upward or downward movement. Favouring the oral route of breathing the levator veli palatini raises the soft palate and the musculus uvulae pull up the uvula. Favouring the nasal route of breathing, the palatoglossus muscle pulls the root of the tongue upward and backward and the palatopharyngeal muscle pulls the wall of the pharynx upwards. Both muscles cause the palatoglossal arches to approach the midline which facilitates swallowing [281]. While the palatoglossal and levator veli palatini muscles have opposing action in terms of route of breathing, they have both been shown to exhibit inspiratory phasic activity and to activate in response to negative pressure stimuli [283, 284], suggesting that these muscles may act to oppose collapse of the pharyngeal airway.

Amongst the anterior pharyngeal muscles the hyoglossus, styloglossus, and palatoglossus tongue muscles originate outside the tongue and are therefore
considered extrinsic tongue muscles. The genioglossus muscle is also an extrinsic tongue muscle, whereas the superior longitudinal, inferior longitudinal, transverse and vertical muscles of the tongue are intrinsic tongue muscles in that they originate and terminate within the tongue [281]. The structure of the tongue has been described in terms of five strata from the base of the tongue (origin of the genioglossus) to the mucosal surface [282]:

1. The stratum closest to the base (origin of the genioglossus) is made up entirely of genioglossus muscle fibres;
2. Moving towards the mucosal surface, the medial section of the next stratum is made up of genioglossus fibres whereas lateral sections contain inferior longitudinal muscle fibres and vertical muscle fibres;
3. The next stratum up, which forms the main body of the tongue, largely consists of a complex interlacing of transverse, vertical, and genioglossus muscle fibres, with styloglossus, palatoglossus and hyoglossus muscle seen laterally;
4. The next stratum up includes superior longitudinal muscle as well as vertical and genioglossus fibres.
5. The final stratum contains no muscle fibres, mainly consisting of mucous epithelium.

In terms of airway patency the hyoglossus and styloglossus muscles both retract the tongue and therefore thought to reduce airway calibre. The palatoglossus muscle pulls the posterior aspect of the tongue upward and backward [281], however as previously mentioned the observation that the palatoglossus muscle increases activity during inspiration and in response to a negative pressure stimuli [283, 284] suggests this muscle may act as a pharyngeal dilator.

5.3. The genioglossus muscle

The genioglossus muscle, which is the largest muscle of the tongue [285] and the only muscle that protrudes the tongue [281], is considered the main pharyngeal dilator muscle [136, 154, 274, 275]. It originates in the anterior portion of the mandible and widens and fans as it extends backwards into the tongue. It inserts
into the lamina propria of the mucous membrane on the dorsum of the tongue and posteriorly runs horizontally to the root of the tongue, the hyoid bone and base of the epiglottis [285, 286]. Its contraction pulls the tongue forward and together with other muscles stiffens and enlarges the pharyngeal airway [134-139]. Consistent with this, direct or indirect stimulation of the genioglossus reduces upper airway resistance and increases flow [137, 287] in a dose response manner [288]. Stimulation also results in a more negative pressure being required for airway closure [136, 289] and decreases severity of OSA [290].

The genioglossus is an inspiratory phasic muscle in that during tidal breathing it contracts prior to and during inspiration, although it maintains some tonic activity during expiration [141, 142, 145, 154, 274, 275, 291, 292]. Its inspiratory phasic activity is thought to be protective against the collapsing forces of negative pressure experienced during inspiration [140]. Its activity is augmented in the supine compared to upright [274] and lateral postures [151], and is thought to be protective against gravitational forces favouring collapse of the airway. Due to its large size, action and accessibility the genioglossus has been the most studied upper airway muscle in terms of its response to negative airway pressure stimuli, and is the muscle examined in the current research.

5.4. Methodology for studying genioglossus activity

5.4.1. Muscle activity measurement

EMG is the standard methodology used to study muscle activity and in particular the electrical signal produced by muscle contraction [293]. In the literature studying the genioglossus muscle activity both surface [146, 148] and intramuscular EMG electrodes [144, 145, 147-149, 151, 153] have been utilised. Horner et al. [146], in one of the first studies to show that negative upper airway pressure led to pharyngeal dilator muscle activation, utilised intra-oral surface electrodes to study the genioglossus activity. While surface electrodes have the advantage of being less invasive than intramuscular electrodes, they are also less selective in terms of targeting a particular muscle and are also known to have a relatively poor signal-to-
noise ratio, and hence many subsequent studies have utilised the intramuscular approach [144, 145, 147-149, 151, 153]. Using the intramuscular approach, pairs of fine insulated wire electrodes, with the ends bared, are inserted into the muscle of interest by means of a hypodermic needle. After insertion the needle is removed and the wires stay in place due to a hook or bend in the fine wire close to the tip [294]. Of those using intramuscular EMG, two main different insertion points have been utilised to study the genioglossus; per-oral [145, 147-149, 151, 153] and per-cutaneous methods [144] have been utilised.

5.4.1.1. Intramuscular EMG methods

Using the per-oral approach the EMG electrodes are inserted into the genioglossus via the mouth, 3–4 mm either side of the frenulum, at an angle of approximately 20°, following surface anaesthesia. Earlier studies inserted to a depth of 22-25 mm [291] however more recent studies have inserted to a depth of 15 mm [149]; ultrasound data from Eastwood et al. [144] suggested a depth of 22-25 mm may have been too deep and placed the electrodes in the geniohyoid muscle. Earlier data [291] also suggested that phasic activity of the genioglossus was more readily observed using the per-oral approach than the per-cutaneous approach, although as emphasised by Eastwood et al. [144] this was only based on two subjects using the per-cutaneous electrodes placed in a more posterior position in the genioglossus than with the per-oral approach. Eastwood et al. [144] subsequently used the per-cutaneous approach to successfully monitor EMG activity during rest and in response to negative pressure pulses, after confirmation of the genioglossus location using ultrasound. That study reported an optimal target insertion depth of 24 ± 4 mm, and an optimal distance from the midline of 3 ± 1 mm. This placed the electrodes at the midpoint of the genioglossus between the superior and inferior margins, and at the midpoint between the midline and the left or right margin of the genioglossus. They also reported that EMG activity was more readily observed when electrodes were placed more anteriorly. As suggested by Sauerland and Harper [291], this may be because anterior recordings are closer to the insertion point of the muscle into the mandible where
there is a greater density of muscle fibres. While the per-cutaneous approach has
the disadvantage of being less direct, in that the insertion must traverse through
the geniohyoid muscle, as suggested by Eastwood et al. [144] using the per-
cutaneous approach the insertion point is more accessible, is likely to better
tolerated and is likely to cause less technical difficulties than if the electrode wires
are required to pass through the mouth. For these reasons, in the current research
the per-cutaneous approach was used, with an insertion point 10 mm from the
inferior margin of the mandible, and 3 mm from either side of the midline, and with
a target depth of ~25-30 mm. The reasoning for the target depth will be discussed
further below.

5.4.1.2. EMG electrode placement, signal sampling, conditioning
and processing

Electrode placement. As mentioned, electrodes in the current study were placed in
the genioglossus muscle according to the method described by Eastwood et al.
[144]; that is needles were inserted in a sagittal plane (90°) under the chin at
10 mm from the inferior margin of the mandible and at a target distance of
approximately 3 mm either side of the midline. This placement was guided by prior
genioglossus muscle contraction by tongue protrusion. The electrodes were
inserted to a target depth of 25-30 mm which is a slightly greater depth than the
average optimal depth reported by Eastwood et al. [144], chosen because the
participants in the current study were expected to be more overweight than the
normal subjects utilised in that study. Correct placement was confirmed by noting
the presence of phasic activity during tidal breathing and hyperventilation and by
observing for an increased response to manoeuvres expected to activate the
genioglossus muscle, such as swallowing and tongue protrusion (although these
manoeuvres have also been shown to activate the geniohyoid muscle, but to a
lesser degree [295]). Additionally in a subset of participants the target depth was
confirmed prior to placement using ultrasound (Figure 5-1).
Signal sampling and conditioning. When recording with intramuscular electrodes faster frequency activity can be recorded compared to surface electrodes, as the signal is not subject to the high frequency filtering effects of the skin and subcutaneous tissues. It has been suggested than one needs to be able to record analogue frequencies in the 0-1,000 Hz bandwidth when using intramuscular electrodes, and since Nyquist Theorem suggests the digital sampling rate must be at least 2 times the maximum recorded frequency component [296], the digital sampling rate must be at least 2000 Hz [297]. In the current study EMG data was digitally sampled at a frequency of 2000 Hz. Additionally in terms of high frequency filters, higher cut-offs are required for intramuscular compared to surface
electrodes in order to prevent degradation of the signal of interest. Raw EMG signals in the current study were band-pass filtered between 10 and 1000 Hz.

**Signal processing.** In order to minimise the influence of EMG response variability, for each individual and stimulus intensity, EMG responses to many presentations of the stimulus were ensemble averaged, time locked to the electrical pulse that triggered the stimulus presentation. In this process there are number of factors regarding data reduction and signal processing that must be considered as outlined below.

1. **Rectification:** Prior to averaging the raw signal it is typical and necessary for the raw signal to be rectified, where the absolute value of each data point is used. Due to the random nature of raw EMG signal which crosses the zero point, if the data is averaged without rectification the mean approaches zero even with increased raw activity and therefore does not reflect the activity of the muscle [293].

2. **Moving time average:** The use of a moving time average is a common technique used in EMG and in particular EMGgg data analysis, whereby for a particular data sample the average of the data in a window around the sample is used to generate a sample value. This serves to smooth the EMG signal thereby reducing the influence of brief transients that may not reflect the general muscle electrical activity. It is important to note that large averaging windows provide increased signal smoothing [293] however if the window is too large important brief fluctuations in muscle activity may be overlooked. For example Eckert *et al.* [145] reported a previously undescribed suppression component in the reflex EMGgg response to negative pressure pulses delivered via a nasal mask to the upper airway. In that study the EMGgg signal was rectified but no moving time average was applied, and the authors suggested that the moving time average used in the majority of prior studies [144, 147, 148, 151, 158] may have obscured
the suppression component. A 100ms moving time average was used in the current experiments to allow comparison with the vast majority of previous studies examining the EMGgg response to negative airway pressure, with consideration of the possibility that this may obscure brief but important muscle responses.

3. **Normalisation.** The EMG signal is a complex signal that may be influenced by a number of factors such as the geometrical relationship between the electrode surface and muscle fibres, the position of the electrode surface relative to the innervation zone, the muscle fibre size and the number a muscle fibres of a motor unit that are close to the electrode recording surface, and hence the signal may differ with different electrode placement [293]. To allow comparisons of EMG signals from task to task, and between subjects and groups a common practice is to normalise the signal by expressing it as a percentage of maximal contraction during manoeuvres aimed to activate the muscle of interest; for the genioglossus muscle this includes swallowing, inspiratory effort against occlusion, maximal voluntary ventilation and tongue protrusion against the maxillary alveolar ridge [142]. In the current experiments a swallow manoeuvre was used to determine the maximum activity, as: (i) it more often produces greater activity compared to other manoeuvres with the recording techniques used in the current research [144, 298], and (ii) this manoeuvre is said to be highly reproducible, which may be related to the fact that once initiated, it is essentially involuntary, whereas other manoeuvres are more effort dependent [284, 298].

4. **Accounting for phasic activity.** As the background EMGgg activity changes throughout the respiratory cycle, EMGgg stimulus response measurements must take into account the phasic activity. This can be achieved by measuring the response relative to a zero load control stimulus. This
adjustment for zero load control has not been previously described in the
literature measuring responses to sudden onset negative pressure stimuli.

5.4.2. Negative pressure stimuli
There is substantial evidence that upper airway dilator muscles, and in particular
the genioglossus muscle, respond to upper airway negative pressure [141, 144-
148, 150, 153-156, 283, 284], and this has been termed the ‘negative pressure
reflex’.

In these studies there are two main ways that upper airway muscle responses to
negative pressure have been studied: (i) using sudden onset, within breath, large
negative pressure pulse stimuli [144-148, 151-153], and (ii) using stimuli or
manipulations presented over multiple breaths such as supra-threshold resistive
loads and negative pressure ventilation [141, 154-156] (Noting some have used
both approaches in a single study).

As the negative pressure pulse has a sudden onset and muscle measurements are
performed soon after the change in negative pressure, this method has the
advantage of examining reflex responses and therefore minimising the influence of
volitional activation of the muscle on findings. However, negative pressure pulses
are often large and are generated by a source external to the subject and it has
been suggested they are non-physiological [156]. In contrast, when the stimuli are
presented over multiple breaths they are, “slow phasic and within the physiological
range (i.e. at normal breathing frequencies and tidal volumes)” [154].

For the current experiments a novel approach was utilised. The main area of
interest in the current research was the reflex response to small negative pressure
stimuli spanning the conscious detection threshold, and thus, sudden onset
inspiratory resistive loads were used as a stimulus. Such a stimulus has the
following advantages:
1. It is has a slower rate of pressure change after stimulus onset than a negative pressure pulse, and hence is more physiological and more akin to what is likely to occur with the collapsing upper airway in OSA.
2. While the negative pressure is initiated when the subject is switched to an external, higher resistance breathing circuit, the pressure change is essentially generated by the subject's diaphragm driven inspiratory activity.
3. As the stimulus has a sudden onset, reflex responses can be assessed while minimising the influence of volitional muscle activity.

In developing the mechanisms for delivering the stimulus, decisions had to be made regarding factors such as the stimulus magnitude, stimulus frequency, stimulus timing, and the route of delivery. These methodological decisions were largely determined by the methodology used to produce RREPs and are discussed in greater detail in chapter 4. Of note however, it has been reported that stimulus timing within the respiratory cycle and route of delivery (nasal vs. oral) do not influence the EMGgg response to negative pressure pulses [299].

5.5. Genioglossus muscle activity and the response to negative pressure

5.5.1. Within-breath sudden onset negative pressure stimuli

5.5.1.1. Negative pressure responses during wakefulness

Horner et al. [146] were the first to show that negative upper airway pressure led to pharyngeal dilator muscle activation. That study presented 500 ms negative pressure pulses at end-expiration, with varying intensities (zero, -2.5, -5, -25 and -35 cmH₂O), to 10 normal, awake, non-obese, supine subjects. Increased EMGgg activation was observed with increased stimulus magnitude. This occurred with both the glottis open and the glottis closed, suggesting that the upper airway had a role in mediating the response, however for larger stimuli, responses were larger with the glottis open suggesting that sub-glottic mechanisms may also be important. Reaction time data, from a visual stimulus to tongue protrusion, was
presented to demonstrate that the EMGgg activation observed (median latency of 34ms) was inconsistent with a volitional response. They reported a median (range) voluntary reaction time of 184 ms (150 – 230 ms). Additionally a visual stimulus reaction time is likely to be faster than the respiratory stimulus reaction time as it does not take into account the time required for mechanoreceptor stimulation and the afferent neural transmission from the airway.

A reduced EMGgg response to negative pressure pulses has been observed during complete upper airway anaesthesia [300] confirming the importance of upper airway receptors, and mucosal receptors in particular, in mediating the EMGgg response to negative pressure. In the same study, during selective anaesthesia, greater reductions in EMGgg responses with nasal cavity and laryngopharynx anaesthesia compared to oropharynx anaesthesia suggested that nasal trigeminal nerves and the internal branches of the superior pharyngeal nerves played a greater role in responses compared to glossopharyngeal nerves [300]. Tests of genioglossus motor function confirmed reductions in EMGgg responses were not due to hypoglossal motor nerve block.

While the majority of studies have only shown activation in response to negative pressure pulses [144, 146-148, 151, 300], a suppression component following activation has also been observed in more recent studies [145, 149, 301]. Eckert et al. [145] speculated that this response may have been previously unobserved due to the lower signal-to-noise ratio of surface electrodes [146, 300, 302] or to moving-time-average smoothing [151, 303]. They also suggested it may be related to inhibition of the respiratory pattern-generator inputs in the same manner as reflex inhibition is observed in inspiratory muscles such as scalene, parasternal intercostals and the diaphragm in response to brief negative pressure stimuli (e.g. Butler et al. [304]). The inhibitory response in inspiratory muscles is thought to be protective against inhalation of a foreign body or collapse of the airway due to increasing negative pressure.
5.5.1.2. Negative pressure responses during sleep

There is some conjecture regarding whether genioglossus responses to sudden onset negative pressure are diminished in sleep, although as suggested by Malhotra et al. [151], at least some of the discrepancy may be related to posture. Early studies reported an attenuated response of upper airway muscles to negative pressure pulses during stable NREM sleep [147, 302]. Shea et al. [158] extended these findings by examining responses to negative pressure at sleep onset and in REM sleep. They suspected that the diminished response to negative pressure would be more apparent at sleep onset and in REM, where respiratory events are more common; however while they observed a diminished response during REM sleep there was only a trend for a reduction during sleep onset. As a trend for a reduction was observed the authors suggested that the negative pressure reflex may decline gradually as sleep deepens. However, Malhotra et al. [151] reported increased EMGgg activity in stable supine NREM sleep compared to wakefulness in response to negative pressure pulses delivered in early inspiration, whereas a diminished response was observed during lateral NREM sleep. This is consistent with previously mentioned studies in that these studies were all conducted with participants in a lateral posture. The finding was supported by a later study which found that activation in response to pressure pulses delivered in early inspiration was unaffected by sleep, where participants were studied in a supine posture [145]. Malhotra et al. postulated that upper airway muscles become more responsive when the pharynx is vulnerable to collapse, when tongue protrusion is likely to facilitate airway patency or when the genioglossus muscle is at a mechanical advantage. In contrast to the posture dependent nature of genioglossus activation in NREM sleep, in REM sleep earlier findings of reduced REM sleep activation that were observed in a lateral position [158] have also been observed in the supine position [145].

With regard to the influence of sleep on the subsequent suppression phase of the response to negative pressure, Eckert et al. [145] reported a larger suppression during NREM sleep compared to wakefulness, however the pharyngeal negative
pressure stimulus was also more negative during sleep, confounding the observation.

5.5.1.3. Negative pressure responses in OSA patients
Few studies have examined pharyngeal muscle EMG responses to sudden onset negative airway pressure in OSA patients during wakefulness. Studies that have examined these responses have shown inconsistent findings with reports of increased [148], decreased [284], and no difference [149] in pharyngeal muscle activation in OSA patients compared to control subjects. These studies are difficult to compare due to numerous methodological differences (Table 5-1). However only the studies of Eckert et al. [149] and Berry et al. [148], which did not show deficits in OSA patients, examined the genioglossus muscle; whereas the only study to have shown a deficit in pharyngeal muscle responses in OSA patients studied responses to levator palatine and palatoglossus muscles [284]. Data also suggest no difference between OSA patients and controls in the suppression phase of the genioglossus response to negative pressure pulses [149].

While a deficit in genioglossus muscle responses to negative pressure pulses in OSA patients appears unlikely during wakefulness, as yet responses in sleep remain inadequately tested.
Table 5-1: Summary of studies comparing upper airway EMG response to negative pressure pulses in OSA participants vs. controls during wakefulness.

<table>
<thead>
<tr>
<th>Article</th>
<th>Subjects</th>
<th>Muscle</th>
<th>Position</th>
<th>Stimulus route</th>
<th>Stimulus onset</th>
<th>EMG electrode</th>
<th>EMG conditioning</th>
<th>Measurement</th>
<th>Response</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eckert [149]</td>
<td>12 untreated OSA patients (mean ± S.E. AHI: 36 ± 6) and 13 controls (4 ± 1)</td>
<td>Genioglossus</td>
<td>Not specified</td>
<td>Nasal</td>
<td>250ms NPP, -15 cmH2O;</td>
<td>Early inspiration (~250–300 ms after onset)</td>
<td>Per-oral intramuscular</td>
<td>Rectified, ensemble averaged; No MTA</td>
<td>Peak EMG in unspecified window relative to 100ms pre-stimulus baseline</td>
<td>Activation then suppression in GG. Activation only in TP</td>
</tr>
<tr>
<td>Berry [148]</td>
<td>11 OSA (AHI &gt; 40); 11 controls (Long Beach). 14 OSA (AHI &gt; 25); 14 controls (Boston). OSA subjects mostly treated.</td>
<td>Genioglossus</td>
<td>Seated (Long Beach)</td>
<td>Supine (Boston)</td>
<td>Nasal</td>
<td>Early inspiration (150 ms after insp. Onset - Long Beach; not specified Boston)</td>
<td>Intraoral surface (Long Beach) or per-oral intramuscular (Boston).</td>
<td>Average; Rectified; MTA 50ms time constant; %max (Long Beach).</td>
<td>Maximum EMG within 150ms relative to %max.</td>
<td>Activation</td>
</tr>
<tr>
<td>Mortimore [284]</td>
<td>16 OSA (some treated); 16 controls. AHI not provided.</td>
<td>Levator palatine; palatoglossus</td>
<td>Seated</td>
<td>NPP 2.5, 5, 7.5, 10 and 12.5 cmH2O plus 0 cmH2O</td>
<td>Nasal and oral</td>
<td>End expiration</td>
<td>Per-oral intramuscular</td>
<td>Averaged; rectified;100ms MTA</td>
<td>EMG amplitude @100ms post stimulus, relative to %max</td>
<td>Activation</td>
</tr>
</tbody>
</table>

Abbreviations: Pepi: NPP: Negative pressure pulse; MTA: moving time average; %max: Percentage of maximal EMG activity; GG: genioglossus; TP: tensor palatini.
5.5.2. Negative pressure stimuli presented over multiple breaths

As has been previously mentioned negative pressure pulse stimuli have been criticised as being non-physiological [156]. An alternative approach to investigate EMGgg responses to negative pressure has been to examine activity during basal breathing or challenges to basal breathing, where the stimulus is presented over multiple breaths. Thus responses to negative airway pressure changes that are slow and in phase with the respiratory cycle, and in the physiological range, can be studied. There is a significant body of literature that has used this approach with considerable contribution to the understanding of how the genioglossus muscle functions and how it responds to negative pressure. This literature is summarised below.

5.5.2.1. Basal activity of the genioglossus muscle

The genioglossus is an inspiratory phasic muscle during basal breathing, meaning that activity increases with inspiration; it also maintains some activity during expiration [142, 150, 274, 275, 291]. As suggested by Pillar et al. [227] there are a number of possible mechanisms that contributed to this activity including (i) the brainstem respiratory pattern generator, (ii) voluntary contraction, (iii) mechanoreceptive reflexes activated in response to the collapsing forces generated during inspiration, and (iv) chemoreceptive reflexes. That central respiratory pattern generating neurons are at play is evidenced by the fact that the genioglossus muscle is activated prior to inspiratory flow [154, 227]. That negative pressure driven mechanoreceptive mechanisms are involved has been supported by a number of observations using various methodologies and these will be discussed further below:

1. *Basal breathing.* A tight and significant correlation has been observed between $P_{epi}$ and peak EMGgg observed during inspiration on a within breath basis during wakefulness [227].

2. *CPAP.* The addition of CPAP reduces the upper airway resistance and therefore the negative pressure generated in the airway. Consistent with
the importance of negative pressure on EMGgg activity, CPAP reduces EMGgg activity during wakefulness [298].

3. **Resistive loading:** Resistive loading generally serves to increase negative airway pressure and reduce airflow. Malhotra et al. [155] demonstrated a close correlation between peak epiglottic negative pressure and peak genioglossus activity generated during resistive loading in healthy subjects during wakefulness. In that study various supra-threshold resistive loads were administered (5, 10, 15, and 25 cmH$_2$O·L$^{-1}$·s$^{-1}$) by varying the calibre of the inspiratory circuit, with each load administered over multiple breaths. Linear regression across the loading conditions revealed strongest correlations between peak EMGgg activity and nadir $P_{epi}$. Peak flow and pharyngeal resistance at peak flow did not correlate with EMGgg activity, emphasising the importance of $P_{epi}$ as the key stimulus. As recognised by the authors however, while $P_{epi}$ was identified as a key stimulus modulating phasic EMGgg activity, the study was not able to distinguish whether genioglossus activation was influenced by central mechanisms (e.g. the central pattern generator providing simultaneous activation of genioglossus and diaphragmatic activity) or whether only local mechanisms were involved.

4. **Negative pressure ventilation (NPV):** Non-invasive NPV via an ‘iron lung’ serves two main purposes. Firstly, entrainment of respiration to negative pressure mechanical ventilation serves to minimise the influence of the central mechanisms (e.g. central pattern generator, chemoreceptor or vagal influences) on genioglossus activity. Evidence of entrainment includes the elimination of pre-activation of genioglossus activity prior to inspiratory flow and minimisation of diaphragmatic EMG activity [157]. Secondly, the pressure settings can be adjusted to manipulate the negative pressure stimulus delivered to the airway. Using this model it has been demonstrated that when the influence of the central respiratory drive is minimised, $P_{epi}$ continues to be closely related to EMGgg activity, both across different stimulus levels and on a within breath basis.
[154, 227]. Akahoshi et al. [154] also demonstrated that manipulation of $P_{ETCO_2}$ did not impact on genioglossus activity, suggesting that chemoreceptive reflexes were not important. Additionally, the task of entraining to the negative pressure ventilation likely reduces the influence of volitional activation of the EMGgg, although volitional activation cannot be completely excluded with this model.

5. **Anaesthesia.** Anaesthesia has been shown to lead to a decrement in peak phasic genioglossus activity and this has been observed during basal breathing, negative pressure ventilation, when the influence of central mechanisms are minimised, and during inspiratory resistive loading [227]. With anaesthesia the tightness of the relationship between Pepi and EMGgg is also diminished. This not only suggests that local mechanoreceptors are involved, but that mucosal receptors are important. This is consistent with the anaesthesia studies using negative pressure pulse stimuli [300]. The observation that upper airway resistance increases when local (anaesthesia) and when central mechanisms (negative pressure ventilation) are minimised [227] suggests that both play a part in activation of the genioglossus, however as phasic activity remains in the presence of anaesthesia and negative pressure ventilation, there remains the possibility of involvement of other mechanisms, the presence of deeper intramuscular receptors for example. Alternatively, incomplete anaesthesia or incomplete passivity during negative ventilation may explain this observation.

6. **Tracheostomised patients.** Tracheostomised patients have been used to demonstrate that mechanisms local to the upper airway contribute to the phasic genioglossus activity observed during basal breathing. The tracheostomy allows the upper airway to be bypassed, preventing exposure to respiratory stimuli such as negative pressure changes. Malhotra et al. [303] studied the EMGgg activity of 5 tracheostomised patients with OSA, while breathing via a nasal mask compared to breathing via their tracheostomy. There was a decrement in EMGgg
activity when breathing via the tracheostomy, supporting the concept that local receptor mechanisms in the upper airway contribute to genioglossus activity.

In summary, EMGgg activity increases during inspiration. This activity can be manipulated experimentally by techniques that alter airway pressure; for example EMGgg activity is augmented with increased negative airway pressure induced by resistive loading and NPV. Evidence from studies using NPV, anaesthesia, $P_{ET\,CO_2}$ manipulation, and tracheostomised patients suggest that local mechanoreceptor mechanisms are important in mediating this activity, that central mechanisms are also involved, but that chemoreceptive reflexes are of lesser importance.

5.5.2.2. Genioglossus activity in sleep

In normal individuals sleep onset is associated with an abrupt reduction in ventilation, a rise in upper airway resistance and a fall in genioglossus, diaphragm and intercostal EMG activity. However, by the fifth breath after sleep onset, EMGgg largely recovers to stable waking levels, and during stable NREM sleep EMGgg activity is comparable to that seen during wakefulness [143, 157, 305]. Also, there is no difference in the within-breath slope of the relationship between EMGgg and $P_{epi}$ between wakefulness and stable NREM sleep [157]. These observations may suggest that local negative pressure mechanoreceptive responses to negative pressure during stable sleep are preserved. However, the application of NPV, which minimises the influence of central mechanisms on genioglossus activity, results in a marked reduction in EMGgg activity and reduced slope of the within-breath relationship between EMGgg and $P_{epi}$ in stable sleep but not wakefulness [157]. This suggests that central mechanisms may be responsible for preservation of EMGgg activity in stable sleep during basal breathing, and thus local negative pressure mechanoreceptive mechanisms are diminished. Regardless of whether the mechanism causing EMGgg to increase after initially falling at sleep onset is central or local, negative pressure is clearly important as the addition of CPAP, which prevents the rise in resistance and increase in negative pressure usually seen at the wake-sleep transition, also prevents the increase in EMGgg activity.
[143]. With NPV, the reductions in EMGgg seen are greater during stable NREM compared to sleep onset, suggesting local negative pressure responses may diminish slowly over time during sleep. This hypothesis is consistent with the pressure pulse findings of Shea et al. [158], where a only trend for a reduction was observed in the EMGgg activation response to negative pressure pulses at sleep onset compared to wakefulness, whereas a statistically significant reduction was observed in REM sleep.

If the reduction in the negative pressure reflex is a gradual process with sleep progression as has been suggested, it seems there must be additional mechanisms that lead to the initial sudden decrement of EMGgg activity at sleep onset. Lo et al. [292] explored whether loss of a ‘wakefulness’ stimulus, independent of changes in the negative pressure reflex and central pattern generator output with sleep, made a contribution. This was achieved with a positive pressure ventilation (PPV) model. Similar to the use of NPV, entrainment to inspiratory PPV serves to minimise the influence of central mechanisms on genioglossus activity, however the addition of expiratory PPV minimises the influence of negative pressure reflex changes across sleep onset. In that study EMGgg activity fell from wakefulness to sleep, suggesting that wakefulness has an important independent effect on upper airway dilator muscle activity that is unlikely to be mediated through central respiratory control or local upper airway reflex mechanisms. From this and other findings the authors postulated that the reduction in EMGgg during sleep onset is contributed to by a combination of factors but is largely due to a loss of the wakefulness drive, with only minor contributions from a decreased negative pressure reflex and reduced respiratory control (central pattern generator) input [292].

It is worth noting that evidence as to whether the negative pressure reflex is diminished in sleep is somewhat conflicting between studies using a fast onset negative pressure pulse stimulus and those studies examining responses over multiple breaths. Pressure pulse studies suggest that the presence of a decrement
in the negative pressure reflex is posture dependent, with no decrement observed in the supine position [151], whereas slower multi-breath studies, also conducted in a supine posture, suggest a deficit that increases as sleep deepens [157]. Reasons for this discrepancy remain unclear although one possibility is that it may due to an increased threshold for negative pressure reflex elicitation in sleep compared to wakefulness.

5.5.2.3. Genioglossus activity in OSA patients

Phasic EMGg activity is greater during wakefulness in OSA patients versus controls during basal breathing [141-143]. This is also the case under numerous conditions that manipulate the negative pressure in the airway, such as during resistive loading, negative pressure ventilation, and during basal breathing with reduced gas density, and is accompanied by increased resistance and negative airway pressure [141]. However, this augmentation does not appear to be due to increased sensitivity to negative pressure, as the slope of the within breath and between condition relationship between $P_{epi}$ and EMGg is tight and not significantly different between OSA patients and control subjects [141, 149]. Thus the increased activity is largely thought to be due to a neuromuscular response to increased resistance and negative airway pressure [141], caused by smaller and more collapsible upper airway. Consistent with this view CPAP reduces upper airway resistance and EMGg activity in OSA patients and normal controls, and to a larger degree in OSA patients [143]. However, in OSA patients it remains higher after equalisation of negative airway pressure, at least compared to young controls, suggesting mechanisms other than the negative pressure reflex may be involved [143].

At sleep onset, EMGg activity falls to a greater degree in OSA patients than in healthy controls [142, 143], however in a similar fashion to healthy controls the genioglossus muscle responds quickly, and by the 5th breath following sleep onset EMGg activity is equivalent to pre-sleep levels [143]. This suggests genioglossus negative pressure responses remain active, although to what degree remains
unclear, however the response in OSA patients is unable to reopen the occluded airway. The reason for the larger drop in EMGgg activity at sleep onset is also unclear; specific sleep onset and ventilatory drive effects have been suggested as possibilities [143] although as previously mentioned Lo et al. [292] has suggested that the loss of wakefulness drive is important in the drop in EMGgg activity at sleep onset.

5.5.2.4. Responses to negative pressure in wakefulness in OSA patients
As mentioned above there does not appear to be a defect in the response to negative pressure in OSA patients during wakefulness, when the stimulus is presented over multiple breaths. There is a tight relationship between $P_{\text{epi}}$ and EMGgg activity in both healthy individuals and OSA patients and no difference has been observed in the slope of the within-breath and between condition relationship between $P_{\text{epi}}$ and EMGgg when comparing OSA patients to controls [141, 149].

5.5.2.5. Responses to negative pressure in sleep in OSA patients
Data from a study by Jordan et al. [159] suggests that there is no defect in genioglossus muscle responses in OSA patients during sleep. They used a device with the ability to deliver continuous positive or negative pressure and prior to stimulus delivery held participants at a pressure just greater than the pressure where flow limitation would occur in the airway. They then dropped pressure to varying degrees (2-10 cmH$_2$O) over multiple breaths and examined the EMGgg. In that study EMGgg responses were similar between groups; however control subjects were able to restore ventilation without cortical arousal.

McGinley et al. [160] compared EMGgg responses in OSA patients and controls during sleep using step wise sustained pressure drops (over 5-10 minutes) from a holding pressure (above the pressure where flow limitation occurred), to the cycling threshold (a pressure where breathing instability starts to occur). The mechanical loads and ventilatory parameters were similar between OSA and control groups at
the cycling threshold suggesting comparable stimuli. In that study tonic but not phasic EMG activity was greater in control subjects compared to OSA patients; the authors argued that since phasic activity is largely influenced by local reflex mechanisms, that local reflex mechanisms remain intact in OSA.

An additional point worth noting is that both of these studies demonstrate that a delay occurs in the activation of EMG after negative pressure stimuli delivery during sleep, with little change in EMG activation observed after pressure drops from holding pressure in the first 3-5 breaths [159, 160].

5.6. Summary and conclusions

OSA is a sleep disorder characterised by recurrent collapse of the pharyngeal airway, where the pharynx is prone to collapse because it lacks rigid support. One of the key forces promoting collapse is the negative airway pressure generated by the diaphragm during inspiration, whereas one of the key forces opposing collapse is the activity of pharyngeal muscles that dilate the airway. Of the pharyngeal dilator muscles the genioglossus of the tongue is the most studied because of its action, size and accessibility. Its contraction pulls the tongue forward and stiffens and enlarges the pharyngeal airway. The genioglossus is an inspiratory phasic muscle, meaning that it contracts during inspiration, an action thought to be protective against the collapsing force of inspiratory negative pressure. At sleep onset its activity diminishes but then increases within the first 3-5 breaths post sleep onset.

There is substantial evidence that upper airway dilator muscles, and in particular the genioglossus muscle, activate in response to upper airway negative pressure [141, 144-148, 150, 153-156, 159, 160, 283, 284], and this has been termed the ‘negative pressure reflex’. There are two main methodologies that have been used to study responsiveness of the genioglossus muscle to negative pressure: (i) using large, sudden onset, within-breath negative pressure pulses and (ii) using stimuli delivered over multiple breaths. Being sudden onset, negative pressure pulses
have the advantage of being able to assess local reflex muscle responses while
minimising volitional muscle activation, however they have been criticised as being
non-physiological. Stimuli presented over multiple breaths can be presented at
frequencies and volumes within the physiological range; however during
wakefulness the influence of volitional activity cannot be excluded.

During sleep it is likely that EMGgg responses to negative pressure are diminished,
particularly during REM sleep. While pressure pulse studies suggest that EMGgg
responses may be preserved in stable supine sleep [145, 151], perhaps the best
evidence for a diminished local response comes from the multi-breath study of
Fogal et al. [157]. That study demonstrated that entrainment to negative pressure
ventilation, designed to minimise the influence of central mechanisms on
genioglossus activity, resulted in a marked reduction in EMGgg activity and
reduced slope of the within-breath relationship between EMGgg and Pepi in stable
sleep but not wakefulness. Thus local reflex mechanisms may be more important
in producing the EMGgg activation response to negative pressure in awake
compared to sleep. The increased EMGgg activity seen after the first few breaths
of sleep onset or after negative pressure drops in stable sleep may be mediated by
central mechanisms rather than local reflex responses.

In wakefulness, although a tight relationship have been observed between Pepi
and EMGgg in multi-breath studies [154, 156], suggesting a moment to moment
modulation of EMGgg in response to negative airway pressure, no differences
have been observed in EMGgg sensitivity between OSA patients and control
subjects [141, 143]. Additionally, no deficits in EMGgg responses to negative
pressure pulses have been observed in OSA patients [148, 149]. Multi-breath
studies in sleep also suggest little difference in EMGgg responsiveness between
OSA patients and control subjects [159, 160]. From these findings it has been
suggested that OSA results from an anatomically compromised airway in
combination with normal reduction in EMGgg activity and responsiveness during
sleep [30, 143]. One aspect that has not been assessed experimentally, however,
is the threshold negative pressure stimulus required to elicit an EMGgg response, and no studies have specifically examined EMGgg responses to small sudden onset stimuli targeted to cross the conscious detection threshold. This may be an important factor in the pathogenesis of OSA, as failure to respond to minor threats to airway patency may lead to worsening collapse which is difficult to remedy by later muscle recruitment, ultimately requiring cortical arousal for restoration of ventilation, with repetition of the cycle on resumption of sleep.

Driven by the aim to examine EMGgg responses to small negative pressure stimuli, for the current research it was decided to take a novel approach to stimulus delivery using sudden onset inspiratory resistive loads. Sudden onset respiratory resistive loads have not previously been used in studies examining EMGgg responses to negative pressure. Such a stimulus has the following advantages: (i) small negative pressure stimuli can be easily applied, (ii) there is a slower rate of pressure change than a negative pressure pulse, and hence it is a more physiological stimulus, (iii) while the stimulus may be moderated by an external device, the pressure change is generated internally by the participant’s respiratory pump driven inspiratory activity. This is opposed to a negative pressure pulse which is entirely externally generated and therefore less physiological and, (iv) as the stimulus is sudden onset reflex responses can be assessed, while minimising the influence of volitional activity.

A following experimental chapter (Chapter 8) utilises sudden onset resistive loads, with an aim to explore whether genioglossus activation in response to threshold negative pressure stimuli is impaired in OSA. It was hypothesised that OSA patients would have impaired EMGgg responsiveness to threshold inspiratory resistive loads during wakefulness, when compared to normal healthy subjects.
6. Experiment 2: Relationship between conscious detection of respiratory stimuli and the respiratory related evoked potential

6.1. Preface

As mentioned in the thesis preface, this experimental chapter is prepared in a style suitable for publication. As such: (i) the chapter contains an abstract summarising the experiment, (ii) the chapter does not refer to supporting chapters and therefore material from supporting chapters may be repeated in summarised form, and, (iii) abbreviations are defined in full at first use in even if they have previously been defined in the thesis.
6.2. Abstract

Objective/Background: Respiratory load sensory detection studies show that loads clearly above the conscious detection threshold produce a cortical response, the respiratory related evoked potential (RREP), whereas loads clearly below do not. From this it has been hypothesised that a subcortical gating mechanism exists, preventing arrival of information at the cortex. This study examined this concept by comparing RREPs produced by consciously detected vs. undetected loads, near the detection threshold.

Participants/Methods: Participants (n=10; all male) had EEG recorded and wore a nasal mask connected to a resistive loading manifold. A range of mid-inspiratory resistive loads (approx. 1.2, 2.2, 3.0, 6.2 cmH₂O·L⁻¹·s) plus a zero load control were presented 90 times each, in random block design. Participants were cued prior to the stimulus and signalled detection by button press.

Results: There were statistically significant differences in peak-to-peak amplitude of the P1 RREP peak for detected (mean ± standard deviation; 3.86 ± 1.45 µV; P = 0.020) and undetected loads (3.67 ± 1.27 µV; P = 0.002) vs. control (2.36 ± 0.81 µV), although baseline-to-peak differences were not significantly different. In contrast peak-to-peak P3 amplitude was significantly greater for detected (5.91 ± 1.54 µV; P < 0.001) but not undetected loads (3.33 ± 0.98 µV; P = 0.189) vs. control (3.69 ± 1.46 µV), with the same pattern observed for baseline-to-peak measurements.

Conclusions: The P1 peak, thought to reflect arrival of somatosensory information, appeared to be present in response to both detected and undetected loads, but the later P3 peak, thought to reflect cognitive processing, was present for detected loads only. This suggests that for sub-threshold loads sensory information may reach the cortex, but is not cognitively processed. This argues against subcortical gating of sensory information.
6.3. Introduction
Sensory perception or detection of respiratory loads is important in respiratory pathology such as asthma and obstructive sleep apnoea (OSA). In asthma, conscious perception of increased respiratory load caused by airway narrowing is necessary if patients are to initiate treatment early during exacerbations. Additionally, there is evidence that ‘poor perceivers’ are at greater risk of serious exacerbations and hospitalisations [205, 206, 247]. In OSA, sensory detection of the increased load imposed by a narrow airway may be important in initiating compensatory upper airway dilator muscle responses. There is also some evidence of blunted upper airway sensation in OSA [172, 186, 222, 224].

Sensory detection of respiratory loads has classically been studied using psychophysical techniques; in particular the conscious detection threshold of respiratory resistive loads has been examined. The conscious detection threshold is defined as the added resistance ($\Delta R$) detected on 50% of presentations ($\Delta R_{50}$) [163, 167, 174, 212, 306].

More recently respiratory related evoked potentials (RREPs) have been used to study sensory detection of respiratory loads. RREPs are the averaged electroencephalographic (EEG) response to multiple presentations of a respiratory stimulus. They have been recorded in response to a number of respiratory stimuli, including inspiratory resistive loads [173, 174, 192, 193, 198], and are made up of a number of positive and negative components. The most recent studies have reported Nf, P1, N1, P2, and P3 components, in order of latency from stimulus onset, although the P1 and P3 components have most commonly been the targets of RREP research. P1 is thought to represent arrival of the primary afferent information at the somatosensory cortex whereas P3 is thought to reflect cognitive processing of the respiratory signal [173, 174, 187, 189, 191]. This is largely based on studies suggesting a P1 source location within the somatosensory cortex [173, 191, 196, 232-235], and on component characteristics. While both P1 and P3 increase in amplitude with increased stimulus magnitude [173, 174, 192, 193], only
P3 is influenced by cognitive factors such as whether or not attention is paid to the stimulus [174, 191, 239].

A relationship exists between conscious detection of respiratory loads and the RREP. From this it has been suggested that the RREP is a neural indicator of cortical sensory information processing, related to sensory detection of respiratory loads [174, 198]. In particular, it has been reported that a $\Delta R$ clearly above the conscious detection threshold elicits the RREP, whereas a $\Delta R$ well below the conscious detection threshold (~30-50% of the threshold load) does not [174, 198]. For loads above the threshold component amplitudes are correlated with magnitude estimation [173, 193]. Furthermore, RREPs are influenced by background resistance ($R_0$) in a similar way to conscious detection. In an important study Wiley and Zechman [167] demonstrated that the conscious detection threshold for resistive loads was related to the $R_0$, which incorporates the resistance of the breathing circuit and the participant’s intrinsic airway resistance. They reported that the detection threshold occurred at a common ratio of $\Delta R_{50}$ to $R_0$ ($\Delta R_{50}/R_0$) of approximately 0.3. More recently, Chou and Davenport [198] reported that an RREP produced by a $\Delta R$ that was above the conscious detection threshold ($\Delta R/R_0 > 1.5$) could be abolished by increasing the background resistance so that the same $\Delta R$ was below the conscious detection threshold ($\Delta R/R_0 < 0.15$).

These observations have been used to support the concept of a subcortical gating process [197, 198, 230, 266], where the “gate” is considered as a filter receiving and evaluating sensory stimuli, allowing attention to be directed to essential physiological functions such as breathing [266], while protecting cognitive processes from being flooded with redundant sensory stimuli. Since awareness or sensation of basal breathing is possible if attention is directed to it, it follows that mechanosensory afferent transmission occurs during basal breathing, but that the information is ‘gated out’ from cognitive processing. Similarly, sub-threshold
respiratory stimuli may result in mechanosensory afferent transmission, but the afferent transmission may be gated out from cognitive processing. For a supra-threshold load, respiratory mechanosensory afferent activity passes to or is ‘gated in’ to allow cognitive processing, resulting in conscious awareness of the stimulus. Although the anatomical site of the gating mechanism is unknown, the thalamus has been suggested as a possible candidate [187, 230]. This is related to observations that: (i) the thalamus is considered an essential relay point in sensory processing [269], (ii) the thalamus has been implicated in sensory gating of other sensory modalities [270, 271] and, (iii) in animal models there is evidence that neural pathways exist from respiratory muscle nerves to the somatosensory cortex via thalamic neurons [264, 272, 273].

This study aimed to examine the relationship between conscious detection and the RREP in closer detail than has been examined previously, by targeting respiratory resistive loads at, or close to, the conscious detection threshold, rather than loads well above or below the threshold as has occurred in previous studies [174, 198]. By definition, at the conscious detection threshold 50% of stimuli will be consciously detected and 50% will not. Therefore this study planned to generate and compare RREPs produced by detected vs. undetected resistive loads of similar magnitude. This may provide insight into the proposed gating mechanisms [197, 198, 230, 266]. In support of sensory gating of respiratory stimuli occurring as a subcortical process, it was hypothesised that RREP components would be present in response to consciously detected respiratory loads but absent or substantially altered in response to undetected loads.

6.4. Methods

6.4.1. Participants

Seventeen healthy individuals participated in this study. Participants were control participants from a separate study investigating RREP responses in OSA patients compared to healthy controls (Chapter 7). Participants were excluded if an occlusion load did not produce a clear RREP, if the presented resistive loads did
not span the conscious detection threshold, or if the resistive loads presented were not sufficiently close in magnitude to the conscious detection threshold, resulting in a low number of stimulus presentations contributing to the ensemble average (<35). This number was chosen to maximise the number of study participants while also attempting to maximise the signal-to-noise ratio. The selection of trials into analyses is described in greater detail below (Section 6.4.4).

All participants were male, and free from diagnosed respiratory, neurological, psychiatric, cardiovascular, and sleep disorders. Exclusion criteria included current intake of psychoactive medications, as well as alcohol or recreational drug abuse or tobacco use.

Power calculations were derived from control participant data from the separate study investigating RREP responses in OSA. P1 component amplitude was considered to be primary outcome. From this data it was calculated that to have 90% likelihood of detecting a 1 standard deviation difference at the \( P = 0.05 \) level required 13 subjects. Additional subjects were recruited to allow for excluded data or other technical issues.

Spirometry (JLab software version 5.2, PFTpro with whole body plethysmograph, Jaeger, Carefusion GmbH, Wurzberg, Germany or Vmax Spectra 62J body plethysmograph, Sensormedics, Yorba Linda, CA, USA) confirmed normal lung function in all participants. Participants were recruited from the community and were required to have an apnoea hypopnoea index (AHI) less than 15\,h\(^{-1}\) using ‘Chicago’ criteria for scoring respiratory events [13], as determined using full polysomnography (PSG). In a suspected OSA population a Chicago AHI of 15\,h\(^{-1}\) has been estimated to be approximately equivalent to 4\,h\(^{-1}\) using the American Academy of Sleep Medicine (AASM) 2007 [9] ‘recommended’ criteria and 9\,h\(^{-1}\) using the AASM ‘alternative’ criteria [16], which is most similar to the most recent recommendations [12].
The study conformed to the standards set by the Declaration of Helsinki and was approved by the Austin Health Human Research Ethics Committee. All participants gave informed written consent to participate in the study.

6.4.2. Preliminary visit

An initial screening visit included the in-laboratory overnight PSG as well as lung function testing the following morning. In addition to spirometry, total airways resistance ($R_{aw}$) was measured by plethysmography, via the oral route with a mouthpiece, as well as via the nasal route with a modified non-vented nasal mask (Profile Lite, Philips Respironics, Murrysville, PA, USA), both before and following nasal decongestant (0.05% oxymetazoline hydrochloride). Following administration of oxymetazoline hydrochloride solution, local vasoconstriction usually occurs within five to ten minutes, persists for five to six hours, and then gradually declines over the next six hours [307].

6.4.3. Experimental protocol and equipment

Following the screening visit, participants attended the laboratory at 8am for experimental testing, having abstained from alcohol and caffeine for at least 12 hours.

Participants were instrumented with surface electrodes for EEG recording (positioned at $F_Z$, $C_Z$ and $P_Z$ referenced to linked mastoid) and a vertical electrooculogram (EOG). EEG and EOG signals were amplified and band-pass filtered (S-Series, Compumedics, Abbotsford, Victoria, Australia) between 0.3 and 100 Hz for EEG and 0.3 and 35 Hz for EOG. EEG signals were later software filtered with a low pass 35 Hz filter. Prior to the testing session EEG and EOG were calibrated using a signal generator (Equipment Calibrator, Compumedics).

Following nasal decongestant (0.05% oxymetazoline hydrochloride) a custom-made air-perfused catheter was inserted as previously described [308]. A pressure
transducer (S-Series, Compumedics) attached to the proximal end of the catheter was used to monitor epiglottic pressure ($P_{epi}$).

Participants breathed via a modified non-vented nasal mask (Profile Lite, Philips Respironics) connected to a non-rebreathing valve (series 2600, Hans Rudolph, Kansas City, MO, USA). The static dead space of this mask and valve combination was approximately 125 ml. An additional pressure transducer (S-Series, Compumedics, Abbotsford, Victoria, Australia) was connected to the mask to monitor mask pressure ($P_{mask}$). A pneumotachograph (Fleisch No. 3) in the inspiratory line, connected to a differential pressure transducer (DP45, Validyne Engineering, Northridge, CA, USA), was used to monitor inspiratory flow.

Both $P_{mask}$ and $P_{epi}$ pressure transducers were simultaneously calibrated against a water manometer prior to each testing session. The pneumotachograph was calibrated over a range of flows using a 3 litre syringe using customised software (Spike2, Cambridge Electronic Design, Cambridge, England) based on the method described by Tang et al. [309].

All signals were digitally sampled at 1000 Hz (Micro1401 mkII, Cambridge Electronic Design) and were visually displayed and recorded throughout the experimental session (Spike2). The same software was also used to automate stimulus delivery and for data analysis.

A custom-made manifold, used to provide respiratory stimuli, was situated in an adjacent room to the participant and connected to the inspiratory side of the non-rebreathing valve, via reinforced tubing passing through the adjoining wall. The manifold was manufactured from clear acrylic (10 mm, Plasticut, Campbellfield, Victoria, Australia) with external dimensions of 21 cm x 21 cm x 47 cm and was subdivided by clear acrylic partitions with cut-outs of various diameter; each covered with a perforated stainless steel sheet (thickness: 0.9 mm; perforation diameter: 0.1 mm; open area: 3%; ActionLaser, Hornsby, New South Wales,
Australia). A port containing a fast actuating balloon valve (9340 series, Hans Rudolph, Shawnee Mission, KS, USA) was located between each pair of partitions. Bypass of the manifold (between stimulus breaths) was achieved with another balloon valve located on a t-piece (8250 series, Hans Rudolph) at the manifold entrance. Application of stimuli was achieved by activation of the t-piece balloon valve, directing inspiratory flow through the manifold, and simultaneous activation of various combinations of the manifold port balloon valves via a balloon valve controller (2430 series, Hans Rudolph). This allowed presentation of various resistive loads, with good linearity characteristics, spanning the detection threshold (≈1.2, 2.2, 3.0, 6.2 cmH₂O·L⁻¹·s) above a background circuit resistance of approximately 2 cmH₂O·L⁻¹·s. Manifold characteristics were determined with a 3-litre syringe activated at various flow rates. The manifold could also be completely occluded. Port balloon valves were also activated during a control condition (no added resistance above background circuit) to avoid any confounding influences of their activation. Control of stimulus presentations was performed using custom software (developed using Spike2) with stimuli presented in semi-random order (block design) every 2-4 breaths during mid-inspiration with a target of 90 presentations of each stimulus. The stimulus continued until end inspiration for all stimuli except for the occlusion stimulus which, for participant comfort, was presented for 800 ms. To maintain attention the participant was cued via headphones at end inspiration on the breath prior to stimulus presentation with an automatically generated message: “next breath”. Background music of the participant’s choice served to mask experimental sounds. Forced decision conscious detection of the presented stimulus (Yes/No) was signalled with a button press.

Participants were seated in an upright position in a dentist’s chair with back and arms supported to allow relaxation of postural muscles. Standardised information and instructions were given to each participant to sit quietly and comfortably, breathe as normally as possible via the nasal mask and avoid falling asleep. Additionally, for the target (stimulus presentation) breath they were instructed to
keep their eyes open and to avoid eye movement and blinking as much as possible.

Immediately prior to the experimental session biological checks were conducted to ensure signal quality; these included eyes open, closed, left, right, up and down. This was followed by a 5 minute familiarisation session.

During the experimental session participants were allowed a 5 minute break every 20-30 minutes. The entire visit, including equipment set-up, familiarisation and the experimental protocol lasted approximately 3.5-4.5 hours depending on respiratory rate.

Three control participants attended on a separate occasion to the main experimental testing to test the influence of the loading manifold on component latencies. On that occasion comparison was made between RREP responses to occlusion of the apparatus with and without the loading manifold in circuit.

**6.4.4. Data analysis**

For each participant, individual resistive load presentations were separated into those that were consciously detected and those that were not. Individual trials were only included if the target breath was free from eye movement and blink artefact, movement artefact, and if a 20 second window around the stimulus (viewed in a 30 second epoch) was free from sleep, defined as any theta frequency or slower activity observed in the central EEG derivation. The presence of sleep was determined by an experienced polysomnographic scientist. Separate ensemble averages were then generated from the detected and undetected loads, time-locked to the electrical pulse causing balloon valve inflation and stimulus presentation, with the proviso that equal numbers from each resistive load magnitude contributed to the separate detected and undetected ensemble averages. To achieve this, if a particular load was below the conscious detection threshold and therefore had a greater number of undetected than detected loads,
all of its detected loads would be used in the ‘detected’ ensemble average. An equal number of the undetected loads would be used in the ‘undetected’ ensemble average, selected in order of presentation, and the remainder of the undetected loads would be disregarded. Conversely, for resistive loads above the conscious detection threshold, all undetected loads were included in the ‘undetected’ ensemble average, however only a subset of detected loads contributed to the ‘detected’ ensemble average, equal to the total number of undetected loads for that resistive load magnitude. This methodology allowed: (i) production of ‘detected’ and ‘undetected’ ensemble averages that were matched for both presentation numbers and stimulus magnitude, (ii) for resistive load magnitudes closer to the conscious detection threshold to make a greater contribution to the ensemble average, and (iii) maximisation of the number of individual load presentations contributing to the ensemble averages. For each participant, separate ensemble averages were also created for the occlusion stimulus and the zero load control; the number of individual stimulus presentations contributing to these were also matched to the numbers contributing to the ‘detected’ and ‘undetected’ ensemble averages.

The manifold occlusion RREP was used to create participant specific 50 ms RREP component detection windows for Nf and P1 components, and 80 and 150 ms windows for the broader N1 and P3 components respectively. For each individual participant these windows were set around the component latencies on the EEG channel where the component was maximal. This methodology was employed to ensure objective RREP component measurements, and to allow for potential differences in component latencies caused by study specific stimulus characteristics (stimulus rise time in particular [189, 190]) and individual participant differences. When determining the component detection windows Nf was defined as the first negative RREP peak, P1 as the first positive peak, N1 as the second negative peak, and P3 as the subsequent large positive peak. P2 was only occasionally discernible and therefore was not included in the analysis. Stimulus onset, for detection window and subsequent RREP component latency
measurements, was defined as the onset of the sudden decrement in the ensemble averaged $P_{\text{mask}}$ following balloon valve activation. For the zero load control condition, in the absence of a $P_{\text{mask}}$ decrement, stimulus onset was set to the average stimulus onset of all other stimulus conditions for that participant.

Baseline-to-peak amplitude within each component detection window was then objectively measured from the ensemble average produced for each of the four conditions: zero load control, undetected loads, detected loads, and occlusion, where baseline was defined as the average EEG activity in a 200 ms window prior to the stimulus. Nf, P1, N1 and P3 components are known to be recorded maximally from frontal, centro-parietal, central and parietal EEG channels respectively [173, 191, 232, 234, 235]. Peak measurements were generally made from known maximal sites (Nf at F$_Z$, N1 at C$_Z$ and P3 at P$_Z$). However, P1 was measured from C$_Z$ as P1 amplitudes were greater at C$_Z$ rather than P$_Z$ using the current equipment (Chapter 7). As component amplitude may be influenced by prior components, peak-to-peak amplitudes were also recorded with a focus on the P1 and P3 components (i.e. Nf amplitude subtracted from P1 amplitude at C$_Z$ and N1 amplitude subtracted from P3 amplitude at P$_Z$).

Flow and pressure channels were also ensemble averaged for each condition, time locked to the stimulus generating electrical pulse which triggered balloon valve inflation. Prior to ensemble averaging of the Pepi channel, the Pepi signal was offset to account for a small pressure generated by air perfusion of the catheter.

The conscious detection threshold was defined as the added resistive load detected with a 50% probability ($\Delta R_{50}$) and was calculated using the logit transformation method described by Killian et al. [166]. For this calculation, to avoid floor and ceiling effects, a stimulus intensity was only included if it was detected on more than 5% and less than 95% of individual trials. The detection threshold was
also calculated with the added resistance expressed as a proportion of background resistance \( \Delta R_{50} / R_0 \) (Weber fraction).

6.4.5. Statistical analysis

Stimulus characteristics between detected and undetected loads were compared using paired sample \( t \)-tests.

For each of the RREP component windows (Nf, P1, N1, P3), the baseline-to-peak amplitude of the four conditions (zero load, detected, undetected, occlusion) were initially compared using a one-way ANOVA for repeated measures. Where appropriate planned contrasts then compared each of the loaded conditions against the zero load control, as well as detected vs. undetected loads. The same comparisons were conducted for the peak-to-peak amplitudes and latency measurements.

Results are presented as mean ± standard deviation unless otherwise stated. A \( P \)-value of <0.05 was considered statistically significant.

6.5. Results

6.5.1. Participant characteristics

Of the original 17 participants considered for inclusion, one was excluded from analysis due to a poorly defined RREP in response to occlusion, three were excluded due to the presented loads not spanning the conscious detection threshold, and three were excluded due to a low number of stimulus presentations contributing to the ensemble averages.

Participant characteristics of the remaining 10 participants are shown in Table 6-1.
Table 6-1: Participant characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>M:F</td>
<td>10:0</td>
</tr>
<tr>
<td>Age (y)</td>
<td>38.0 (34.0, 45.8)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 (1.70, 1.81)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.0 (72.8, 90.8)</td>
</tr>
<tr>
<td>BMI (kg·m$^{-2}$)</td>
<td>25.9 (25.1, 27.9)</td>
</tr>
<tr>
<td>Neck Circumference (cm)</td>
<td>39.0 (38.6, 39.9)</td>
</tr>
<tr>
<td><strong>Sleep parameters</strong></td>
<td></td>
</tr>
<tr>
<td>ESS</td>
<td>3.5 (2.3, 5.8)</td>
</tr>
<tr>
<td>AHI ($\text{h}^{-1}$)</td>
<td>3.5 (2.0, 5.5)</td>
</tr>
<tr>
<td>%TST&lt;90%SpO$_2$</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
<tr>
<td><strong>Respiratory function parameters</strong></td>
<td></td>
</tr>
<tr>
<td>FEV1 (L BTPS)</td>
<td>4.1 (3.5, 5.2)</td>
</tr>
<tr>
<td>FEV1 (%Pred)</td>
<td>106.0 (97.3, 113.5)</td>
</tr>
<tr>
<td>FVC (L BTPS)</td>
<td>5.5 (4.8, 6.2)</td>
</tr>
<tr>
<td>FVC (% Pred)</td>
<td>106.5 (102.5, 113.8)</td>
</tr>
<tr>
<td>FER (%)</td>
<td>77.5 (74.3, 81.5)</td>
</tr>
<tr>
<td>FER (%Pred)</td>
<td>98.0 (92.3, 101.3)</td>
</tr>
<tr>
<td>FRC (L BTPS)</td>
<td>3.3 (3.3, 3.8)</td>
</tr>
<tr>
<td>FRC (% Pred)</td>
<td>95.5 (81.8, 101.5)</td>
</tr>
<tr>
<td>TLC (L BTPS)</td>
<td>7.2 (6.4, 7.9)</td>
</tr>
<tr>
<td>TLC (% Pred)</td>
<td>107.5 (102.0, 113.5)</td>
</tr>
<tr>
<td>RV (L BTPS)</td>
<td>1.7 (1.6, 2.0)</td>
</tr>
<tr>
<td>RV (%Pred)</td>
<td>90.5 (81.5, 96.5)</td>
</tr>
<tr>
<td>Raw (cmH$_2$O·L$^{-1}$·s)</td>
<td></td>
</tr>
<tr>
<td>Oral route</td>
<td>2.1 (1.6, 2.3)</td>
</tr>
<tr>
<td>Nasal route (Pre-decongest)</td>
<td>4.2 (3.3, 5.7)</td>
</tr>
<tr>
<td>Nasal route (Post-decongest)</td>
<td>3.2 (2.8, 4.5)</td>
</tr>
<tr>
<td>Nasal (Pre-decongest) – oral route</td>
<td>1.6 (1.4, 3.6)</td>
</tr>
</tbody>
</table>

Values are median (inter-quartile range). Abbreviations: BMI: Body Mass Index; ESS: Epworth Sleepiness Scale; AHI: Apnoea Hypopnoea Index derived using AASM Chicago hypopnoea definition [13]; %TST<90%SpO$_2$: % of total sleep time with oxygen saturation less than 90%; FEV1: forced expiratory volume in 1 second; FER: forced expiratory ratio; FVC: forced vital capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity. Raw: airway resistance measured using body box plethysmography; % Pred: percent predicted; BTPS: body temperature and pressure, saturated.

6.5.2. Stimulus characteristics

The mean number of stimulus presentations contributing to each ensemble average was 57 ± 13. There were no significant differences between detected and
undetected resistive loads in pre-stimulus circuit resistance, stimulus $\Delta R$, or $\Delta R/R_0$ (Weber fraction) (Table 6-2). There was a small but statistically significant difference in pre-stimulus $P_{\text{mask}}$ ($P_{\text{mask}0}$) and $P_{\text{epi}}$ ($P_{\text{epi}0}$) between detected and undetected loads (Table 6-2; Figure 6-1). However stimulus $P_{\text{mask}}$ change ($\Delta P_{\text{mask}}$), $\Delta P_{\text{mask}}/P_{\text{mask}0}$, stimulus $P_{\text{epi}}$ change ($\Delta P_{\text{epi}}$) and $\Delta P_{\text{epi}}/P_{\text{epi}0}$ were not significantly different between detected and undetected loads (Table 6-2; Figure 6-1).

Table 6-2: Comparison of stimulus conditions between detected and undetected loads.

<table>
<thead>
<tr>
<th></th>
<th>Detected</th>
<th>Undetected</th>
<th>Difference</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline ($\text{cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$)</td>
<td>1.96 (0.12)</td>
<td>2.00 (0.13)</td>
<td>0.03 (0.06)</td>
<td>0.095</td>
</tr>
<tr>
<td>$\Delta$ ($\text{cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$)</td>
<td>2.26 (0.42)</td>
<td>2.24 (0.42)</td>
<td>-0.01 (0.04)</td>
<td>0.402</td>
</tr>
<tr>
<td>$\Delta R_0$</td>
<td>0.44 (0.18)</td>
<td>0.44 (0.19)</td>
<td>0.00 (0.01)</td>
<td>0.255</td>
</tr>
<tr>
<td><strong>$P_{\text{mask}}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline ($\text{cmH}_2\text{O}$)</td>
<td>-1.08 (0.22)</td>
<td>-1.03 (0.21)</td>
<td>0.05 (0.06)</td>
<td><strong>0.017</strong></td>
</tr>
<tr>
<td>$\Delta$ ($\text{cmH}_2\text{O}$)</td>
<td>-0.79 (0.20)</td>
<td>-0.76 (0.20)</td>
<td>0.03 (0.07)</td>
<td>0.176</td>
</tr>
<tr>
<td>$\Delta P_{\text{mask}0}$</td>
<td>0.74 (0.20)</td>
<td>0.75 (0.18)</td>
<td>0.00 (0.06)</td>
<td>0.887</td>
</tr>
<tr>
<td><strong>$P_{\text{epi}0}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline ($\text{cmH}_2\text{O}$)*</td>
<td>-2.85 (1.77)</td>
<td>-2.57 (1.61)</td>
<td>0.28 (0.29)</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>$\Delta$ ($\text{cmH}_2\text{O}$) *</td>
<td>-0.42 (0.44)</td>
<td>-0.49 (0.41)</td>
<td>-0.07 (0.10)</td>
<td>0.072</td>
</tr>
<tr>
<td>$\Delta P_{\text{epi}0}$*</td>
<td>0.23 (0.21)</td>
<td>0.29 (0.22)</td>
<td>0.05 (0.07)</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Notes: Values are mean (standard deviation). Baseline values are prior to stimulus presentation. Abbreviations: $\Delta$: Change; $R_0$: Background resistance incorporating circuit and airway resistance measured using plethysmography. $P_{\text{mask}}$: Mask pressure; $P_{\text{mask}0}$: Baseline mask pressure; $P_{\text{epi}}$: Epiglottic pressure; $P_{\text{epi}0}$: Baseline epiglottic pressure. $P$-values derived using paired $t$-tests. *: $n = 9$
Figure 6-1: Stimuli presented in the study. Panels from top to bottom are: (i) Flow, (ii) mask pressure, and, (iii) epiglottic pressure. Traces are the grand average (n=10 for $P_{\text{mask}}$ and flow; n=9 for $P_{\text{epi}}$), with the various stimulus conditions overlayed on each other. Abbreviations: $P_{\text{mask}}$: mask pressure; $P_{\text{epi}}$: epiglottic pressure.

6.5.3. Conscious detection threshold

The mean conscious detection threshold was 2.22 (0.76) cmH$_2$O·L$^{-1}$·s and was within the range of presented resistive loads for the 10 participants. When
expressing the $\Delta R$ as a Weber fraction, accounting for $R_0$, the conscious detection threshold was 0.44 (0.25) (Table 6-3).

Table 6-3: Conscious detection thresholds for each participant, expressed in raw resistance and as a Weber fraction.

<table>
<thead>
<tr>
<th>Participant</th>
<th>$\Delta R$ (cmH$_2$O·L$^{-1}$·s)</th>
<th>$\Delta R/R_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>2.9</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>1.4</td>
<td>0.27</td>
</tr>
<tr>
<td>7</td>
<td>1.2</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>3.1</td>
<td>0.65</td>
</tr>
<tr>
<td>9</td>
<td>3.3</td>
<td>1.05</td>
</tr>
<tr>
<td>10</td>
<td>1.7</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Mean (SD) 2.22 (0.76) 0.44 (0.25)

Abbreviations: $\Delta R$: change in resistance; $R_0$: Background resistance, incorporating intrinsic airways resistance, measured using plethysmography, and background circuit resistance.

6.5.4. RREP component amplitudes

6.5.4.1. Nf

There was a statistically significant difference in baseline-to-peak Nf amplitude between stimulus conditions (Table 6-4). Planned contrasts revealed that the baseline-to-peak Nf amplitude was significantly more negative for detected resistive loads ($P = 0.023$) and occlusion ($P = 0.002$) but not for undetected loads ($P = 0.081$), when compared to the zero load control. Baseline-to-peak Nf amplitudes were not significantly different between detected and undetected loads ($P = 0.248$). Visual inspection of the grand averaged RREP at $F_z$, suggested Nf was present for occlusion and detected loads but not undetected loads or the zero load control (Figure 6.2).
Table 6-4: RREP component amplitudes (µV).

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Undetected</th>
<th>Detected</th>
<th>Occlusion</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline-to-peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nf @ Fz</td>
<td>-0.75 (1.01)</td>
<td>-1.61 (1.09)</td>
<td>-1.96 (1.48)*</td>
<td>-2.77 (1.16)*</td>
<td>0.003</td>
</tr>
<tr>
<td>P1 @ Cz</td>
<td>1.82 (1.22)</td>
<td>2.26 (1.25)</td>
<td>2.61 (1.68)</td>
<td>3.15 (1.91)</td>
<td>0.215</td>
</tr>
<tr>
<td>N1 @ Cz</td>
<td>-0.30 (1.45)</td>
<td>0.06 (0.98)</td>
<td>-0.54 (1.73)</td>
<td>-1.80 (2.54)</td>
<td>0.068</td>
</tr>
<tr>
<td>P3 @ Pz</td>
<td>3.17 (1.75)</td>
<td>3.08 (0.99)</td>
<td>5.48 (2.14)</td>
<td>8.35 (3.09)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak-to-peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1@Cz-Nf@Cz</td>
<td>2.36 (0.81)</td>
<td>3.67 (1.27)*</td>
<td>3.86 (1.45)*</td>
<td>5.50 (2.12)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P3@Pz-N1@Pz</td>
<td>3.69 (1.46)</td>
<td>3.33 (0.98)</td>
<td>5.91 (1.54)*</td>
<td>9.07 (2.15)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). P-values are from one-way repeated measures ANOVA. Symbols: * P < 0.05 from planned comparison vs. control load. † P < 0.05 from planned comparison vs. undetected load. See text for planned comparison P-values.
Figure 6-2: Grand average (n = 10 participants) RREPs from Fz electrode site. Horizontal panels represent responses to occlusion, detected loads, undetected loads and the zero load control. Grey vertical panel represents the 50 ms component detection window for Nf, defined by the latency of Nf in response to the occlusion stimulus. Symbols: * = Baseline-to-peak measurement significantly different ($P < 0.05$) from zero load control.

**6.5.4.2. P1**

There was no statistically significant difference in baseline-to-peak P1 amplitude between stimulus conditions (Table 6-4). However, there was a significant difference in peak-to-peak P1 amplitude between stimulus conditions. Planned contrasts revealed that the peak-to-peak P1 amplitude was significantly greater for detected resistive loads ($P = 0.020$), undetected loads ($P = 0.001$) and occlusion ($P = 0.001$), when compared to the zero load control. Peak-to-peak P1 amplitudes were not significantly different between detected and undetected loads ($P = 0.372$).
Visual inspection of the grand average RREP at C2 suggested P1 was present for the occlusion, detected and undetected loads but not the zero load control (Figure 6-3).

6.5.4.3. **N1**

There was no statistically significant difference in baseline-to-peak N1 amplitude between stimulus conditions (Table 6-4).

![Grand average RREPs from C2 electrode site.](image)

Figure 6-3: Grand average (n = 10) RREPs from C2 electrode site. Horizontal panels represent responses to occlusion, detected loads, undetected loads and the zero load control. Grey vertical panel represents the 50 ms and 80 ms component detection window for P1 and N1 respectively, defined by the latency of components in response to the occlusion stimulus. Symbols: † = Peak-to-peak P1 measurements significantly different (P < 0.05) from zero load control.
6.5.4.4.  P3

There was a statistically significant difference in baseline-to-peak P3 amplitude at $P_Z$ between stimulus conditions (Table 6-4). Planned contrasts revealed that the baseline-to-peak amplitude in the P3 component detection window was significantly greater for detected resistive loads ($P = 0.006$) and occlusion ($P < 0.001$) but not undetected loads ($P = 0.431$), when compared to the zero load control. Baseline-to-peak P3 amplitudes were significantly greater for detected compared to undetected loads ($P < 0.001$). There was also a significant difference in peak-to-peak P3 amplitude between stimulus conditions (Table 6-4). Similar to the baseline-to-peak measurements, planned contrasts revealed that the peak-to-peak P3 amplitude was significantly greater for detected resistive loads ($P < 0.001$) and occlusion ($P < 0.001$), but not undetected loads ($P = 0.189$) when compared to the zero load control. Peak-to-peak P3 amplitudes were significantly greater for detected compared to undetected loads ($P < 0.001$). Visual inspection of the grand averaged RREP at $P_Z$ indicated P3 was present for occlusion and detected loads but not undetected loads or the zero load control (Figure 6-4).
6.5.5. RREP Component Latencies

There were no statistically significant differences between stimulus conditions in the latency from stimulus onset of any component peaks (Nf, P1, N1, and P3) (Table 6-5).
Table 6-5: RREP peak latencies (ms)

<table>
<thead>
<tr>
<th>Component</th>
<th>Undetected</th>
<th>Detected</th>
<th>Occlusion</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 @ Fz</td>
<td>87 (26)</td>
<td>85 (14)</td>
<td>90 (16)</td>
<td>0.710</td>
</tr>
<tr>
<td>P1 @ Cz</td>
<td>166 (15)</td>
<td>166 (20)</td>
<td>158 (22)</td>
<td>0.392</td>
</tr>
<tr>
<td>N1 @ Cz</td>
<td>228 (26)</td>
<td>204 (27)</td>
<td>212 (21)</td>
<td>0.196</td>
</tr>
<tr>
<td>P3 @ Pz</td>
<td>378 (54)</td>
<td>373 (37)</td>
<td>384 (31)</td>
<td>0.877</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). Values are the latency from stimulus onset to the maximal RREP amplitude measured within the component detection window of interest. P-values are from one-way repeated measures ANOVA.

6.6. Discussion

In this study a unique methodology was used to examine the relationship between the RREP and conscious detection of respiratory loads. Respiratory loads spanning the conscious detection threshold were separated into those that were consciously detected and those that were not, then separate ensemble averages were created from these two groups of stimulus presentations that were matched for stimulus intensity and number of stimulus presentations. In opposition to the hypothesis that predicted that RREP components would not be produced in response to undetected loads, this analysis suggested that undetected loads may produce early but not late RREP components. In contrast detected loads matched for intensity elicit early components and late components. There was no difference in latency of any component between detected and undetected loads.

For the early P1 component the peak-to-peak measurements and visual inspection of grand average RREPs (Figure 6-2) in the present study suggest that the P1 component is present for both detected and undetected resistive loads. Thus load-related sensory information may reach the somatosensory cortex for undetected loads close to the conscious detection threshold, but is not cognitively processed. Previous studies have suggested P1 peak is only elicited for respiratory loads above the conscious detection threshold [174, 198]. However, the P1 peak may not have been elicited by sub-threshold loads in previous studies as they were considerably below the conscious detection threshold at approximately 30-50% of
its value [174, 198]. It should be noted however that for baseline-to-peak P1 measurements no significant differences were found between stimulus conditions. Given the peak-to-peak P1 amplitude results, and considering that there was no difference in baseline-to-peak P1 amplitude between the occlusion stimulus and the zero load control, this finding likely indicates a substantial influence of prior components, or that that the study was insufficiently powered to detect baseline-to-peak P1 amplitude differences.

For the Nf component, baseline-to-peak amplitude for detected resistive loads was more negative compared to the zero load control, but this was not the case for undetected loads. In combination with visual inspection of the grand averages, this may suggest the presence of an Nf component in response to detected but not undetected loads. Casting some doubt on this interpretation, it must be noted that there was no statistically significant difference in baseline-to-peak Nf amplitude between detected and undetected loads. Additionally there was a trend for greater Nf amplitude in response to the undetected load compared to the control load, with the mean undetected Nf amplitude being approximately 2 times that of the control load. The role of Nf in the sensory processing of respiratory stimuli is not known and so the significance of this finding is uncertain. However because of its early latency and localisation being consistent with a supplementary motor cortex source [233] it has been suggested that the Nf peak may reflect a predictive process in cognitive functioning [187] or some form of preparatory motor response [173]. While the results of this study do not elucidate the role of Nf, the fact that P1 and Nf may respond differently in the current study is consistent with the dipole modelling study of Logie et al. which proposes that P1 and Nf are likely generated by separate radial dipoles.

The most robust finding from this study was that detected resistive loads produced the P3 component of the RREP whereas undetected loads did not. This finding was observed when baseline-to-peak and peak-to-peak measurements were made, and this finding was clearly evident on visual inspection of the grand
average RREPs (Figure 6-4). This result is consistent with previous studies suggesting that only loads above the conscious detection threshold produce a P3 component [174]. The results from the current study suggest that P3 may act as an index of the perceptual detection of increased airflow resistance, adding to the evidence indicating that the P3 peak is related to the cognitive processing of respiratory stimuli. Other observations supporting the proposal that P3 is related to cognitive processing include: (i) P3 amplitude is related to attention; it is diminished or absent if attention is not directed to the stimulus [174, 191, 239], (ii) P3 amplitude is influenced by stimulus context, for example larger amplitudes are observed with smaller stimulus probability [240], (iii) P3 amplitude has a direct correlation with stimulus magnitude estimation [173], and, (iv) P3 is dampened in both healthy and asthmatic poor perceivers [242].

The observed results have implications on theories relating to threshold gating of respiratory stimuli. Gating of respiratory stimuli has been proposed by Davenport and colleagues [174, 187, 198, 230, 266], where the “gate” is considered as a filter receiving and evaluating sensory stimuli. If current data is interpreted to indicate that Nf and P3 peaks are absent in response to undetected loads but present in response to detected loads then this is consistent with a proposed subcortical threshold gating process [197, 198, 230, 266], whereby if stimulus magnitude is sufficient the subcortical gate allows respiratory information to be transmitted to the cortex. However in contrast, the P1 data from the current study could be taken to indicate that the P1 component is present in response to detected and undetected loads, which is more supportive of a cortical mechanism affecting this component. This is supported by the observation that P1 to undetected loads was not substantially altered compared to detected loads. The mean peak-to-peak P1 amplitude in response to undetected loads was close to 100% of the mean P1 amplitude in response to detected loads. This is in contrast to the frequency gating study of Chan et al. [197] which examined the RREP response to paired 150 ms occlusions in a single breath, separated by a 500 ms interval. That study reported peak Nf, P1 and N1 amplitudes for the second presentation that were
approximately 80-85%, 60-65%, and 45% of the first presentation respectively, suggested by the authors to indicate attenuation of signals arriving in the somatosensory cortex [197].

It has been suggested that N1 peak may be important in the gating process, linking the primary sensory neural information and the attention related cognitive processes [174, 187, 197, 230]. This suggestion is related to its dependence on attention [174, 191] and its temporal location. Chan et al. [197] emphasised the importance of the N1 peak in frequency gating, highlighting that it was attenuated to a greater degree than other peaks. Whilst in the present study a trend for increasing N1 amplitude was observed, from undetected to detected to occlusion stimuli, there was no statistically significant difference from the zero load control for any stimulus condition. While there is a possibility the N1 result is a type-II error, the P1 and N1 results in the present study taken together suggests a distinction between threshold gating of respiratory stimuli and frequency gating.

More generally, the observation in the current study that P1 may be present in response to undetected loads but not other components, suggests that different processes may be involved in the gating of the different components and therefore that respiratory sensory gating is a multi-stage (cortical and sub-cortical) and multi-component process. This is similar to proposals for other sensory modalities [267].

The methodology of the present study allowed a close matching of stimulus intensity and the number of stimulus presentations contributing to the RREPs in response to detected and undetected loads. While the stimuli were closely matched they were not identical, with very small (and of questionable physiological significance) but statistically significant differences observed, possibly providing insight into the process of stimulus detection. Although the $R_0$ and $\Delta R$ were well matched, detected loads had slightly more negative baseline $P_{\text{mask}}$ and $P_{\text{epi}}$ and therefore greater inspiratory flow, suggestive of greater ventilatory drive prior to stimulus presentation. This observation is consistent with the findings of Burdon et
showing increased magnitude estimation with increased ventilatory drive induced by exercise, hypercapnia, and hypoxia. In that study the authors proposed that the close relationship between magnitude estimation and mouth pressure was indicative of an important role of inspiratory muscle force in sensing respiratory loads.

Some caution should be exercised in interpretation of the results from the present study. The analysis used 10 of 17 healthy control participants who were part of a larger study comparing OSA patients to healthy controls in their threshold RREP response to inspiratory resistive loads (Chapter 7). That study aimed to target loads around the conscious detection threshold but not necessarily at the conscious detection threshold. This meant that for the current analysis participants were excluded if presented loads were not sufficiently close to the conscious detection threshold, resulting in a low number of stimulus presentations contributing to the ensemble averages. The small number of participants may have contributed to the inability to find significant baseline-to-peak P1 and N1 amplitude differences between stimulus conditions, particularly considering a priori power calculations predicted that 13 subjects were required to have 90% likelihood of detecting a 1 standard deviation difference in P1 amplitude at the $P = 0.05$ level. Increased noise due to low number of stimulus presentations may have also made it difficult to find statistically significant differences in the baseline-to-peak amplitude of smaller components such as P1 and N1 compared to the zero load control. It is recognised that this study may have benefited by increasing the number of load presentations and therefore increasing the signal-to-noise ratio of the RREP. Increasing the number of load presentations however, was not feasible given the already lengthy and monotonous task assigned to participants and because signal-to-noise ratio only increases as a function of the square root of the number of trials [249]. Nevertheless, future studies using similar methodology would benefit by specifically targeting resistive loads at or very close to the conscious detection threshold. This could be achieved using a threshold tracking
procedure similar to that described by Zechman and Burki [168], and would serve to maximise the number of stimulus presentations.

In this study there was no reliance on subjective peak detection. Instead an automated process was utilised, measuring peak amplitudes in a predefined component detection window, based on a large stimulus, occlusion, to guide where components would be expected to occur. This was possible as component latency does not change with stimulus magnitude [173, 174]. Using a control stimulus accounted for any noise within the ensemble average and for any deflections not related to presentation of the stimulus. The importance of zero load control is highlighted by the grand average RREP at P$_Z$ which demonstrated a small positive deflection which may be related to the detection task required for participants in this study (Figure 6-4).

Creating component detection windows was also important as components in the current study were delayed compared to previous studies. In the present study, P1 latencies were reported at approximately 160 ms, whereas mean P1 latencies have previously been reported in the range of ~60-145 ms [173, 174, 190, 236]. As has been previously suggested [190], it is proposed that this was a result of the nature of the stimulus, in particular the rate of pressure change, which is a function of the apparatus used to deliver the stimulus, as well as respiratory drive. Indeed, Davenport et al. [189] noted that individuals with reduced rate of pressure change had longer component latencies and that mouth pressure change at 0.1 s was inversely correlated with the latency of the RREP P1 peak. To assess this contention, three control participants attended on a separate occasion to the main experimental testing and a comparison was made between RREP responses to occlusion of the apparatus with and without the loading manifold in circuit. Removing the loading manifold resulted in maximal rate of pressure change increase in the order of 70 cmH$_2$O·s$^{-1}$ and a reduction in P1 latency in the order of 60 ms on average. Thus longer RREP peak latencies may be due to a delay between stimulus onset and respiratory mechanoreceptor activation. Importantly,
the faster rate of pressure change only reduced component latency and did not change the pattern of components observed.

In the current study there was no significant difference between detected, undetected loads and occlusion in the latency of any of the RREP components. These latency findings are consistent with previous observations that latency of components is not influenced by stimulus magnitude [173, 174], although one must consider that latencies were constrained by component detection windows in the current study.

6.7. Conclusion
Using a unique methodology, this study examined the relationship between conscious detection of respiratory loads and the RREP. The study tested the notion that only loads above the conscious detection threshold produce RREP components [174, 198] by comparing ensemble averages from detected and undetected loads close to the conscious detection threshold, matched for stimulus intensity and number of stimulus presentations. The results from the present study indicate that the P1 component of the RREP, but not other components, may be present in response to sub-threshold loads. Considering that P1 is thought to reflect arrival of somatosensory information at the cortex, and P3 is thought to reflect cognitive processing, this result suggests that normal load-related sensory information reaches the somatosensory cortex for sub-threshold loads, but is not cognitively processed. If replicated in future studies, this observation has implications for the threshold gating of respiratory stimuli, indicating involvement of subcortical and cortical mechanisms and suggesting a multi-stage and multi-component process.

6.8. Postscript
This experimental thesis chapter seized on an opportunity recognised to use methodology and techniques developed for the key experimental chapters, to improve understanding of the sensory detection of respiratory stimuli.
As the study was conducted with normal control subjects it had little bearing on the key thesis aims (which were to explore if there were impairments in OSA patients in the detection of, and neuromuscular compensation for, negative pressure respiratory load stimuli close to the conscious detection threshold). Nevertheless, this study was an important thesis chapter as it improved understanding of the RREP, which was the primary outcome measure for the key experimental chapter examining sensory detection in OSA (Chapter 7).
7. Experiment 3: Cortical responses to respiratory loads in awake severe obstructive sleep apnoea patients and healthy controls

7.1. Preface

As mentioned in the thesis preface, this experimental chapter is prepared in a style suitable for publication. As such: (i) the chapter contains an abstract summarising the experiment, (ii) the chapter does not refer to supporting chapters and therefore material from supporting chapters may be repeated in summarised form, and, (iii) abbreviations are defined in full at first use in even if they have previously been defined in the thesis.
7.2. Abstract

Objective/Background: This study utilised respiratory related evoked potentials (RREPs) to investigate whether sensory detection of small negative pressure respiratory stimuli, close to the conscious detection threshold, was impaired in obstructive sleep apnoea (OSA). It was reasoned that impaired detection of a minor challenge to airway patency may lead to further collapse that is difficult to remedy by later muscle recruitment.

Participants/Methods: Comparison was made between 16 severe OSA and 17 healthy control participants in their RREP response to mid-inspiratory resistive loads of varying intensity (≈1.2 to 6.2 cmH$_2$O·L$^{-1}$·s), delivered while participants were awake via a nasal mask. Stimulus intensity was expressed as change in epiglottic pressure relative to background conditions, and the response as the amplitude of the P1 component of the RREP, which reflects arrival of somatosensory information at the cortex.

Results: There was no significant difference between control and OSA participants in the threshold (mean [95% confidence intervals]: -0.01 [-0.22, 0.19] vs. -0.24 [-0.72, 0.24]; $P = 0.392$) or the sensitivity (4.18 [2.63, 5.73] vs. 2.60 [0.04, 5.16]; $P = 0.268$) of the relationship between P1 amplitude and stimulus intensity. Additionally no significant differences were found between control and OSA participants in the latency of the P1 component, or the threshold, sensitivity or latency of the later P3 component of the RREP.

Conclusions: This study is unique in its focus on the cortical response to small respiratory loads and the results do not support the concept that a sensory deficit contributes to the pathogenesis of OSA.
7.3. Introduction

Obstructive sleep apnoea (OSA) is characterised by the repetitive narrowing or collapse of the pharyngeal (upper) airway during sleep, resulting in reduction or cessation of airflow, despite ongoing ventilatory effort. Adverse consequences include daytime somnolence [5], impaired cognition [47], reduced quality of life [48], increased motor vehicle accident risk [49], cardiovascular disease [57], metabolic impairment [51], and increased mortality risk [55, 56]. While anatomy is considered an important factor in OSA pathogenesis, it has also been recognised that anatomy does not fully explain its incidence and severity [30, 35, 99, 100], and therefore that other factors must be involved.

It has been demonstrated that upper airway dilator muscles activate in response to the collapsing forces of upper airway negative pressure [145, 146], the activation response being termed the ‘negative pressure reflex’. Following from this it has been postulated that impaired sensory function, and in particular impaired sensation of negative airway pressure, may contribute to OSA pathogenesis as negative pressure may fail to lead to an upper airway muscle response that is adequate to prevent airway collapse [172, 178, 186, 221-224].

Previous investigators have shown impaired upper airway sensory function in awake OSA subjects, using conscious detection of various stimuli; more specifically, studies have shown reduced upper airway temperature sensitivity [221] and two-point discrimination [222, 223], as well as reduced upper airway conscious detection thresholds for vibration [222] and endoscopic air pulses [224]. Studies examining conscious detection of increased respiratory resistance, arguably a more physiologically relevant stimulus, have shown mixed results; Clerk et al. [178] found no difference between OSA subjects, snorers and controls, whereas an earlier study found an increased detection threshold for OSA subjects compared to controls [186]. Another study demonstrated reduced magnitude estimation of large increases in respiratory resistance in OSA subjects [172].
Studies involving conscious detection of a stimulus are potentially influenced by impairments in attention, often a prominent feature of OSA [84]. A more objective measure of sensory function is the respiratory related evoked potential (RREP), which is the averaged cortical electroencephalographic (EEG) response to multiple presentations of a respiratory stimulus [187]. The RREP is made up of a number of positive and negative components; P1 in particular is thought to represent arrival of the primary afferent information at the cortex and is not influenced by attention [174, 191], as opposed to the later P3 component, which is thought to reflect cognitive processing of the respiratory signal [174, 187, 189, 191, 196, 197]. In OSA, a number of studies have examined the RREP during wakefulness in response to large respiratory stimuli such as airway occlusion [190, 199, 200] or negative pressure pulses during inspiration [149, 201] or expiration [202], with inconsistent results. However of those, only two have suggested an amplitude reduction of the early P1 component in OSA [201, 202]; the majority suggesting that there is preservation of the amplitude of the P1 component [149, 190, 199, 200]. Delayed latency of the early Nf but not P1 has been reported in both adults [199] and children [236]. Grippo et al. [202] also reported that all control, but less than half of OSA participants showed a cortical response to a relatively small negative pressure pulse (-1 cmH₂O), suggestive of a raised threshold for eliciting the RREP in OSA.

The majority of OSA studies to date, however, have not examined RREP responses to small respiratory stimuli, and none have attempted to quantitate thresholds for eliciting RREP components. This may be an important omission, as failure to detect and respond to minor threats to airway patency may lead to worsening collapse which is difficult to remedy by later upper airway muscle recruitment. The P1 and P3 components lend themselves to threshold analysis, given that for these components amplitude has been shown to increase with increasing stimulus magnitude [173, 174, 192, 193].
Furthermore, OSA studies to date have not taken the contribution of the background respiratory load into consideration. Load thresholds for both conscious detection and for eliciting RREPs have been shown to be influenced by the background resistance ($R_0$) [167, 198], incorporating the resistance of the subject’s airways and the breathing circuit. Indeed, both conscious detection and RREPs can be abolished by a large enough increase in background resistance [167, 198]. This is important when examining OSA subjects, as they have been shown to have increased intrinsic resistance [175-179].

The aim of the current study was to explore whether sensory detection of small negative pressure respiratory stimuli, close to the conscious detection threshold, was impaired in OSA. It was hypothesised that OSA patients would have an increased threshold and reduced sensitivity for eliciting the P1 component of the RREP in response to small respiratory stimuli during wakefulness, when compared to age matched healthy control subjects.

### 7.4. Methods

This study was approved by the Austin Health Human Research Ethics Committee and conformed to the standards set by the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants gave informed written consent to participate in the study.

#### 7.4.1. Participants

Sixteen untreated severe OSA patients and 17 healthy control subjects participated. All were male, and free from respiratory, neurological, psychiatric, cardiovascular, and sleep disorders (other than OSA for the patient group). Exclusion criteria for both groups included current intake of psychoactive medications, as well as alcohol or recreational drug abuse or tobacco use.

Spirometry confirmed normal lung function in all participants (JLab software version 5.2, PFTpro with whole body plethysmograph, Jaeger, Carefusion GmBH, 164
Wurzberg, Germany or Vmax Spectra 62J body plethysmograph, Sensormedics, Yorba Linda, CA, USA). The groups were matched for age, and attempts were made to minimise body mass index (BMI) differences between groups by restricting the BMI of participants to less than 35 kg·m\(^{-2}\) and by selectively recruiting controls that were overweight or at the high end of the healthy range. OSA participants were recruited following clinical investigation for suspected OSA, where severe OSA was defined as an apnoea hypopnoea index (AHI) greater than 30·h\(^{-1}\) using Chicago criteria [13], plus at least 5% of sleep time with oxygen saturation less than 90%. Healthy control participants were recruited from the community and were required to have an AHI less than 15·h\(^{-1}\) using Chicago criteria for scoring respiratory events, confirmed using polysomnography (PSG). A Chicago AHI of 15·h\(^{-1}\) has been estimated to be approximately equivalent to 4·h\(^{-1}\) using the American Academy of Sleep Medicine (AASM) 2007 [9] ‘recommended criteria’ and 9·h\(^{-1}\) using AASM 2007 ‘alternative’ criteria [16], which is most similar to the most recent recommendations [12].

7.4.2. Preliminary visit

For control participants an initial screening visit included the overnight PSG as well as a range of lung function tests the following morning. For the OSA group the screening visit incorporated lung function testing only. In addition to spirometry, total airways resistance (Raw) was measured by plethysmography, via the oral route with a mouthpiece, as well as via the nasal route with a modified nasal mask (Profile Lite, Philips Respironics, Murrysville, PA, USA), both before and following nasal decongestant (0.05% oxymetazoline hydrochloride). Following administration of oxymetazoline hydrochloride solution, local vasoconstriction usually occurs within five to ten minutes, persists for five to six hours, and then gradually declines over the next six hours [307]. The difference between the pre-decongestant nasal and oral resistance estimates was considered to reflect the nasal component of upper airway resistance. Lung volumes via the oral route, neck circumference and Epworth Sleepiness Scale (ESS) were also measured to characterise the participant characteristics.
7.4.3. Experimental protocol and equipment

Participants attended the laboratory at 8am for experimental testing on a separate day following the screening visit, having abstained from alcohol and caffeine for at least 12 hours.

The equipment used for physiological recordings, the instrumentation applied to the patients, as well as the signal conditioning and calibration methodologies were identical to that used in experiment 2 (Chapter 6) and have been previously described in section 6.4.3. In brief the following physiological measurements were undertaken: EEG, electrooculogram (EOG), epiglottic pressure ($P_{\text{epi}}$), mask pressure ($P_{\text{mask}}$), and airflow.

Participants were required to breathe via a breathing circuit which was also identical to that used in experiment 2 and has also been previously described in section 6.4.3. In brief this circuit included: a modified sealed nasal mask (Profile Lite, Philips Respironics), a non-rebreathing valve (series 2600, Hans Rudolph, Kansas City, MO, USA), which was connected via tubing to a custom-made manifold, used to provide respiratory stimuli, situated in an adjacent room to the participant. The manifold allowed presentation of various resistive loads, with good linearity characteristics, spanning the detection threshold ($\approx 1.2, 2.2, 3.0,$ and $6.2 \text{ cmH}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}$) above a background circuit resistance of approximately $2 \text{ cmH}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}$ as well as a control condition (no added resistance above background circuit) and a complete occlusion. Pilot testing with the manifold revealed that RREP component latencies were variable between participants and were delayed in the current study compared to previous studies; for example the first negative peak at the frontal site occurred at average latencies of approximately $90$ ms in the current study compared to latencies in the range of $25-80$ ms in past studies [173, 174, 190, 236]. This meant that average latencies of approximately $160$ ms were observed for the first positive component in the current study.
compared to latencies in the order of ~60-145 ms in past studies [173, 174, 190, 236]. Confidence was gained with the component labelling as, although delayed, the RREP morphology was comparable to previous studies, and the reason for the delay in components is likely related to the stimulus characteristics (the reduced rate of stimulus pressure change in particular), resulting in a delay in stimulus transmission in the order of 80 ms, and this is elaborated on in the Discussion. Due to the delay the manifold occlusion stimulus was used to assist in detecting and locating RREP peaks, by creating component detection windows for each participant, as described in the Data Analysis section. Control of stimulus presentations was performed using custom software, with all stimuli (control, occlusion and various resistive loads) presented in semi-random order (block design) every 2-4 breaths during mid-inspiration with a target of 90 presentations of each stimulus. The stimulus continued until end inspiration for all stimuli except for the occlusion stimulus which, for participant comfort, was presented for 800 ms. To maintain attention the participant was cued via headphones at end inspiration on the breath prior to stimulus presentation with an automatically generally message “next breath”. Background music of the participant’s choice served to mask experimental sounds. Forced decision conscious detection of the presented stimulus (Yes/No) was signalled with a button press.

Participants were seated in an upright position in a dentist’s chair with back and arms supported to allow relaxation of postural muscles. Standardised information and instructions were given to each participant prior to the experimental session. They were instructed to sit quietly and comfortably, breathe as normally as possible via the nasal mask and avoid falling asleep. Additionally, for the target breath they were instructed to keep the eyes open and to avoid eye movement and blinking as much as possible.

Immediately prior to the experimental session biological checks were conducted to ensure signal quality; these included eyes open, closed, left, right, up and down. This was followed by a 5 minute familiarisation session.
During the experimental session participants were allowed a 5 minute break every 20-30 minutes. The entire visit, including equipment set-up, familiarisation and the experimental protocol lasted approximately 3.5-4.5 hours depending on respiratory rate.

To test the influence of the loading manifold on component latencies three control participants attended on a separate occasion to the main experimental testing. On that occasion comparison was made between RREP responses to occlusion of the apparatus with and without the loading manifold in circuit. Additionally, a further three control participants returned for a repeat testing session, where negative pressure pulses were delivered.

7.4.4. Data analysis

The EEG signal was ensemble averaged separately for each participant at each stimulus intensity, time locked to the electrical pulse which triggered balloon valve inflation. Individual trials were included in the average if the target breath was free from eye movement and blink artefact, movement artefact, and if a 20 second window around the stimulus (viewed in a 30 second epoch) was free from sleep, defined as any theta frequency or slower activity observed in the central EEG derivation. The presence of sleep was determined by an experienced polysomnographic scientist.

The particular stimuli used in this study resulted in relatively long and variable (between participants) component latencies. Only components that occurred within a specified window were accepted for measurement, with component windows defined by the latency of components observed in response to the occlusion stimulus, which, being the largest stimulus, most reliably produced RREP components. For P1, the detection window was defined as the interval 25 ms before and after the P1 peak identified for the occlusion stimulus, whereas for the broader P3 component the detection window was defined as the interval 75 ms
before and after the P3 peak identified for the occlusion stimulus. The EEG channel in which the component was maximal was used in determining the window location and separate component windows were created for each participant.

When determining the location of the component detection window from the occlusion stimulus data, P1 was identified as the first positive RREP component peak and P3 was identified as the largest subsequent positive RREP peak.

Stimulus onset was defined as the onset of the sudden decrement in the ensemble averaged Pmask recording following balloon valve activation. For the zero load control condition, in the absence of a Pmask decrement, stimulus onset was set to the average stimulus onset of all other stimulus conditions for that individual.

Subsequently, the ensemble averaged EEG for each stimulus intensity was assessed in random order with the assessor blinded to the stimulus magnitude. The assessor determined whether a component existed in the component window; if present the peak amplitude was measured relative to the average amplitude in the component window of the zero load control ensemble average; if absent the peak amplitude was recorded as zero. Where a component was present its latency was measured from stimulus onset to its peak.

Flow and pressure channels were also ensemble averaged, time locked to the stimulus generating electrical pulse which triggered balloon valve inflation. Prior to ensemble averaging of the Pepi channel the Pepi signal was offset to account for a small pressure generated by air perfusion of the catheter.

The conscious detection threshold was calculated as previously described in section 6.4.4.

7.4.5. Statistical analysis

The statistical analysis was conducted using SPSS (version 18; Chicago, IL, USA).
Initial analysis explored differences between OSA and control participant characteristics using Mann Whitney U tests, as not all parameters were normally distributed. Results are presented as median (inter-quartile range).

Additionally, the impact of participant group (OSA vs. Control) and stimulus condition (intensity) on stimulus ventilatory characteristics were explored using two-way mixed between-within group ANOVAs. Where appropriate, results were further examined using Bonferroni post hoc comparisons. Results are presented as mean ± standard deviation.

Our primary analysis compared OSA and control participants in both the sensitivity and threshold of the relationship between stimulus intensity and P1 amplitude. Linear mixed effect regression models (LMM) were utilised for this analysis [311], with models allowing for random patient intercepts. Initial analysis yielded a statistical comparison of: (i) the slope of the relationship between P1 amplitude and stimulus intensity (P1 sensitivity), and (ii) P1 amplitude at zero stimulus intensity (y-axis intercept) between groups. Rather than the y-axis intercept, the key interest of the current study was the x-axis intercept (the point where the relationship between P1 amplitude and stimulus intensity crossed the stimulus intensity (x) axis) or, in other words, the largest intensity at which the P1 component was not detectable (P1 threshold). To determine this, the same LMMs as above were applied to OSA and control participants separately so that the linear equation could be solved for y = 0. For each group this analysis yielded model estimates of: (i) the slope of the relationship between P1 amplitude and stimulus intensity, (ii) P1 amplitude at zero stimulus intensity (y-axis intercept), and, (iii) the variance and covariance associated with these. This allowed calculation of the x-axis crossing and estimation of the associated variance. Subsequent independent-samples t-tests were conducted to compare P1 threshold differences between OSA and controls.
In the above analysis the key measure of stimulus intensity was considered to be Pepi change ($\Delta$Pepi) as a proportion of background Pepi ($P_{\text{epi}_0}$). $P_{\text{epi}_0}$ was defined as the nadir Pepi prior to stimulus onset.

For completeness similar analyses were conducted using added resistance and mask pressure, relative to background conditions, as the measure of stimulus intensity. Secondary outcome measures included:

1. The P3 RREP component threshold and sensitivity analysed using the same methods as for the P1 component.
2. Latency of the P1 and P3 components, also analysed using LMM, and,
3. Conscious detection threshold differences analysed using independent-samples $t$-tests.

### Results

#### 7.5. Participant characteristics

There were no statistically significant differences between control and OSA groups in age, height, weight, forced expiratory volume in 1 second (FEV1) and forced expiratory ratio (FER). OSA patients had larger BMI and neck circumference, reduced absolute total lung capacity (TLC), absolute and percent predicted functional residual capacity (FRC) and absolute forced vital capacity (FVC), and elevated Raw (measured via oral route as well as nasal route, pre- and post-decongestant) compared to controls. The nasal component of upper airway resistance (nasal resistance – oral resistance) was not statistically significantly different between groups. As expected OSA patients had increased ESS, AHI and percentage sleep with oxygen saturation ($\text{SpO}_2$) less than 90%, compared to controls (Table 7-1).
Table 7-1: Participant characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSA</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>M:F</td>
<td>16:0</td>
<td>17:0</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>43.5 (38.8, 48.0)</td>
<td>38.0 (33.0, 48.0)</td>
<td>0.295</td>
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<tr>
<td>Height (m)</td>
<td>1.73 (1.69, 1.79)</td>
<td>1.78 (1.70, 1.80)</td>
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<tr>
<td>Weight (kg)</td>
<td>91.5 (82.8, 99.0)</td>
<td>83.0 (75.0, 93.0)</td>
<td>0.126</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>31.4 (27.6, 32.4)</td>
<td>26.0 (25.3, 28.4)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Neck Circumference (cm)</td>
<td>44.0 (40.8, 45.3)</td>
<td>39.5 (38.5, 40.0)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Sleep parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS</td>
<td>11.0 (8.5, 14.3)</td>
<td>5.0 (2.0, 6.0)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>AHI (h⁻¹)</td>
<td>54.8 (38.8, 70.5)</td>
<td>4.4 (2.6, 5.8)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>%TST&lt;90%SpO₂</td>
<td>9.2 (5.0, 19.0)</td>
<td>0.0 (0.0, 0.1)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Respiratory parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (L BTPS)</td>
<td>3.6 (3.4, 4.0)</td>
<td>4.1 (4.0, 4.5)</td>
<td>0.056</td>
</tr>
<tr>
<td>FEV₁ (%Pred)</td>
<td>93.0 (85.5, 102.8)</td>
<td>103.0 (97.0, 111.0)</td>
<td>0.146</td>
</tr>
<tr>
<td>FVC (L BTPS)</td>
<td>4.6 (4.2, 5.0)</td>
<td>5.3 (4.9, 5.9)</td>
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<tr>
<td>FVC (% Pred)</td>
<td>92.5 (86.8, 104.3)</td>
<td>106.0 (100.0, 110.0)</td>
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</tr>
<tr>
<td>FER (%)</td>
<td>78.0 (74.8, 81.5)</td>
<td>79.0 (74.0, 80.0)</td>
<td>0.736</td>
</tr>
<tr>
<td>FER (%Pred)</td>
<td>98.5 (95.3, 105.3)</td>
<td>97.0 (92.0, 102.0)</td>
<td>0.402</td>
</tr>
<tr>
<td>FRC (L BTPS)</td>
<td>2.8 (2.1, 3.0)</td>
<td>3.3 (2.9, 3.7)</td>
<td><strong>0.011</strong></td>
</tr>
<tr>
<td>FRC (% Pred)</td>
<td>75.0 (61.3, 84.8)</td>
<td>84.0 (78.0, 98.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>TLC (L BTPS)</td>
<td>6.1 (5.6, 6.6)</td>
<td>6.8 (6.3, 7.9)</td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td>TLC (% Pred)</td>
<td>95.0 (90.8, 110.8)</td>
<td>105.0 (99.0, 110.0)</td>
<td>0.168</td>
</tr>
<tr>
<td>RV (L BTPS)</td>
<td>1.6 (1.2, 1.8)</td>
<td>1.6 (1.3, 2.0)</td>
<td>0.683</td>
</tr>
<tr>
<td>RV (%Pred)</td>
<td>87.0 (72.3, 88.3)</td>
<td>87.0 (71.0, 95.0)</td>
<td>0.510</td>
</tr>
<tr>
<td>Raw (cmH₂O·L⁻¹·s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral route</td>
<td>3.1 (2.7, 4.0)</td>
<td>2.3 (2.0, 2.8)</td>
<td><strong>0.026</strong></td>
</tr>
<tr>
<td>Nasal route (Pre)</td>
<td>6.2 (5.7, 7.6)</td>
<td>5.1 (3.7, 6.3)</td>
<td><strong>0.048</strong></td>
</tr>
<tr>
<td>Nasal route (Post)</td>
<td>4.7 (4.1, 5.6)</td>
<td>4.0 (2.8, 5.0)</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Nasal (Pre) – oral route</td>
<td>3.2 (1.9, 4.5)</td>
<td>2.3 (1.4, 3.5)</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Values are median (inter-quartile range). Abbreviations: BMI: body mass index; ESS: Epworth Sleepiness Scale; AHI: apnoea hypopnoea index derived using AASM Chicago hypopnoea definition [13]; %TST<90%SpO₂: % of total sleep time with oxygen saturation less than 90%; FEV₁: forced expiratory volume in 1 second; FER: forced expiratory ratio; FVC: forced vital capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity. Raw: airway resistance measured using body box plethysmography; Pre/Post: pre or post decongestant (oxymetazoline); % Pred: percent predicted; BTPS: body temperature and pressure, saturated. P-values derived from Mann Whitney U tests.

### 7.5.2. Stimulus characteristics

There was no statistically significant difference in the number of stimulus presentations per stimulus condition for OSA participants (97.8 ± 4.8) compared to
controls (97.2 ± 8.8; \( P = 0.807 \)). In addition, as per the study block design the main effect of stimulus condition (\( P = 0.283 \)) and the interaction (\( P = 0.313 \)) did not reach statistical significance.

Of the individual stimuli presented, on average 65.8 ± 15.6% were considered suitable to be included in the ensemble average with no statistically significant difference between stimulus conditions (\( P = 0.660 \)) or participant groups (\( P = 0.269 \)) and no statistically significant interaction effect (\( P = 0.701 \)).

On average participants failed to make a forced choice as to whether they could detect a load on 2.5 ± 5.0% of stimulus presentations. There was no statistically significant main effect of group (\( P = 0.454 \)) on response failure; nor was there a statistically significant interaction effect (\( P = 0.973 \)). There was a statistically significant stimulus condition main effect (\( P = 0.046 \)) with, in general, response failure observed in a larger proportion of lower intensity stimuli (e.g. 3.9 ± 6.6% vs. 0.7 ± 2.1% for the control condition and occlusion condition respectively; \( P = 0.037 \)).

Fewer stimulus presentations were available for the Pepi ensemble average due to technical issues with the Pepi signal, likely related to catheter blockages or the catheter tip contacting the airway wall; no Pepi measurements were available for one control participant due to these technical problems and this participant was excluded from the analysis. Of the individual stimuli presented, 40.4 ± 19.0% were of sufficient quality to be included in the Pepi ensemble average, with no statistically significant difference between stimulus conditions (\( P = 0.561 \)) or participant groups (\( P = 0.682 \)) and no statistically significant interaction effect (\( P = 1.000 \)).

Stimulus presentation resulted in a reduction in flow, as well as more negative \( P \)mask and Pepi (see a participant example in Figure 7-1). There was no statistically significant difference between OSA and control participants in \( \Delta \)Pepi in
response to the stimulus; however $P_{epi_0}$ was more negative in OSA compared to control participants. This meant that $\Delta P_{epi}$ was greater in control compared to OSA participants for a given stimulus when expressed as a proportion of $P_{epi_0}$ (Table 7-2).

Figure 7-1: Example of the various stimuli presented in the study. Panels from top to bottom are: (a) Flow, (b) mask pressure, and, (c) epiglottic pressure. Traces are the ensemble average in a single control participant, with the various stimulus intensities overlayed on each other. Note the stimulus is presented during mid-inspiration. Abbreviations: $P_{mask}$: mask pressure; $P_{epi}$: epiglottic pressure.
Table 7-2: Stimulus characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSA</th>
<th>Control*</th>
<th>Group P-value</th>
<th>Interaction P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆Pepi/Pepti₀</td>
<td></td>
<td></td>
<td>0.010</td>
<td>0.125</td>
</tr>
<tr>
<td>load 1</td>
<td>0.08 (0.08)</td>
<td>0.16 (0.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>0.15 (0.12)</td>
<td>0.26 (0.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>0.19 (0.15)</td>
<td>0.34 (0.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>0.31 (0.19)</td>
<td>0.56 (0.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>0.80 (0.43)</td>
<td>1.47 (0.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆Pepi (cmH₂O)</td>
<td></td>
<td></td>
<td>0.603</td>
<td>0.919</td>
</tr>
<tr>
<td>load 1</td>
<td>-0.36 (0.46)</td>
<td>-0.35 (0.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>-0.61 (0.52)</td>
<td>-0.53 (0.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>-0.86 (0.95)</td>
<td>-0.73 (0.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>-1.41 (1.23)</td>
<td>-1.21 (0.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>-3.68 (2.90)</td>
<td>-3.24 (1.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepti Nadir (cmH₂O)</td>
<td></td>
<td></td>
<td>0.002</td>
<td>0.141</td>
</tr>
<tr>
<td>load 0</td>
<td>-5.16 (2.51)</td>
<td>-2.65 (1.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 1</td>
<td>-5.39 (2.56)</td>
<td>-2.93 (1.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>-5.56 (2.47)</td>
<td>-3.15 (1.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>-5.95 (2.79)</td>
<td>-3.25 (1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>-6.39 (3.06)</td>
<td>-3.81 (1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>-8.67 (4.44)</td>
<td>-5.82 (1.83)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). Abbreviations: Pepi: Epiglottic pressure; ∆Pepi: Change in epiglottic pressure; Pepti₀: Background epiglottic pressure. P-values derived using two-way mixed between-within group ANOVAs. Added resistive load intensities: load 0 = 0.0 cmH₂O·L⁻¹·s; load 1 = 1.2 cmH₂O·L⁻¹·s; load 2 = 2.2 cmH₂O·L⁻¹·s; load 3 = 3.0 cmH₂O·L⁻¹·s; load 4 = 6.2 cmH₂O·L⁻¹·s.

*: n = 16 for controls

### 7.5.3. Conscious detection threshold

The conscious detection threshold was within the range of presented resistive loads for the majority of participants. The conscious detection threshold could not be determined for one OSA patient in whom the threshold would have been greater than the largest delivered resistive load stimulus. The calculated detection threshold was below the lowest delivered resistive load stimulus for two controls and one OSA participant; and above the largest delivered resistive load stimulus for one OSA and one control participant. There was no statistically significant
difference in calculated conscious detection threshold between OSA
(3.1 ± 1.8 cmH\textsubscript{2}O·L\textsuperscript{-1}·s) and control participants (2.4 ± 1.6 cmH\textsubscript{2}O·L\textsuperscript{-1}·s; \( P = 0.292 \)).

When expressing the added resistance as a Weber fraction, accounting for background conditions, there was also no difference in conscious detection threshold between OSA (0.46 ± 0.33) and controls (0.47 ± 0.39; \( P = 0.966 \)).

7.5.4. P1 amplitude
An example of the RREP produced during the study is shown in Figure 7-2.
Figure 7-2: Respiratory related evoked potential examples.
Notes: Horizontal panels represent ensemble average RREPS at the Cz electrode site, in a single control participant in response to various stimuli. Grey vertical panel represents component detection window for the first positive peak, defined by the component latency observed in response to the occlusion stimulus.
Previous studies have reported the P1 component amplitude to be maximal at centro-parietal [191, 232, 234] or parietal [173, 196, 233] regions of the cortex. Preliminary analysis examining P1 amplitude in response to the largest stimulus, occlusion, revealed a trend for larger P1 amplitude in central (2.23 ± 2.03 µV) vs. parietal recordings (1.77 ± 1.50 µV; \( P = 0.052 \)). All subsequent P1 measurements were therefore made from the central EEG site.

There was no significant difference between control and OSA participants in the threshold of the relationship between P1 amplitude vs. stimulus intensity when stimulus intensity was expressed as \( \Delta \text{Pepi}/\text{Pepi}_0 \) (Table 7-3; Figure 7-3). This was also the case when the stimulus intensity was expressed in terms of nadir Pepi and \( \Delta \text{Pepi} \).

The sensitivity (slope) of the relationship between P1 amplitude and stimulus intensity was greater in control compared to OSA participants when the stimulus was expressed as \( \Delta \text{Pepi} \), however there was no statistically significant difference between groups when the stimulus was expressed as nadir Pepi, nor as \( \Delta \text{Pepi}/\text{Pepi}_0 \) (Table 7-3; Figure 7-3).
Table 7-3: The threshold and sensitivity of the relationship between each of P1 and P3 amplitude (µV) vs. stimulus intensity.

<table>
<thead>
<tr>
<th>Component</th>
<th>Parameter</th>
<th>Stimulus</th>
<th>Control*</th>
<th>OSA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Threshold</td>
<td>ΔPepi/Pepi₀</td>
<td>-0.01 (-0.22, 0.19)</td>
<td>-0.24 (-0.72, 0.24)</td>
<td>0.392</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΔPepi (cmH₂O)</td>
<td>0.11 (-0.39, 0.62)</td>
<td>1.38 (-1.01, 3.77)</td>
<td>0.309</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepi nadir (cmH₂O)</td>
<td>-1.39 (-3.09, 0.32)</td>
<td>-1.19 (-5.87, 3.48)</td>
<td>0.939</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>ΔPepi/Pepi₀</td>
<td>4.18 (2.63, 5.73)</td>
<td>2.60 (0.04, 5.16)</td>
<td>0.268</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΔPepi (cmH₂O)</td>
<td>-1.78 (-2.54, -1.02)</td>
<td>-0.50 (-0.97, -0.03)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepi nadir (cmH₂O)</td>
<td>-0.76 (-1.35, -0.18)</td>
<td>-0.24 (-0.45, -0.02)</td>
<td>0.132</td>
</tr>
<tr>
<td>P3</td>
<td>Threshold</td>
<td>ΔPepi/Pepi₀</td>
<td>-0.10 (-0.35, 0.16)</td>
<td>-0.12 (-0.37, 0.12)</td>
<td>0.901</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΔPepi (cmH₂O)</td>
<td>0.15 (-0.33, 0.63)</td>
<td>0.53 (-0.40, 1.46)</td>
<td>0.480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepi nadir (cmH₂O)</td>
<td>-0.98 (-2.59, 0.63)</td>
<td>-0.87 (-4.49, 2.75)</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>ΔPepi/Pepi₀</td>
<td>5.43 (2.88, 7.98)</td>
<td>6.72 (2.33, 11.10)</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΔPepi (cmH₂O)</td>
<td>-2.74 (-3.92, -1.56)</td>
<td>-1.52 (-2.29, -0.74)</td>
<td><strong>0.045</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepi nadir (cmH₂O)</td>
<td>-1.02 (-1.70, -0.33)</td>
<td>-0.41 (-0.69, -0.12)</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Values are model estimates (5th and 95th confidence intervals) from linear mixed models conducted separately for each group. P-values for threshold measurements are from t-tests following separate group mixed models, whereas sensitivity P-values are from a single linear mixed model comparing groups. Abbreviations: Pepi: Epiglottic pressure; ΔPepi: Change in epiglottic pressure; Pepi₀: Background epiglottic pressure. *: n = 16 for controls.
Figure 7-3: P1 amplitude vs. stimulus intensity, expressed as \( P_{\text{epi}} \) change relative to background \( P_{\text{epi}} \).
Notes: The sensitivity and threshold were not statistically different between OSA and control participants (\( P = 0.392 \) and 0.268 for threshold and sensitivity respectively). Abbreviations: \( \Delta P_{\text{epi}} \): Epiglottic pressure change; \( P_{\text{epi}} \): background epiglottic pressure. \( n = 16 \) for controls.
Dashed line indicates linear mixed model (LMM) estimate. Note that the LMM estimate takes into account repeated measures at different stimulus intensities.

7.5.5. P1 latency

Mean latency from stimulus onset to P1 peak for all participants and stimulus intensities combined was 158 ± 24 ms. With the stimulus expressed as a \( \Delta P_{\text{epi}} \) relative to \( P_{\text{epi}} \), LMM model analysis revealed there was no statistically significant change in latency with stimulus intensity change (slope) for control participants (\( P = 0.561 \)). Also, there was no statistically significant difference between OSA and controls in the slope (\( P = 0.627 \)) or the intercept (\( P = 0.179 \)) of the relationship between P1 peak latency and stimulus intensity.
7.5.6. P3 amplitude

Preliminary analysis examining P3 amplitude in response to the largest stimulus, occlusion, revealed no statistically significant difference in peak amplitude between central and parietal sites ($P = 0.177$). The P3 component has generally been reported to be maximal in parietal regions of the cortex [173, 191, 232, 240]. P3 measurements were therefore taken from the parietal EEG site.

There was no statistically significant difference between control and OSA participants in the threshold of the relationship between P3 amplitude and stimulus intensity where the stimulus intensity was expressed as $\Delta\text{Pepi}/\text{Pepi}_0$ (Table 7-3). This was the also the case when the stimulus was expressed as nadir Pepi or $\Delta$Pepi.

There was a statistically significant different between control and OSA participants in the sensitivity of the relationship between P3 amplitude and stimulus intensity when the stimulus was expressed as $\Delta$Pepi, however this was not the case when the stimulus intensity was expressed in terms of nadir Pepi nor $\Delta$Pepi/$\text{Pepi}_0$ (Table 7-3).

7.5.7. P3 latency

Mean latency from stimulus onset to P3 peak for all participants and stimulus intensities combined was $374 \pm 52$ ms. With the stimulus expressed as a $\Delta$Pepi/$\text{Pepi}_0$ LMM model analysis revealed there was no statistically significant change in latency with stimulus intensity change (slope) for control participants ($P = 0.536$). Also, there was no statistically significant difference between OSA and controls in the slope ($P = 0.542$) or the intercept ($P = 0.554$) of the relationship between P3 peak latency and stimulus intensity.
7.6. Discussion

The main finding from this study was that during wakefulness there was no difference between OSA and control participants in the threshold or sensitivity of the relationship between the P1 component amplitude of the RREP and the intensity of a negative pressure stimulus, where the stimulus was delivered via the nasal route and expressed in terms of $\Delta P_{\text{epi}}/P_{\text{epi}}^0$. Additionally, there was no difference between OSA and control participants in the latency of the P1 component. This is the first study to conduct such an analysis focusing on small stimuli spanning the threshold of conscious detection. The absence of deficits in this intensity range is contrary to the concept of a sensory deficit contributing to the pathogenesis of OSA, or alternatively that OSA contributes to a sensory deficit.

The results are consistent with the majority of RREP studies suggesting intact sensory processing of respiratory stimuli during wakefulness in adults [149, 190, 199, 200] although some studies suggest a blunted cortical response [201, 202]. However comparisons are difficult between the current study and previous studies due to stimulus differences, with most previous studies utilising large stimulus intensities. While studies in adults [199] and children [236] have reported a delay in the Nf component in OSA, consistent with the current study no delays in the P1 component have been reported [190, 199, 236]. Grippo et al. [202] is the only study to have suggested a raised threshold for eliciting the RREP; they reported that all control but less than half of OSA participants showed a cortical response to a relatively small negative pressure pulse (-1 cmH\textsubscript{2}O). The difference in findings could be contributed to by a number of methodological differences between the two studies. For example, Grippo et al. applied an extrinsic pressure pulse via the oral route during expiration whereas in the current study an intrinsic (participant generated) resistive load stimulus was applied via the nasal route during inspiration. Compared to a pressure pulse the present stimulus is likely to be a more naturalistic physiological stimulus and more akin to respiratory changes occurring during sleep apnoea. Additionally the current study accounted for
background conditions whereas with the study of Grippo et al. it is unknown whether differences in intrinsic airways resistance between subject groups may have contributed to findings.

One must consider that the current findings may have been influenced by difficulty in picking component peaks close to the eliciting threshold where they may be small relative to background noise. Attempts were made to standardise the selection of components by creating component windows for each individual participant, based on a large stimulus, occlusion, to guide where components would be expected to occur, thereby reducing the influence of individual differences in peak latencies on the selection process. This was possible as latency does not change with stimulus magnitude [173]. At the same time attempts were made to limit subjectivity in peak identification by blinding the assessor to stimulus intensity. While it is recognised that this study may have benefited by increasing the number of load presentations and therefore increasing the signal-to-noise ratio of the RREP, doing so was not feasible given the already lengthy and monotonous task assigned to participants and because signal-to-noise ratio will only increase as a function of the square root of the number of trials [249].

Creating component detection windows contributed in a second way as component latencies were later in the current study compared to previous studies. In the current study a model estimate P1 latency of approximately 158 ms was reported, whereas mean P1 latencies have previously been reported in the range of ~60-145 ms [173, 174, 190, 198, 236]. While the identified P1 is approaching the previously reported latency of the P2 component in studies with comparable electrode configuration (170-220 ms) [171, 191, 232, 242], the first positive component was most likely to be P1 for the following reasons: (i) All components were delayed in the current study; for example the first negative peak in the current study occurred at an average latency of 89 ± 19 ms, compared to past studies in the range of 25-80 ms, (ii) the RREP morphology was preserved in comparison to past studies. In particular, consistent with P1 but not P2 the first positive component in the current
study was followed by a negative component (e.g. Webster and Colrain [191]), and, (iii) in the current study the first positive component increased in amplitude with increasing stimulus magnitude, which is also consistent with a P1 component but not P2. P2 amplitude has been shown to be independent of stimulus magnitude [173]. As suggested by Gora et al. in a similar context [190], the delayed latency is likely to be a result of the nature of the stimulus, in particular the reduced rate of pressure change, which is a function of the apparatus used to deliver the stimulus, as well as respiratory drive. While the rate of pressure change is generally not reported in RREP studies in the current study it was estimated that the range of \( \approx 15-30 \text{ cmH}_2\text{O} \cdot \text{s}^{-1} \) was in the order of 2-10 times slower than studies with the most comparable methodology [171, 174, 190, 198]. Indeed, Davenport et al. [189], presenting occlusion stimuli from onset of inspiration, noted that individuals with reduced rate of pressure change had longer component latencies and that mouth pressure change at 0.1 s was inversely correlated with the latency of the RREP P1 peak. To assess the possibility that slower change in pressure contributed to longer component latencies, 3 control participants attended on a separate occasion to the main experimental testing and RREP responses to occlusion of the apparatus with and without the loading manifold in circuit were compared. Removing the loading manifold resulted in maximal rate of pressure change increase from approximately 15 cmH\(_2\)O\cdot s\(^{-1}\) to 85 cmH\(_2\)O\cdot s\(^{-1}\) and a reduction in P1 latency in the order of 60 ms on average to an average latency of 97 ms. Negative pressure pulses were delivered to a further three participants resulting in a maximal rate of pressure change of approximately 300 cmH\(_2\)O\cdot s\(^{-1}\) and a reduction in P1 latency to approximately 80 ms. Thus longer RREP peak latencies may be due to a delay between stimulus onset and respiratory mechanoreceptor activation, and from these observations it was estimated that this delay was in the order of 80 ms for P1. Importantly, the faster rate of pressure change only reduced component latency and did not change the pattern of components observed. Given the middle-aged population examined in the current study, age may also be a factor contributing to longer component latencies. Harver et al. [239] reported
longer RREP component latencies in older (mean age ± standard deviation: 61.5 ± 3.6 years) vs. younger (26.5 ± 2.5) subjects in the range of 50-70 ms; for P3 in particular they reported an increase in latency with age of around 2 ms per year. However the median age of participants in the current study was approximately 40 years old and therefore age is unlikely to be a major contributor to the latency delays observed.

Given the task assigned to participants the influence that sleepiness and attention would have on results was considered. Counter measures taken included: (i) studying participants in an upright position, (ii) cuing participants prior to stimulus presentation, and, (iii) not including stimulus presentations during or adjacent to recorded micro-sleep. However most importantly, the current study focused on the initial P1 component of the RREP, which is thought to reflect arrival of somatosensory information at the cortex [174, 187, 189, 191, 196, 197] and has been shown not to be influenced by attention [174, 191]. Additionally, the P3 component was not adversely impacted by reduced attention expected in OSA patients. The finding that there were very few occasions where participants failed to respond to the stimulus and that there was no difference in response failure rate between control and OSA participants, also argues against sleepiness and attention influencing the current results.

Taking background conditions into consideration was of particular importance in the current study due to the small magnitude stimuli studied and because OSA patients had greater intrinsic resistance, measured via both the oral and nasal routes using plethysmography. The importance of doing so is highlighted by the finding that statistically significant OSA vs. control differences in the sensitivity of relationship between component amplitude and stimulus intensity were abolished after taking into account the background conditions. It has long been recognised that the smallest difference between two stimuli that can be consciously perceived increases with background stimulus intensity (known as Weber’s Law) [213]. Wiley and Zechman [167] were the first to demonstrate this applied to respiratory stimuli,
however more recently Chou and Davenport [198] have shown a similar relationship with RREPs; they demonstrated that larger respiratory resistive loads are required to elicit an RREP with increasing background resistance (incorporating extrinsic breathing circuit resistance and intrinsic resistance) and that the RREP can be abolished by increasing background resistance. Our finding of increased intrinsic resistance in OSA is in agreement with a number of previous studies using various methodologies to assess intrinsic resistance, such as catheters to measure upper airway resistance [177], and plethysmography to measure total airway resistance [178]. However, other studies have reported no difference [180, 181] or increased resistance in a supine but not a seated position [185]. Increased resistance may be due to a smaller upper airway observed in OSA; related to increased soft tissue volume [92, 96] or craniofacial differences [215]. Inflammation-related oedema may also be a factor in decreasing airway patency and increasing resistance [216]. Increased nasal resistance in particular has been recognised as a potential etiological factor in OSA [180] as flow limitation in the nasopharynx would lead to more negative pressure in the pharyngeal airway, favouring collapse. Current thinking however is that nasal resistance only plays a minor role in OSA pathogenesis [175, 217, 218] or that it may be important in association with certain pharyngeal conditions [217, 219]. In the current study while the mean difference between airways resistance measured via nasal and oral routes was greater in OSA vs. control participants, reflecting greater nasal resistance, the difference was not statistically significant.

In the current study, OSA participants demonstrated more negative baseline $P_{epi}$, possibly related to elevated intrinsic resistance. Thus, while there was no statistically significant difference in the stimulus $P_{epi}$ drop between groups, control participants had a greater $P_{epi}$ drop relative to background conditions. While stimulus conditions were not always equivalent between groups in the current study, this was not a concern as differences were accounted for in the statistical analysis. One of the advantages of the linear mixed model is that it is able to
account for uneven spacing of repeated measures, negating the need for matching of stimulus conditions between groups [311, 312].

While the specific stimulus for eliciting the RREP is unknown, given that upper airway receptors have been identified as a probable source of the RREP [261, 262], it was felt that $P_{\text{epi}}$ measurements were the most physiologically relevant stimulus measure. Alternative stimulus measures include changes in resistance or $P_{\text{mask}}$, with and without correction for background conditions. Importantly P1 data was analysed using these alternative stimulus measures without alteration of the findings. In the current study it was also noted that the calculated threshold for P1 elicitation was a negative value of $\Delta P_{\text{epi}}/P_{\text{epi}}_0$ (Table 7-3; Figure 7-3), which does not make intuitive sense. It should be noted however that confidence intervals included zero, allowing for a positive threshold. An alternative possibility is that relationship between stimulus intensity and P1 amplitude may be non-linear. To account for such a possibility a log transformation was applied to the stimulus measurement, however the model fit was not superior to the analysis using untransformed data. Nevertheless, it is worth noting that analysis following data transformation did not alter the findings or interpretations.

While the current results are not supportive of a deficit in sensory processing of respiratory stimuli in OSA it is important to recognise that it does not preclude the existence of a sensory deficit. There are numerous potential RREP sources in the respiratory system including the upper airway, as well as lower sources such as the lung, chest wall and diaphragm, thus a sensory deficit in one location may be compensated for others and there may be redundancy in sensory detection of respiratory loading. Current evidence suggests a contribution from the upper airway to the RREP [261, 262], possibly from intramuscular sources as upper airway anaesthesia does not alter the RREP [246, 261], and from respiratory pump muscle afferents [248], but not pulmonary mechanoreceptors, innervated by the vagus nerve [235, 262]. However, the finding that bypassing much of the upper airway with a laryngeal mask substantially reduced the cortical response to
negative pressure pulses [261], at least argues against redundancy of the upper airway in RREP production. It also remains possible that a raised threshold for eliciting the early components of the RREP may be important in some individuals. The current thinking about OSA pathogenesis is that it is multi-factorial, with some factors being more important in some individuals than others [30, 35, 313]. This may also explain the larger variation in stimulus characteristics observed in OSA participants (Table 7-2).

Also, given this study was conducted during wake, it remains possible that compensatory mechanisms exist during wake that are lost during sleep. While studies have reported impaired cortical evoked responses in OSA during sleep [190, 199], responses to threshold respiratory loads have not been examined and hence warrant further investigation. Such investigation would be made more difficult in a severe OSA population as used in the current study, however, due to persistent airway closure, hence previous studies examining cortical responses in sleep have used mild [190] or moderate [199] patient groups. In the current study, using a severe OSA population served to maximise the chance of finding group differences.

In addition, attempts were made to minimise confounders in the current study by restricting the BMI of participants to less than 35 kg·m\(^{-2}\) and selectively recruiting control participants with increased BMIs. While this meant groups were similar in BMI, the OSA group still had significantly greater BMI compared to the control group. This may have contributed to the lower lung volumes, increased resistance and therefore the differences in background pressures seen in OSA participants, however as previously mentioned, background conditions were accounted for in the analysis. It is possible that reducing the confounding influence of BMI, as well as excluding participants with co-morbidities that may influence cortical responses, may have resulted in an atypical OSA population sample and therefore care must be taken in extrapolating results to patients with increased BMIs and to patients
with co-morbidities common to sleep apnoea. Nevertheless, studies similar to ours examining such populations are of interest.

In the current study RREPs were recorded from midline electrode derivations (FZ, CZ, and PZ referenced to a liked mastoid), whereas others have recorded from lateral electrode sites (e.g. C3′ and C4′ for P1 measurements [174, 197, 198, 248]). Of interest, while early topographical studies in children report RREP components produced with lateral (e.g. P3, P4, C3, C4) but not midline electrode derivations (e.g. PZ, CZ)[196], topographical measurements in adults have generally not shown differentiation in amplitude between components measured from midline and lateral locations [232-234]. Alternatively, others have reported maximal component amplitudes at the midline [173]. While the dipole modelling study of Logie et al. [233] reported P1 source localisation posterior to the central sulcus, consistent with a location in the primary somatosensory cortex (and consistent with C3′ and C4′ being a suitable recording location) the authors acknowledged a limitation of their study in that a priori knowledge was used to provide starting coordinates for analysis. As the P1 component has been noted to be maximal in amplitude in parietal [173, 196, 233] and centro-parietal [191, 232, 234, 235] regions of the cortex, the current study may have benefited from recording at CPZ, however considering also that both groups were studied under the same conditions and the main interest was comparing groups, there is no indication that using alternative electrode locations would alter the findings or interpretations.

The majority of past RREP studies have delivered the stimulus via the oral route [174, 189, 191, 196-198, 201, 202, 242] while a smaller number, including the current study, have presented the stimulus via the nasal route [149, 171, 199]. This route was chosen as the nose is the normal breathing route during basal breathing [314] and due to participant comfort during the lengthy protocol. Using the nasal route has the potential to effect the RREP due to increased resistance via the nasal route [244, 245] and because increased background resistance is likely to
reduce RREP amplitude [198]. Despite this one study has reported no significant differences in RREPs elicited by occlusion via the nasal route compared to the oral route [246].

Given the negative result of this study one must also consider the possibility of a false negative finding (type-II error) or that the study was underpowered to find a difference, however this seems unlikely given the direction of the threshold results. Although no significant differences were found using the key measure of stimulus intensity, on average OSA participants had lower thresholds in producing the initial P1 component of the RREP, which is the opposite of what would be expected if OSA was associated with impaired sensory function. However, on average greater sensitivities were observed for control participants so that the possibility of a type-II error cannot be excluded.

7.7. Conclusion

The main finding from this study was that there was no statistically significant difference in wakefulness between OSA and control participants in the threshold or sensitivity for eliciting the initial P1 component of the RREP, after background conditions were taken into account. This is the first study to conduct such an analysis focussing on small stimuli spanning the threshold of conscious detection and in general the results do not support the concept of a sensory deficit of small respiratory loads contributing to the pathogenesis of OSA.

7.8. Postscript

This experimental thesis chapter addressed one of the key thesis aims. In particular it explored if there were impairments in OSA patients during wakefulness in the detection of negative pressure respiratory load stimuli close to the conscious detection threshold. In essence, the results do not support the concept of mechanosensory deficit of small respiratory loads contributing to the pathogenesis of OSA. That is, mechanosensory pathways likely remain intact. However, this does not mean that sensory detection is not important in OSA pathogenesis; the
results suggest that in OSA there may be reduced mechanoreceptor activation, related to increased background airway resistance, resulting in reduced sensory detection of small respiratory loads. The increased airway resistance is likely related to anatomical factors, such as a smaller upper airway observed in OSA, increased soft tissue volume or craniofacial differences. Inflammation-related oedema may also lead to decreased airway patency and increased resistance. In sum however, the current findings ultimately lead to the well-known conclusion that anatomy is an important factor contributing to OSA.

Although mechanosensory pathways likely remain intact in OSA, it remains possible that there are deficits in the upper airway neuromuscular response to small respiratory loads. Upper airway anatomy and muscles of the upper airway, the methodology for studying upper airway muscle responses to collapsing negative pressure forces, and what is known about upper airway responses in normal individuals and OSA subjects, was discussed in chapter 5. This then leads onto the experimental chapter that addresses the second key aim of this thesis (Chapter 8), which was to explore if there were impairments in OSA patients during wakefulness in the neuromuscular compensation for negative pressure respiratory load stimuli close to the conscious detection threshold.
8. Experiment 4: EMG response to threshold intensity respiratory loads in awake severe OSA patients and healthy controls

8.1. Preface
As mentioned in the thesis preface, this experimental chapter is prepared in a style suitable for publication. As such: (i) the chapter contains an abstract summarising the experiment, (ii) the chapter does not refer to supporting chapters and therefore material from supporting chapters may be repeated in summarised form, and, (iii) abbreviations are defined in full at first use in even if they have previously been defined in the thesis.
8.2. Abstract

Objective/Background: This study aimed to determine whether the genioglossus neuromuscular responses to small negative pressure respiratory stimuli, close to the conscious detection threshold, were impaired in obstructive sleep apnoea (OSA) patients.

Participants/Methods: A comparison was made between 16 severe OSA and 17 control participants in genioglossus electromyogram (EMGgg) response to mid-inspiratory resistive loads of varying intensity (≈1.2 to 6.2 cmH$_2$O·L$^{-1}$·s), delivered via a nasal mask while the subjects were awake and in a seated upright position. Analysis focused on the relationship between stimulus intensity and the peak EMGgg amplitude in the first 200ms post stimulus. It was hypothesised that OSA patients would have an increased threshold and reduced sensitivity in the relationship between EMGgg activation and stimulus intensity.

Results: There was no significant difference between control and OSA participants in the threshold or the sensitivity of the EMGgg amplitude vs. stimulus intensity relationship, where stimulus intensity was expressed as change in epiglottic pressure relative to background epiglottic pressure.

Conclusions: These results do not support the concept that a deficit in neuromuscular responsiveness to negative upper airway pressure contributes to OSA pathogenesis, however the results are likely influenced by a counterintuitive and novel genioglossus muscle suppression response observed in a significant proportion of participants. This suppression response was observed in both OSA and healthy control participants and may be related to the inhibition seen in inspiratory muscles such as the diaphragm in response to sudden onset negative pressure.
8.3. Introduction

Obstructive sleep apnoea (OSA) is characterised by repetitive narrowing or collapse of the pharyngeal airway during sleep, leading to reduction or cessation of airflow, despite ongoing respiratory effort. One of the key forces promoting collapse is the negative airway pressure generated by the diaphragm during inspiration, whereas a key force opposing collapse is the action of pharyngeal dilator muscles [35]. Of the pharyngeal dilator muscles the genioglossus muscle of the tongue is the most studied because of its size, action and accessibility. Its contraction protrudes the tongue and stiffens and enlarges the pharyngeal airway [134-139]. Consistent with this its stimulation reduces resistance and increases airflow in the upper airway [137, 287] and reduces the apnoea-hypopnoea index in OSA [290].

It has been demonstrated in a number of studies that upper airway dilator muscles, and the genioglossus in particular, respond to negative airway pressure, and this has been termed the ‘negative pressure reflex’ [141, 144-148, 150, 153-156]. Studies have examined this responsiveness to investigate whether it is diminished in OSA and therefore contributes to OSA pathogenesis [141, 148, 149]. In general negative pressure responsiveness has been studied using stimuli presented in two different ways: (i) using large sudden onset within breath negative pressure pulses [144-153] and, (ii) using stimuli presented over multiple breaths [141, 149, 151, 154-156]. Being sudden onset, pressure pulses have the advantage of being able to examine reflex responses while minimizing the influence of volitional muscle activity; however they have been criticised as being non-physiological [156]. On the other hand stimuli presented over multiple breaths can be delivered at respiratory rates and tidal volumes in the physiological range; however during wakefulness the influence of volitional activity cannot be excluded.

To date negative pressure pulse studies have not found a deficit in genioglossus muscle responsiveness in OSA during wakefulness [148, 149]. Multiple breath studies have shown there is a tight relationship between genioglossus activity and epiglottic pressure (Pepi), both within the entire breath and between conditions.
designed to alter negative airway pressure [155, 227], however no differences have been found between OSA and healthy controls [141, 149]. From this data it has been proposed that OSA is a result of a normal reduction in activation and responsiveness in dilator muscle activity in sleep in an anatomically compromised airway [30, 143]. However studies to date have not experimentally examined thresholds of genioglossus responsiveness to negative pressure; nor have they specifically targeted responses to small stimuli close to the conscious detection threshold. Such an omission may be important as failure to respond to a small stimulus may lead to further collapse that is difficult to remedy by later muscle recruitment, or to responses that occur too late to prevent arousal from sleep.

This study aimed to examine whether genioglossus activation was impaired in OSA in response to small negative pressure stimuli, close to the conscious detection threshold. To address this aim a different approach to stimulus delivery was utilised compared to previous studies. Graded, within breath, sudden onset, mid-inspiratory resistive loads were used. Such a stimulus has the following advantages: (i) Compared to a negative pressure pulse it is a more physiological pressure change, both because its slower onset and because the pressure change is generated by the participant’s diaphragm driven inspiratory activity, and (ii) as the stimulus is sudden onset, reflex responses can be assessed, while minimising the influence of volitional activity. Stimulus delivery at peak inspiratory flow was chosen so as to present the stimulus at the point where negative pressure collapsing force was at its maximum. Using such a stimulus the hypothesis of the current study was that participants with OSA would have increased negative pressure thresholds for genioglossus activation and reduced sensitivity of the relationship between genioglossus activation and $P_{epi}$. 
8.4. Methods

8.4.1. Ethical approval

The study conformed to the standards set by the Declaration of Helsinki and was approved by the Austin Health Human Research Ethics Committee. All participants gave informed written consent to participate in the study.

8.4.2. Participants

Sixteen untreated severe OSA patients and 17 healthy controls participated. All participants were male, and free from respiratory, neurological, psychiatric, cardiovascular, and sleep disorders (other than OSA for the patient group). Exclusion criteria for both groups included current intake of psychoactive medications, as well as alcohol or recreational drug abuse or tobacco use.

Spirometry (JLab software version 5.2, PFTpro with whole body plethysmograph, Jaeger, Carefusion GmbH, Wurzberg, Germany or Vmax Spectra 62J body plethysmograph, Sensormedics, Yorba Linda, CA, USA) confirmed normal lung function in all participants. The groups were matched for age, and attempts were made to minimise body mass index (BMI) differences between groups by restricting the BMI of participants to less than 35 kg·m$^{-2}$. Patients were recruited following a recent diagnosis of severe OSA via polysomnography (PSG) (E-series or Somté PSG equipment, Compumedics, Abbotsford, Victoria, Australia), defined as an apnoea hypopnoea index (AHI) greater than 30·h$^{-1}$ using ‘Chicago’ criteria [13], plus at least 5% of sleep time with oxygen saturation less than 90%. Healthy control participants were recruited from the community and were required to have an AHI less than 15·h$^{-1}$ using Chicago criteria for scoring respiratory events, confirmed using PSG. In a suspected OSA population a ‘Chicago’ AHI of 15·h$^{-1}$ has estimated to be approximately equivalent to 4·h$^{-1}$ using the American Academy of Sleep Medicine (AASM) 2007 [9] ‘recommended’ criteria and 9·h$^{-1}$ using AASM 2007 ‘alternative’ criteria [16], which is most similar to most the recent recommendations [12].
8.4.3. Preliminary visit

For control participants an initial screening visit included the overnight PSG as well as lung function testing the following morning. For the OSA group the screening visit incorporated lung function testing only. In addition to spirometry, in both groups total airways resistance ($R_{aw}$) was measured by plethysmography, via the oral route with a mouthpiece, as well as via the nasal route with a modified nasal mask (Profile Lite, Philips Respironics, Murrysville, PA, USA), both before and following nasal decongestant (0.05% oxymetazoline hydrochloride). Following administration of oxymetazoline hydrochloride solution, local vasoconstriction usually occurs within five to ten minutes, persists for five to six hours, and then gradually declines over the next six hours [307]. The difference between the pre-decongestant nasal and oral resistance estimates were considered to reflect the nasal component of upper airway resistance.

8.4.4. Experimental protocol and equipment

Participants attended the laboratory at 8am for experimental testing on a separate day following the screening visit, having abstained from alcohol and caffeine for at least 12 hours.

In addition to electroencephalogram (EEG), electrooculogram (EOG), $P_{epli}$, mask pressure ($P_{mask}$), and airflow recordings, which have been described previously in section 6.4.3, genioglossus electromyogram (EMGgg) measurements were also obtained.

Two pairs of fine-wire nylon-coated intramuscular electrodes (model no. MA318/30, Motion Lab Systems, Baton Rouge, LA, USA) were inserted per-cutaneously into the genioglossus after surface anaesthesia (EMLA cream, AstraZenica, North Ryde, NSW, Australia), using needles as guides, as described by Eastwood and co-workers [144]. Needles were inserted in a sagittal plane ($90^\circ$) under the chin at
10 mm posterior to the inferior margin of the mandible and at a target distance of approximately 3 mm either side of the midline, guided by prior genioglossus muscle contraction via tongue protrusion. The electrodes were inserted to a target depth of 25-30 mm. Target depth was verified by ultrasound (Site Rite 3, Bard Access Systems, Salt Lake City, UT, USA) prior to insertion in ten participants. After removal of the needle, the wires were securely taped into place and attached to spring clips connected to the ends of the amplifier lead wires. Single wires from each pair were connected to provide a EMGgg signal across the midline and the second wire of each pair was used for backup in case of failure of the first. The across-midline electrode pair was referenced to a common electrode on the forehead to yield a bipolar recording.

Raw EMGgg signals were amplified and band-pass filtered between 10 and 1000 Hz (CED1902, Cambridge Electronic Design). EEG and EOG signals were amplified and band-pass filtered (S-Series, Compumedics) between 0.3 and 100 Hz for EEG and 0.3 and 35 Hz for EOG. All signals were digitally sampled at 1000 Hz except for EMGgg which was sampled at 2000 Hz (Micro1401 mkII, Cambridge Electronic Design) and were visually displayed and recorded throughout the experimental session (Spike2, Cambridge Electronic Design, Cambridge, England).

The breathing circuit has also been previously described in section 6.4.3. It included a modified sealed nasal mask (Profile Lite, Philips Respironics), a non-rebreathing valve (series 2600, Hans Rudolph, Kansas City, MO, USA), and a custom-made manifold, used to provide respiratory stimuli, which was situated in an adjacent room to the subject and connected to the inspiratory side of the non-rebreathing valve via reinforced tubing. The manifold allowed presentation of various resistive loads, with good linearity characteristics, spanning the conscious load detection threshold (≈1.2, 2.2, 3.0, and 6.2 cmH$_2$O·L$^{-1}$·s) above a background circuit resistance of approximately 2 cmH$_2$O·L$^{-1}$·s, as well as a control condition (no added resistance above background circuit) and a complete flow occlusion. Control
of stimulus presentations was performed using custom software with stimuli presented in semi-random order (block design) every 2-4 breaths during mid-inspiration with a target of 90 presentations of each stimulus. The stimulus continued until end inspiration for all stimuli except for the occlusion stimulus which, for participant comfort, was presented for 800 ms. To maintain attention the participant was cued via headphones at end inspiration on the breath prior to stimulus presentation with an automatically generated message “next breath”. Background music of the subject’s choice served to mask experimental sounds. Forced decision conscious detection of the presented stimulus (Yes/No) was signalled with a button press.

Subjects were seated in an upright position in a high-backed dentist’s chair with back, arms and head supported to allow relaxation of postural muscles and standardise head position. Standardised information and instructions were given to each participant prior to the experimental session. They were instructed to sit quietly and comfortably, breathe as normally as possible via the nasal mask and avoid falling asleep.

Immediately prior to the experimental session participants performed multiple swallowing and forceful tongue protrusion manoeuvres to determine maximal EMG activity, as well as biological checks to ensure signal quality, including eyes open, closed, left, right, up and down, followed by a 5 minute familiarisation session.

During the experimental session participants were allowed a 5 minute break every 20-30 minutes. The entire visit, including equipment set-up, familiarisation and the experimental protocol lasted approximately 3.5-4.5 hours depending on respiratory rate.

Three control participants returned for repeat testing to test repeatability of EMG response pattern.
8.4.5. Data analysis

All EMGgg measurements were semi-automated and customised using software (Spike2). The EMGgg signal was (i) full wave rectified, (ii) smoothed with a 100 ms moving-time-average, and (iii) ensemble averaged, separately for each stimulus intensity, time locked to the stimulus generating transistor-transistor logic (TTL) electrical pulse which triggered balloon valve inflation. Responses to the different stimulus intensities were normalised by equating the signal averages in a 200 ms pre-stimulus window.

Individual trials were included in the average if the target breath was free from swallows and movement artefact and if a 20 second window around the stimulus (viewed in a 30 second epoch) was free from sleep, defined as any theta frequency or slower activity observed in the central EEG derivation. The presence of sleep was determined by an experienced polysomnographic scientist.

Peak activation within a 200 ms window post stimulus onset was measured separately for each stimulus intensity using an automated algorithm. The measurement was expressed relative to the zero load control, and as a percentage of maximal activation during swallowing. Stimulus onset was defined as the last point preceding the sudden decrement in the ensemble averaged $P_{\text{mask}}$ following balloon valve activation. As there was no sudden $P_{\text{mask}}$ decrement for the control condition, stimulus onset was estimated as the average onset of all other stimulus conditions. The 200 ms response window was chosen to minimise the influence of behavioural responses to the stimulus, and peak activation was measured relative to the control condition to account for EMGgg changes associated with phasic EMGgg activity during unobstructed tidal breathing.

Flow and pressure channels were also ensemble averaged, time locked to the TTL pulse. Prior to ensemble averaging of the $P_{\text{epi}}$ channel the $P_{\text{epi}}$ signal was offset to account for a small pressure generated by air perfusion of the catheter.
Basal EMGgg activity was described according to its tonic and phasic activity based on the expiration and inspiration immediately prior to the stimulus presentation. Tonic activity was defined as the minimal activity, and phasic activity as the maximal activity, during that period.

The conscious detection threshold was defined as previously described in section 6.4.4.

**8.4.6. Statistical analysis**

The initial analysis explored differences between OSA and control participant characteristics using Mann Whitney U tests, as not all parameters were normally distributed. Results are presented as median (inter-quartile range). Differences in maximal, tonic and phasic activity between groups were compared using independent sample t-tests. Additionally, the impact of participant group (OSA vs. Control) and stimulus condition (intensity) on stimulus characteristics were explored using two-way mixed between-within group ANOVAs. Where appropriate, results were further examined using Bonferroni post hoc comparisons. Results are presented as mean ± standard deviation.

The primary analysis utilised linear mixed effect regression models (LMM) [311] to compare the relationship between stimulus intensity and EMGgg activation in the first 200 ms post-stimulus, expressed as a percentage of maximal activation, between groups; with the models allowing for random patient intercepts. In the analysis, due to its likely physiological significance, the key measure of stimulus intensity was considered to be maximal Pepi change in the first 200 ms post stimulus ($\Delta$Pepi) as a proportion of background Pepi ($\text{Pepi}_0$). $\text{Pepi}_0$ was defined as the nadir Pepi immediately prior to stimulus onset.
This analysis yielded a statistical comparison of (i) the slope of the relationship between EMGgg amplitude and stimulus intensity (EMGgg sensitivity), and (ii) EMGgg amplitude at zero stimulus intensity (y-axis intercept) between groups. Rather than the y-axis intercept, the key interest was the point where the relationship between EMGgg amplitude and stimulus intensity crossed the stimulus intensity (x) axis or, in other words, EMGgg activation threshold. To determine this, the same LMMs as above were applied to OSA and control participants separately so that the linear equation could be solved for $y = 0$. For each group this analysis yielded model estimates of: (i) the slope of the relationship between EMGgg amplitude and stimulus intensity, (ii) EMGgg amplitude at zero stimulus intensity (y-axis intercept), and, (iii) the variance and covariance associated with these. This allowed calculation of the x-axis crossing and estimation of the associated variance. Subsequent independent-samples $t$-tests were conducted to compare EMGgg activation threshold differences between OSA and controls.

8.5. Results

8.5.1. Participant characteristics

There were no statistically significant differences between control and OSA groups in age, height, weight, forced expiratory volume in 1 second (FEV1) and forced expiratory ratio (FER). OSA patients had larger BMI and neck circumference, reduced absolute total lung capacity (TLC), absolute and percent predicted functional residual capacity (FRC) and absolute forced vital capacity (FVC), and elevated Raw (measured via oral route as well as nasal route, pre- and post-decongestant) compared to controls. The nasal component of upper airway resistance (nasal resistance – oral resistance) was not statistically significantly different between groups. As expected OSA patients had increased ESS, AHI and percentage sleep with oxygen saturation ($\text{SpO}_2$) less than 90%, compared to controls (Table 8-1).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSA</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>n</td>
<td>M:F</td>
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</tr>
<tr>
<td>Demographics</td>
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<td>16:0</td>
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<td>Age (y)</td>
<td>43.5 (38.8, 48.0)</td>
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<td>Height (m)</td>
<td>1.73 (1.69, 1.79)</td>
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<td>Weight (kg)</td>
<td>91.5 (82.8, 99.0)</td>
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<td>BMI (kg·m$^{-2}$)</td>
<td>31.4 (27.6, 32.4)</td>
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<td>Neck Circumference (cm)</td>
<td>44.0 (40.8, 45.3)</td>
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<tr>
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<td></td>
</tr>
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<td>ESS</td>
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<td>5.0 (2.0, 6.0)</td>
<td>&lt;0.001</td>
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<td>AHI (·h$^{-1}$)</td>
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<td>4.4 (2.6, 5.8)</td>
<td>&lt;0.001</td>
</tr>
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<td>%TST&lt;90%SpO$_2$</td>
<td>9.2 (5.0, 19.0)</td>
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<td>Respiratory parameters</td>
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<td></td>
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<tr>
<td>FEV1 (L BTPS)</td>
<td>3.6 (3.4, 4.0)</td>
<td>4.1 (4.0, 4.5)</td>
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<td>FEV1 (%Pred)</td>
<td>93.0 (85.5, 102.8)</td>
<td>103.0 (97.0, 111.0)</td>
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<tr>
<td>FVC (L BTPS)</td>
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<td>FVC (% Pred)</td>
<td>92.5 (86.8, 104.3)</td>
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<td>FER (%)</td>
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<td>FER (%Pred)</td>
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<td>97.0 (92.0, 102.0)</td>
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<td>FRC (% Pred)</td>
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<td>TLC (L BTPS)</td>
<td>6.1 (5.6, 6.6)</td>
<td>6.8 (6.3, 7.9)</td>
<td>0.023</td>
</tr>
<tr>
<td>TLC (% Pred)</td>
<td>95.0 (90.8, 110.8)</td>
<td>105.0 (99.0, 110.0)</td>
<td>0.168</td>
</tr>
<tr>
<td>RV (L BTPS)</td>
<td>1.6 (1.2, 1.8)</td>
<td>1.6 (1.3, 2.0)</td>
<td>0.683</td>
</tr>
<tr>
<td>RV (%Pred)</td>
<td>87.0 (72.3, 88.3)</td>
<td>87.0 (71.0, 95.0)</td>
<td>0.510</td>
</tr>
<tr>
<td>Raw (cmH$_2$O·L$^{-1}$·s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral route</td>
<td>3.1 (2.7, 4.0)</td>
<td>2.3 (2.0, 2.8)</td>
<td>0.026</td>
</tr>
<tr>
<td>Nasal route (Pre)</td>
<td>6.2 (5.7, 7.6)</td>
<td>5.1 (3.7, 6.3)</td>
<td>0.048</td>
</tr>
<tr>
<td>Nasal route (Post)</td>
<td>4.7 (4.1, 5.6)</td>
<td>4.0 (2.8, 5.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Nasal (Pre) – oral route</td>
<td>3.2 (1.9, 4.5)</td>
<td>2.3 (1.4, 3.5)</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Values are median (inter-quartile range). Abbreviations: BMI: body mass index; ESS: Epworth Sleepiness Scale; AHI: apnoea hypopnoea index derived using AASM Chicago hypopnoea definition [13]; %TST<90%SpO$_2$: % of total sleep time with oxygen desaturation less than 90%; FEV$_1$: forced expiratory volume in 1 second; FER: forced expiratory ratio; FVC: forced vital capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity. Raw: airway resistance measured using body box plethysmography; Pre/Post: pre or post decongestant (oxymetazoline); % Pred: percent predicted; BTPS: body temperature and pressure, saturated. P-values derived from Mann Whitney U tests.
8.5.2. Maximal EMG activity

Maximal EMG activity during swallows (mean ± standard deviation) was not statistically different between OSA and control participants (739 ± 299 µV vs. 761 ± 258 µV; \( P = 0.825 \)).

8.5.3. Basal EMG activity

Inspiratory phasic activity was observed in 16 of 17 control participants. In one control participant, while clear activation was seen during swallowing and tongue protrusion, no phasic activation was observed during basal breathing or hyperventilation. Inspiratory phasic activity was observed in 9 of 16 OSA participants, however in 5 OSA participants expiratory phasic activity was observed and in two participants no phasic activity was observed. Of those with expiratory phasic activity, all showed increased expiratory phasic activity with hyperventilation. Of those without phasic activity, one showed a small amount of inspiratory phasic activation with hyperventilation on the experimental visit. The other participant displayed little phasic activity with hyperventilation, despite clear activation with tongue protrusion and swallowing.

Tonic EMG activity, expressed as a percentage of maximal EMG activity was not different between OSA and control participants (4.3 ± 4.5% vs. 4.3 ± 2.9%: \( P = 0.988 \)), and while the mean difference between tonic and phasic activity was larger for control participants (4.7 ± 5.4%) compared to OSA participants (2.6 ± 3.2%) this difference was not statistically different (\( P = 0.172 \)).

8.5.4. Stimulus characteristics

There was no statistically significant difference in the number of stimulus presentations per stimulus condition for OSA participants (97.8 ± 4.8) compared to controls (97.2 ± 8.8; \( P = 0.807 \)). As per the study block design the main effect of stimulus condition on the number of stimulus presentations (\( P = 0.283 \)) and the interaction (\( P = 0.313 \)) did not reach statistical significance.
Of the individual stimuli presented, on average 81.5 ± 12.4% were considered suitable to be included in the ensemble average with no statistically significant difference between stimulus conditions ($P = 0.427$) or participant groups ($P = 0.427$) and no statistically significant interaction effect ($P = 0.409$).

On average participants failed to make a forced choice as to whether they could detect a load on 2.8 ± 4.8% of stimulus presentations. There was no statistically significant main effect of group ($P = 0.369$) on the number of failed response stimulus presentations nor was there a statistically significant interaction effect ($P = 0.702$). There was a statistically significant main effect of stimulus condition ($P = 0.001$) with, in general, no response observed in a larger proportion of lower intensity stimuli (e.g. 3.8 ± 6.1% vs. 0.6 ± 1.3% for lowest intensity resistive loading condition and occlusion condition respectively; $P = 0.008$).

Fewer stimulus presentations were included in the Pepi ensemble average due to technical issues likely related to catheter blockages or the catheter tip contacting the airway wall. Poor quality Pepi measurements were apparent for the entire recording for one control subject due to these technical issues; and this participant was excluded from the analysis.

Balloon valve activation resulted in reduction in flow, as well as more negative $P_{\text{mask}}$ and $P_{\text{epi}}$ in comparison to the control condition (Figure 8-1; Table 8-2)
Figure 8-1: Example of the various stimuli presented in the study. Panels from top to bottom are: (i) flow, (ii) mask pressure, and, (iii) epiglottic pressure. Traces are the ensemble average in a single control participant, with the various stimulus intensities overlayed on each other. Note the stimulus is presented during mid-inspiration. Abbreviations: $P_{\text{mask}}$: mask pressure; $P_{\text{epi}}$: epiglottic pressure.
There was no statistically significant difference between OSA and control participants in $\Delta P_{\text{epi}}$ in response to the stimulus; however $P_{\text{epi}}_0$ was more negative in OSA (-5.0±2.2 cmH$_2$O) compared to control participants (-2.7 ± 1.2 cmH$_2$O; $P = 0.001$). This corresponded with more negative mask pressure and increased flow in OSA subjects. This meant that $\Delta P_{\text{epi}}$ was greater in control compared to OSA participants when expressed as a proportion of $P_{\text{epi}}_0$ (Table 8-2).

Table 8-2: Stimulus characteristics in first 200ms post stimulus presentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSA</th>
<th>Control*</th>
<th>Group $P$-value</th>
<th>Interaction $P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta P_{\text{epi}}/P_{\text{epi}}_0$</td>
<td></td>
<td></td>
<td>0.009</td>
<td>0.011</td>
</tr>
<tr>
<td>load 1</td>
<td>0.06 (0.06)</td>
<td>0.13 (0.09)</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>0.12 (0.07)</td>
<td>0.19 (0.12)</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>0.15 (0.07)</td>
<td>0.30 (0.37)</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>0.23 (0.10)</td>
<td>0.50 (0.35)</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>0.42 (0.18)</td>
<td>0.72 (0.39)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>$\Delta P_{\text{epi}}$ (cmH$_2$O)</td>
<td></td>
<td></td>
<td>0.365</td>
<td>0.505</td>
</tr>
<tr>
<td>load 1</td>
<td>-0.30 (0.27)</td>
<td>-0.29 (0.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>-0.48 (0.24)</td>
<td>-0.41 (0.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>-0.65 (0.30)</td>
<td>-0.57 (0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>-1.02 (0.36)</td>
<td>-0.86 (0.42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>-1.82 (0.67)</td>
<td>-1.57 (0.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{epi}}$ Nadir (cmH$_2$O)</td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.326</td>
</tr>
<tr>
<td>load 1</td>
<td>-5.31 (2.45)</td>
<td>-2.94 (1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 2</td>
<td>-5.45 (2.41)</td>
<td>-3.12 (1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 3</td>
<td>-5.65 (2.61)</td>
<td>-3.15 (1.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>load 4</td>
<td>-6.02 (2.92)</td>
<td>-3.57 (1.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occlusion</td>
<td>-6.84 (4.41)</td>
<td>-4.26 (2.02)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). Abbreviations: Pepi: Epiglottic pressure; $\Delta$Pepi: Change in epiglottic pressure; $P_{\text{epi}}_0$: Background epiglottic pressure. $P$-values derived using two-way mixed between-within group ANOVAs. Added resistive load intensities: load 1 = 1.2 cmH$_2$O·L$^{-1}$·s; load 2 = 2.2 cmH$_2$O·L$^{-1}$·s; load 3 = 3.0 cmH$_2$O·L$^{-1}$·s; load 4 = 6.2 cmH$_2$O·L$^{-1}$·s. *: n = 16 for controls

![Image of the page content](image_url)
8.5.5. Conscious detection threshold

The conscious detection threshold was within the range of presented resistive loads for the majority of participants. The conscious detection threshold could not be determined for one OSA individual in whom the threshold would have been greater than the largest delivered resistive load stimulus. The calculated detection threshold was below the lowest delivered resistive load stimulus for two control participants; and above the largest delivered resistive load stimulus for one OSA and one control subject. There was no statistically significant difference in calculated conscious detection threshold between OSA ($3.1 \pm 1.8 \text{ cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$) and control participants ($2.4 \pm 1.6 \text{ cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}; P = 0.292$). When expressing the detection threshold as a Weber fraction, accounting for background conditions, there was also no difference in conscious detection threshold between OSA ($0.46 \pm 0.33$) and controls ($0.47 \pm 0.39; P = 0.966$).

8.5.6. EMGgg response

An example of the EMGgg response to the various stimuli is shown in Figure 8-2.
Figure 8-2: Example of the genioglossus EMG response to various stimuli presented during the study in a single control subject. Traces are the ensemble average, with the various stimulus intensities overlayed on each other. Panels from top to bottom are: (i) Rectified, 100ms moving time average EMGgg, (ii) The same signals as in the top panel expressed relative to the zero load control, thus accounting for any background phasic activity observed and, (iii) epiglottic pressure change in response to stimulus presentation. The grey vertical panel represents the 200ms window in which key EMG measurements were made. Vertical dashed line represents stimulus onset, defined as the last point preceding the sudden decrement in the ensemble averaged P\text{mask}. Note the stimulus is presented during mid-inspiration. Abbreviations: EMGgg: genioglossus electromyography. P\text{epi}: epiglottic pressure.
The threshold of the relationship between EMG activation in the first 200ms post stimulus and stimulus intensity, expressed as $\Delta \text{P}_{\text{epi}}/P_{\text{epi}0}$, was not statistically different between OSA patients and control participants (Table 8-3). The same pattern was observed if stimulus intensity was expressed as $\Delta \text{P}_{\text{epi}}$ or the nadir $P_{\text{epi}}$ developed after stimulus onset. The sensitivity (slope) of the relationship between EMG activation and stimulus intensity was not significantly different between OSA patients and control participants when the stimulus was expressed as nadir $P_{\text{epi}}$, $\Delta \text{P}_{\text{epi}}$, or as $\Delta \text{P}_{\text{epi}}/P_{\text{epi}0}$ (Table 8-3).

Table 8-3: The threshold and sensitivity of the relationship between stimulus intensity and the peak genioglossus EMG activation (%max) in the first 200ms post stimulus.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stimulus</th>
<th>Control</th>
<th>OSA</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold</td>
<td>$\Delta \text{P}<em>{\text{epi}}/P</em>{\text{epi}0}$</td>
<td>-75.86 (-8598.08, 8446.36)</td>
<td>-0.37 (-1.38, 0.63)</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>$\Delta \text{P}_{\text{epi}}$ (cmH(_2)O)</td>
<td>281.72 (-58749.90, 59313.34)</td>
<td>2.14 (-4.54, 8.82)</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>$P_{\text{epi min}}$ (cmH(_2)O)</td>
<td>18.60 (-210.82, 248.01)</td>
<td>-15.53 (-38.14, 7.09)</td>
<td>0.772</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>$\Delta \text{P}<em>{\text{epi}}/P</em>{\text{epi}0}$</td>
<td>0.01 (-0.81, 0.83)</td>
<td>0.44 (-0.41, 1.29)</td>
<td>0.379</td>
</tr>
<tr>
<td></td>
<td>$\Delta \text{P}_{\text{epi}}$ (cmH(_2)O)</td>
<td>0.00 (-0.41, 0.41)</td>
<td>-0.08 (-0.28, 0.12)</td>
<td>0.549</td>
</tr>
<tr>
<td></td>
<td>$P_{\text{epi min}}$ (cmH(_2)O)</td>
<td>-0.02 (-0.30, 0.25)</td>
<td>0.02 (-0.03, 0.08)</td>
<td>0.820</td>
</tr>
</tbody>
</table>

Values are model estimates (5th and 95th confidence intervals) from linear mixed models conducted separately for each group. $P$-values for sensitivity are from a linear mixed model comparing groups and $P$-values for threshold are from t-tests after threshold derivation following separate linear mixed models. Abbreviations: $P_{\text{epi}}$: Epiglottic pressure; $\Delta \text{P}_{\text{epi}}$: Change in epiglottic pressure; $P_{\text{epi}0}$: Background epiglottic pressure.

In this study, genioglossus EMG activation was expected in response to negative pressure stimuli. However, EMG suppression was observed in some participants (Figure 8-3) which is likely to have confounded the planned analysis.
Figure 8-3: Example of genioglossus EMG suppression in response to negative pressure stimuli in a single control participant. Traces are the ensemble averages, with the various stimulus intensities overlayed on each other. Panels from top to bottom are: (i) Rectified, 100ms moving time average EMGgg, (ii) The same signals as in the top panel expressed relative to the zero load control, thus accounting for any phasic background activity observed and, (iii) epiglottic pressure change in response to stimulus presentation. The grey vertical panel represents the 200ms window in which key EMG measurements were made. Abbreviations: EMGgg: genioglossus electromyography. Pepi: epiglottic pressure.
To objectively quantify participant response types in the first 200ms and to ascertain if response characteristics were different between OSA and control participants, for each stimulus intensity responses were classified into the following categories; (i) no response, (ii) activation only, (iii) suppression only, and, (iv) activation and suppression, based on whether the EMGgg signal relative to the zero load control deviated outside set limits; which were set at ± 2 times the standard deviation of the EMGgg activity observed in the 200ms baseline window prior to stimulus presentation.

As can be seen from Figure 8-4 there is some suggestion that there is less activation for OSA participants in the 200ms window post stimulus presentation, particularly at lower stimulus intensities. However as has been previously presented, each resistive load stimulus is smaller for OSA participants when expressed as a fraction of their background resistance.
Figure 8.4: Categorised genioglossus EMG responses within 200ms of the various stimuli applied during the study.

Notes: Responses were determined objectively, with a response determined to have occurred if the EMG activity crossed a predetermined threshold of ± 2 standard deviations of the activity observed in a 200ms baseline period prior to stimulus onset.

8.6. Discussion

The aim of this study was to investigate whether upper airway dilator muscle activation, in particular the genioglossus muscle activation, was defective in OSA participants compared to normal control participants, in response to small negative pressure stimuli close to the conscious detection threshold. It was reasoned that responses to small stimuli may be important in OSA pathogenesis as failure to respond to small stimuli may lead to further collapse that is difficult to remedy by later muscle recruitment. The study was unable to establish any significant differences in the threshold or sensitivity of the relationship between EMGgg activation and stimulus intensity between OSA and control participants. Therefore the results are not supportive of the concept that a deficit in neuromuscular
responsiveness to small negative upper airway pressures contributes to OSA pathogenesis.

However the study results are likely confounded by the heterogeneity of genioglossus muscle responses to negative pressure observed; in particular, in some individuals the genioglossus muscle responded to sudden onset of upper airway negative pressure with a counter-intuitive suppression of activity. The suppression response is not expected given that the majority of studies show that the genioglossus muscle activates in response to the collapsing force of negative upper airway pressure [144, 146-148, 151, 158]. The suppression response is not totally unique in the negative pressure reflex literature however [145, 149]; Eckert et al. [145] first reported a previously undescribed suppression component in the reflex EMGgg response to negative pressure pulses delivered via a nasal mask to the upper airway. In that study the EMGgg signal was rectified but no moving time average was applied and the authors suggested that suppression may not have been observed in previous studies due to the moving time average obscuring the suppression component. However, similar to many previous studies, the current study also utilised a 100ms moving time average and suppression was still observed in quite a large portion of participants; for the largest resistive load 56% and 59% for control and OSA participants respectively. Additionally, in the study of Eckert et al. an activation component was noted prior to a suppression component, whereas in the current study suppression was commonly seen in isolation in the first 200ms, or was seen prior to an activation component. Suppression of genioglossus activity has also been observed in response to arousal from sleep in a study examining single motor units [315]. In that study, although not the predominant response, approximately 22% of motor units that were active prior to arousal ceased activity following arousal.

Confidence was obtained that the suppression component was not artifactual in the current study in that, (i) it was observed in multiple participants, (ii) it was load dependent; with suppression increasing with increasing load magnitude, and most
importantly, (iii) it was repeatable. Of three control participants who returned for repeat testing, one who showed activation and two who showed suppression of EMGgg activity on initial presentation, all three showed the same pattern of response on the subsequent visit.

The reason for genioglossus suppression is not clear, however Eckert et al. [145] postulated that the suppression phase may be related to the inhibitory response seen in inspiratory muscles such as scalene, parasternal intercostals and the diaphragm in response to brief negative pressure stimuli [304, 316, 317] and therefore is due to a central pattern generator driven, general respiratory inhibitory response. In this way the suppression response may be designed to protect the airway. For example, if the increase in resistance was due to inhalation of a foreign object it may be counter-productive to attempt to increase flow and reduce resistance by opening the airway with activation of the upper airway dilator muscles. Of interest, anaesthesia studies suggest that, as opposed to the activation response of the genioglossus muscle [300], the inhibitory response of other inspiratory muscles is minimally mediated by mucosal receptors in the upper airway [304].

The fact that the suppression response has not been observed in the majority of previous studies with sudden onset respiratory stimuli may be related to the uniqueness of the stimulus used in the current study. The stimulus used in the current study was of lesser magnitude and rate of pressure change compared to the negative pressure pulses used in prior studies. Additionally, in the current study the stimulus was presented in mid inspiration at the point where negative pressure collapsing force was at its maximum, and where subtle flow limitation would likely first occur in OSA. Previous sudden onset negative pressure studies have presented the stimulus just prior to inspiration or in early inspiration [144-149, 151, 158], when the influence of the central pattern generator on genioglossus muscle activity is minimised. Thus the suppression response seen in the current study may be explained by increased central influence on genioglossus activity.
It must be noted that this suppression response does not occur in all individuals, some responding with activation only and others responding with a suppression and activation and phase in the first 200ms post-stimulus. While there was some suggestion that there were a lesser number of OSA participants showing EMGg activation compared to controls for the same added resistive load stimulus, this may be explained by the increased background resistance observed in OSA participants. This equates to a lesser stimulus delivered for a given resistive load to OSA patients compared to controls. Thus there is no clear evidence that differences in EMGgg activation or suppression responses are a major consideration in OSA pathogenesis overall. However this does not preclude the genioglossus response being important for some OSA individuals; the current thinking about OSA pathogenesis is that it is multi-factorial, with some factors being more important in some individuals than others [30, 35, 313]; this may also be the case for the genioglossus response.

The reason why suppression would occur in some individuals and not others remains unclear although it may be a reflection of the heterogeneity of the genioglossus muscle [144, 318, 319]. Single motor units studies have shown that there is a range of motor unit types in the genioglossus muscle including inspiratory modulated (fire only during inspiration or more so in inspiration), expiratory modulated (fire only in expiration or more so in expiration), tonic (fire in inspiration and expiration to a similar degree) and tonic other (fire in expiration and inspiration with non-respiratory modulation) [318, 319]. Multi-unit recordings, such as those from the current study, are made up of multiple single motor units, which would have a range of the above mentioned discharge patterns. As the different patterns are dispersed throughout the muscle, the pattern of the multi-unit recording will depend on the sample of single motor units recorded. Similar to the heterogeneity of response seen in single motor units to arousal from sleep [315] there may be heterogeneity in how single motor units respond to negative pressure stimuli. For some individuals in the current study the majority of motor units
sampled were predominately of the kind where suppression of activity was the major response. In the current study genioglossus activity was measured using a single EMG channel, and while attempts were made to standardise electrode placement between individuals with the placement occurring close to the genioglossus origin where activity is thought to be maximal, it is also clear that different response patterns are possible in the same individual with different placement [144, 318, 319]. It was reassuring however that that same pattern of response was observed in the three control individuals that were retested on a separate occasion to the experimental testing. Nevertheless, future studies may benefit from sampling genioglossus activity simultaneously from multiple locations.

This heterogeneity of genioglossus muscle activity also reflected in phasic activity observed with 5 of 16 OSA individuals showing expiratory phasic activity during basal breathing. This activity is unexpected given that multi-unit EMG studies have largely shown inspiratory phasic activity in the genioglossus muscle [141, 142, 144, 145, 150, 154, 274, 275, 291, 292]. Also, in single motor unit studies only 7-15% of motor units have shown expiratory modulated activity, compared to approximately 50-80% showing inspiratory modulated activity [318, 319], with no difference in the proportions of the different activity patterns between control and OSA subjects [319]. Thus, by random chance, in some OSA patients the predominant pattern of the sampled motor units was increased activity during expiration. Confidence in electrode placement can be gained from the fact that in all these individuals expiratory phasic activity increased with hyperventilation; where phasic activation with hyperventilation has been suggested as a check for correct electrode placement in the genioglossus muscle [144]. Additionally manoeuvres designed to activate the genioglossus muscle such as tongue protrusion and swallowing resulted in increased EMGgg in all these participants. Although speculative, why this pattern is not commonly seen in previous studies may be related to posture; in the current study participants were in an upright posture so that they would maintain attention and minimise micro-sleeps, thus allowing assessment of conscious detection thresholds in participants that were subjected to a lengthy and
often monotonous protocol. This is in contrast to the majority of past multi-unit studies where patients are often studied in a lateral posture, or a supine posture where gravitational forces favour collapse of the upper airway [144, 145, 149, 151, 300]. Posture may also explain why baseline tonic and phasic activity was not greater in OSA participants compared to controls in this study, as has been observed previously [141-143]; supine posture is known to increase genioglossus activity [151, 295]. It is unknown whether similar heterogeneous responses seen in the current study would also be observed in a supine posture. While Malhotra et al. [151] reported no difference in EMGgg responses to negative pressure pulses between lateral and supine positions during wakefulness, similar to many other studies with a sudden onset negative pressure stimulus, no inhibitory responses were observed in that study.

Given the unusual responses to negative pressure and the phasic expiratory activity observed in some study participants, it is important to comment further on electrode placement in the current study. A number of observations suggest that in the majority of participants electrode placement was in the genioglossus muscle. Firstly, ultrasound measurements were performed in a subset of participants, and in the majority of these the electrodes measurement suggest the tip would have been placed well within the genioglossus muscle, whereas in a small minority the electrode was likely to be closer to the interface between the genioglossus and geniohyoid muscle. Secondly, the electrodes were inserted to a target depth approaching 30 mm which is a greater depth than the average optimal depth reported by Eastwood et al. [144], chosen because the patients in the current study were expected to be more overweight than the normal participants utilised in that study. Thirdly, while ultrasound measurements were not performed in all participants, the observation that maximal activation of the genioglossus muscle during swallow was similar between groups may suggest equivalent placement of the electrodes. Nevertheless, it remains possible that in some individuals electrodes were placed in the geniohyoid muscle in the current study; however this is unlikely to explain the current observations, given the suppression activity was
observed in individuals with both robust and modest inspiratory phasic activity during basal breathing as well as one individual with slight expiratory phasic activity. Additionally, even if electrodes were placed in the geniohyoid the pattern of phasic activity and muscle activity suppression that was observed in the current study is not expected, given that the predominant activity of the geniohyoid muscle during basal breathing is inspiratory phasic and because it is thought that the geniohyoid muscle may also act as a upper airway dilator [295].

Few studies have examined EMGgg responses to sudden onset negative airway pressure in OSA during wakefulness. Those that have [148, 149] are difficult to compare due to the heterogeneity of responses to negative pressure in the current study and due to numerous methodological differences. Methodological differences include posture (upright vs. supine), electrode placement (per-cutaneous vs. per-oral), electrode type (intramuscular vs. surface), timing of stimulus delivery (mid inspiratory vs. early inspiratory), and signal conditioning (with or without moving time average), however most notably, the current study focussed on responses to small negative pressure stimuli close to the conscious detection threshold whereas others have examined responses to large negative pressure pulses [148, 149]. Nevertheless, no deficits in genioglossus activation have been observed in OSA participants in response to negative pressure pulses [148, 149] and in fact in one study increased activation was observed in some situations for participants with OSA [148]. Thus to date, including the current study, no deficit in the protective response of the genioglossus muscle to negative pressure has been found. Similarly, when examining EMGgg activity over multiple breaths during wakefulness, there is a tight relationship between Pepi and EMGgg activity in both normal individuals and OSA patients and no difference has been observed in the slope of the within-breath and between condition relationship between Pepi and EMGgg between OSA and controls [141, 149]. Data also suggests no difference between OSA patients and controls in the suppression phase of the genioglossus response to negative pressure pulses [149].
In the current study responses were examined in a single upper airway muscle however it is recognised that the pharynx is made up of a complex arrangement of muscles that may act to increase or decrease airway calibre or that may act to change compliance of the airway. This is related to the fact that the upper airway muscles not only have a role in respiration; they also play a role in functions such as swallowing and phonation. Thus, while the genioglossus muscle is considered a key upper airway dilator muscle [136, 154, 274, 275] it is possible that other upper airway dilator muscles would behave differently. Indeed one study examining palatal muscle responses to negative pressure [284] reported impaired response in OSA.

The method of expressing EMG activity relative to the percentage maximal activity may be challenged as it can be affected by electrode placement and variable effort during calibration. For this reason attempts were made to standardise electrode placement as much as possible, as well as use a swallowing manoeuvre to determine maximal activity. Swallowing is one of the manoeuvres which often produces greater activity compared to other manoeuvres with the recording techniques used in the current study [144, 298], and this manoeuvre is said to be highly reproducible, which may be related to the fact that once initiated, it is essentially involuntary, whereas other manoeuvres are more effort dependent [284, 298].

Additionally it should be noted that EMG is a measure of electrical activity in the muscle and may not reflect the force generated or the functional outcome of the changes in muscle activity, however these considerations do not alter the interpretation of the findings.

In an attempt to minimise the influence of behavioural responses to the stimulus, the current results focus on the response of the genioglossus muscle in the initial 200 ms post stimulus presentation. This is despite there often being additional recruitment post 200 ms. Horner et al. [146] have reported a median (range)
voluntary reaction time from visual stimulus to protrude the tongue of 184 ms (150 – 230 ms), however a visual stimulus does not take into account the time required from stimulus onset to mechanoreceptor stimulation and the afferent neural transmission from the airway and hence is likely an underestimation of the voluntary reaction time to a respiratory stimulus. Zechman et al. [169] have reported reaction time from a large, inspiration onset respiratory stimulus to button press in the order of 450 ms and reaction times to stimuli close to the conscious detection threshold of over 1 second, well past the 200 ms time window used in the current study. Additionally, in the same group of participants in the current study it was observed that the P1 component of the respiratory related evoked potential (RREP) in response to negative pressure stimuli, which is thought to reflect arrival of somatosensory information at the cortex, was delayed at approximately 160 ms (Chapter 7) compared to similar studies that have reported latencies in the order of ~60-145 ms [173, 174, 190, 236]. It was postulated that this was related the nature of the stimulus, in particular a slow rate of pressure change, leading to a delay between stimulus onset and respiratory mechanoreceptor activation. This observation in particular offered confidence that the influence of behavioural activation was minimised in a 200 ms window. In studies examining the inhibitory response of inspiratory muscles, reaction time of greater than 100ms from a tap on the chest wall to rapid inspiration were reported [304] but again this stimulus does not take into account any delay from start of negative pressure change to mechanoreceptor stimulation, which is likely to be important when comparing to the current study.

Taking background conditions into consideration was of particular importance in the current study due to the small magnitude stimuli presented and because OSA patients had greater intrinsic resistance, measured via both the oral and nasal routes using plethysmography. Sensory detection of respiratory stimuli, both measured subjectively using conscious detection [167] and measured more objectively using RREPs [198] have been shown to be influenced by background conditions; Chou and Davenport [198] demonstrated that larger respiratory
resistive loads are required to elicit an RREP with increasing background resistance (incorporating extrinsic breathing circuit resistance and intrinsic resistance). Thus it follows that neuromuscular responses are likely to be influenced in a similar fashion and should take into account background stimulus conditions.

Possibly related to elevated intrinsic resistance, during experimental testing in the current study, OSA participants demonstrated more negative baseline $P_{	ext{epi}}$, which meant that control participants had a greater $P_{	ext{epi}}$ drop relative to background conditions. While stimulus conditions were not always equivalent between groups in the current study, this was not a concern as differences were accounted for in the statistical analysis. One of the advantages of the linear mixed model is that it is able to account for uneven spacing of repeated measures, opposing the need for matching of stimulus conditions between groups [311, 312].

Also, given this study was conducted during wake, it is yet unknown how findings would translate to sleeping individuals. While there was no change in the initial activation morphology and amplitude with sleep in response to negative pressure pulses, Eckert et al. [145] reported a larger suppression during non rapid eye movement sleep and a further decline in rapid eye movement sleep compared to wakefulness in response to negative pressure pulses. However, in that study the pharyngeal negative pressure stimulus was also more negative during sleep, confounding the observation. Studies examining EMG activation responses during sleep over multiple breaths have failed to show a deficit in OSA [159, 160] however, EMG responses to sudden onset negative pressure during sleep have not been examined in OSA patients and so further investigation in this area is warranted. Such an investigation would be made more difficult in a severe OSA population as used in the current study, due to repetitive airway closure. In the current study, using a severe OSA population served to maximise the chances of finding group differences.
In addition attempts were made to minimise confounders in the current study by restricting the BMI of participants to less than 35 kg·m$^{-2}$ and by excluding participants with co-morbidities that may influence results. Care must therefore be taken in extrapolating results to patients with increased BMIs and to patients with co-morbidities common to sleep apnoea. Nevertheless, similar studies to the current one examining such populations are of interest.

8.7. Conclusion

This study aimed to determine whether there was a deficit in OSA in genioglossus muscle activation in response to small negative pressure stimuli, close to the conscious detection threshold. The main finding was that there was no statistically significant difference in wakefulness between OSA and control participants in the threshold or sensitivity of the relationship between EMGgg activation and stimulus intensity, after background conditions were taken into account. These results do not support the concept that a deficit in neuromuscular responsiveness to negative upper airway pressure contributes to OSA pathogenesis. However, the results are likely influenced by a counterintuitive and unexpected genioglossus muscle suppression response observed in a significant proportion of participants. While there was no clear difference in the proportion of OSA and control participants that showed the suppression response, this does not preclude it being important in some individuals. The suppression response may be a general respiratory inhibitory response to protect the airway from inhalation of a foreign object, related to the inhibition seen in inspiratory muscles such as the diaphragm in response to sudden onset negative pressure.

8.8. Postscript

This experimental thesis chapter addressed one of the key thesis aims. In particular it explored if there were impairments in OSA patients during wakefulness in the neuromuscular response to negative pressure respiratory load stimuli close to the conscious detection threshold. Opposed to the hypothesis the study was unable to demonstrate a deficit in neuromuscular response to threshold respiratory
loading in OSA. However this negative result was largely contributed by an unexpected suppression of muscle activity, likely confounding the analysis which focussed on an expected neuromuscular activation. These results highlight the complexity of the upper airway musculature and point to the fact that even in the most studied upper airway muscle, the genioglossus, further research is required to fully understand its structure, physiology and function.

Taken together with the results exploring sensory function in OSA (Chapter 7), these studies have not demonstrated deficits in mechanosensory detection or in the neuromuscular response to collapsing forces in the upper airway. This suggests that other factors may be more important in OSA pathogenesis. However, little is known about the observed neuromuscular suppression observed in this experimental chapter, and therefore further work is required in this area to fully understand whether this response contributes to OSA pathogenesis.
9. Overall summary and conclusions

9.1. Summary of results, implications and limitations

The key aims of this thesis were to explore if there were impairments in awake OSA patients in the detection of, and neuromuscular compensation for, negative pressure respiratory load stimuli close to the conscious detection threshold. Thresholds for cortical detection of respiratory loads, and thresholds of neuromuscular compensation in response to respiratory loads have not been previously examined in OSA during wakefulness or during sleep. It was reasoned that this may be an important factor in the pathogenesis of OSA, as failure to detect and respond to minor threats to airway patency may lead to worsening collapse which is difficult to remedy by later muscle recruitment, or that dilator muscle recruitment occurs too late to prevent arousal, with repetition of the cycle on resumption of sleep. It was hypothesised that OSA patients would have impaired detection of, and neuromuscular compensation for, threshold respiratory loads during wakefulness, when compared to normal healthy individuals.

Sensory detection of respiratory loads was examined using the RREP (Chapter 7), which is the averaged cortical response to multiple presentations of a respiratory stimulus. This is considered a more objective measure of sensory detection compared to conscious detection, as the early peaks of the RREP in particular are not influenced by attention. This is an important consideration when studying those with OSA, as EDS and associated impaired attention are prominent features of OSA. The early P1 peak of the RREP was the focus of this study as it is thought to reflect arrival of somatosensory information at the cortex.

Neuromuscular compensation was tested using intramuscular genioglossus EMG (Chapter 8). The genioglossus muscle was used due to its importance as an upper airway dilator muscle (it is known to activate in response to the collapsing force of negative pressure), because of its size and accessibility, and because it is the best studied upper airway muscle.
To test the key hypotheses a group with severe OSA was compared to a healthy control group without OSA. Common procedures were used to test for differences in sensory detection and neuromuscular response. In particular the stimuli were a range of mid-inspiratory resistive loads, designed to cross the conscious detection threshold. By increasing the resistance to flow, resistive loads lead to a more negative airway pressure, where negative pressure is a key collapsing force in OSA. This stimulus had the following features: (i) it was a more subtle stimulus magnitude compared to negative pressure pulses that have been used in past research, (ii) it was sudden onset which is a requirement to elicit an RREP, and is important to assess reflex neuromuscular response while minimising volitional influences, and (iii) it is more naturalistic compared to a negative pressure pulse in that the pressure change is generated by the participant’s diaphragm driven inspiratory activity. Additionally, mid-inspiratory stimulus delivery has been shown to elicit RREPs of greater amplitude compared to stimuli delivered in early inspiration or end expiration.

No significant difference was found between awake OSA and control participants in the threshold or sensitivity of the relationship between the P1 component amplitude and stimulus intensity. The absence of deficits using a low intensity stimulus is in agreement with the majority of previous studies that have utilised larger stimuli, and taken together the results are contrary to the concept of a sensory deficit of respiratory stimuli being a major contributor to OSA pathogenesis.

Additionally, there were no significant differences between OSA and control participants in the threshold or sensitivity of the relationship between EMG activation and stimulus intensity. These results using low intensity stimuli, taken together with those that have utilised large negative pressure pulses, are also not supportive of the concept that a deficit in neuromuscular response to negative upper airway pressure is a major contributor to OSA pathogenesis. However, the neuromuscular results of the current research were likely confounded by an
unexpected and novel finding, in that a large portion of participants exhibited a counter-intuitive suppression of EMGgg in response to negative pressure stimuli. This response is counter-intuitive as suppression of EMGgg would lead to increasing upper airway collapse in the face of the collapsing force of negative pressure. Suppression of EMGgg activity has previously only been seen in pressure pulse studies that, unlike the current one, have not used smoothing techniques to remove the influence of brief transients in the EMG signal. The reason for this suppression is unclear although it has been postulated that this may be a central pattern generator driven response, similar to what is seen in other respiratory muscles such as scalene, parasternal intercostals, and the diaphragm in response to negative pressure stimuli. The inhibition in these muscles is thought to protect the airway by preventing inhalation of a foreign object. It may not have been seen in previous negative pressure pulse studies as they have targeted stimulus delivery at end expiration or start of inspiration to minimise the influence of central mechanisms on muscle responses. To test this hypothesis, future studies could deliver resistive load stimuli with healthy participants entrained to negative pressure ventilation, which would serve to minimise the influence of central mechanisms on EMG responses. Also, to improve understanding of this phenomenon, future studies examining single motor units in the genioglossus muscle would also benefit by studying the response to sudden onset mid-inspiratory stimuli.

As mentioned, it was originally hypothesised that sensory and neuromuscular responses would be impaired in response to small negative pressure stimuli close to the conscious detection threshold. A secondary hypothesis was that any abnormalities observed would not be corrected after treatment of OSA for 6 months. As no pre-treatment abnormalities were apparent, this analysis was not undertaken.

While the current results are not supportive of a deficit in sensory processing of respiratory stimuli in OSA, or of a deficit in neuromuscular response to respiratory
stimuli, it is important to recognise that it does not preclude deficits being important in some individuals. As has been previously mentioned, the current thinking about OSA pathogenesis is that it may be multi-factorial, with some factors being more important in some individuals than others.

It is also important to recognise that it is possible that results may differ under different conditions. For example, for the current research participants were studied in an upright posture to ensure attention was paid to the stimulus and to minimise the chances of participants falling asleep. Thus if subjects were studied in different postures, such as a supine posture where the airway is more prone to collapse, it is unknown if similar results would be obtained. Further work examining the effect of posture on sensory and neuromuscular responses to sudden onset negative pressure is warranted.

Also, although deficits were not found in wakefulness, it is unknown whether these findings would translate to sleeping individuals. This is important given that OSA is a sleep state dependent disorder. Sensory and neuromuscular responses to small respiratory loads have not been studied in sleep and so this is an area that requires further investigation. Such an investigation would be made more difficult in a severe OSA population, as used in the current research, due to persistent airway closure. However, one way to overcome this problem and to equate background loading between OSA and control groups, would be to deliver continuous positive or negative airway pressure to participants to a holding level just above the pressure at which flow limitation occurs. Small dial-downs in pressure could then be delivered and the sensory and neuromuscular responses measured.

A caveat should also be made about the type of participants that were recruited for the current research. To maximise chances of finding differences between groups a comparison was made between a severe OSA population and a group without OSA. However to minimise confounders recruitment was restricted to those without common co-morbidities. Additionally attempts were made to minimise the influence
of obesity as a co-founder by restricting recruitment of OSA participants to those with BMI of less than 35 kg·m$^{-2}$ and by selectively recruiting overweight control subjects. It is possible then that the OSA subjects recruited would be less representative of the OSA population than if a random sample had been obtained. Although it was not intentional, the selection criteria for the current research, which combined severe OSA with a restricted BMI, also resulted in a sampled OSA population that included male but not female participants. This likely relates to the preponderance of central obesity in males compared to peripheral obesity in females, which would increase the chances of severe OSA at a lower BMI. Given that male gender is a strong risk factor for OSA this observation does not diminish the importance of the current findings, however further work is clearly required to confirm the current results are applicable to both genders.

It also should be mentioned that for this research there was an unspoken presumption that the common sensory mechanisms were used in producing the cortical and neuromuscular responses to negative pressure. However it is possible that disparate sensory mechanisms are utilised. This is supported by the fact that past studies show that upper airway anaesthesia results in reduction in the EMG response to negative pressure but not the RREP. This suggests that the upper airway mucosa is important for sensory detection for the negative pressure reflex but not in producing the RREP. Studies combining the neuromuscular response, the RREP and anaesthesia or tracheostomised participants may be beneficial in elucidating afferent sources.

In the lead up to conducting the key experiments, a question arose relating to the diagnosis of OSA. OSA is typically diagnosed with in-laboratory PSG, which involves the simultaneous monitoring of numerous physiological variables during sleep; however the use of PM devices with a restricted number of signal recordings is becoming more common. An experiment was conducted to examine the impact of using two abbreviated signal montages on the accuracy, precision and interscorer reliability of PSG sleep and arousal scoring, compared to a standard
reference montage, in individuals being investigated for OSA (Chapter 2). One abbreviated montage incorporated 2 signals dedicated to sleep and arousal scoring and the other incorporated a single signal, whereas in the past 4 signals had been considered the minimum required. Using abbreviated montages resulted in changes in the distribution of sleep stages, a reduction in the arousal index and resultant reductions in sleep and arousal scoring agreement. The changes were greater when using 1 signal compared to 2 signals. Additionally, using the 1-signal montage, there were reductions in precision of summary statistics including TST and the amount of REM sleep scored, and reductions in the inter-scorer reliability of REM sleep and arousal scoring. Some of these findings have important implications for OSA diagnosis as for example, reduced TST precision is likely to impact the AHI because it is used as its denominator. Additionally, due to the reduction in arousals scored, a reduction in AHI is likely as some hypopnoea scoring is dependent on association with cortical arousals. These results were used to guide project procedures and ultimately resulted in the use of a PM device that had equivalent recording capabilities to those used in full PSG. More broadly these results also provide useful information in the development of future standards for the diagnosis of OSA.

In conducting the key experiments an opportunity was recognised to improve understanding of sensory detection of respiratory stimuli and in particular the relationship between conscious detection of negative pressure stimuli and the RREP. In a group of healthy participants a novel approach was used to create RREPs from consciously detected and undetected resistive loads, that were close to the conscious detection threshold and were closely matched for stimulus intensity and number of stimulus presentations (Chapter 6). This analysis indicated that the early P1 component of the RREP, thought to reflect arrival of somatosensory information, was present in response to detected and undetected load presentations. In contrast, the later P3 component, thought to reflect cognitive processing of the stimulus, was present for detected but not undetected load presentations. This suggests that for loads below the conscious detection
threshold, afferent sensory information reaches the somatosensory cortex, but is not cognitively processed. The findings have implications for the gating of respiratory stimuli, where the “gate” is considered as a filter receiving and evaluating sensory stimuli, allowing attention to be directed to essential physiological functions, while protecting cognitive processing from being flooded with redundant sensory stimuli. The findings argue against subcortical gating of respiratory somatosensory information, which has been previously proposed by other authors, and argues for a cortical process.

9.2. Conclusions

The key experiments conducted for this thesis are the first to examine whether a deficit exists in OSA in the sensory detection of, and upper airway neuromuscular response to, small respiratory loads close to the conscious detection threshold. In opposition to the hypothesis, no significant differences were found between a group with severe OSA compared to a healthy control group, suggesting that these factors may not be important contributors to the pathogenesis of OSA. However the neuromuscular results were confounded by a new and unique observation that a proportion of individuals respond to the collapsing force of negative upper airway pressure with a counter-intuitive suppression of muscle activity. This neuromuscular suppression should be a target of future research to improve understanding of upper airway muscle function in the face of a challenge to airway patency.

In the lead up to the key experiments a question arose relating to the diagnosis of OSA. An investigation found that using PSG devices with a restricted number of channels resulted in reduced accuracy, precision and reliability of PSG scoring. This finding has implications for future standards and recommendations for PSG recording and analysis in the diagnosis of OSA.

Additionally by using a unique analysis methodology a further experiment improved the understanding of sensory detection of respiratory stimuli, which is not only
important in OSA, but is also important in other respiratory pathology such as asthma. Opposing previous views, the findings suggested that sensory information from respiratory stimuli below the conscious detection threshold reaches the somatosensory cortex but is then filtered or gated from cognitive processing.
10. Bibliography


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Author/s:
RUEHLAND, WARREN

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