THE EFFECTS OF HYPOXIA ON RESPIRATORY SENSATION AND REFLEXES IN HEALTHY SUBJECTS: Implications for Sleep and Respiratory Disease

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ABSTRACT

Hypoxia is a common feature of many respiratory disorders including acute severe asthma, chronic obstructive pulmonary disease and pneumonia. Hypoxia also occurs during sleep-disordered breathing in conditions such as sleep hypoventilation syndrome and sleep apnea. In most respiratory diseases hypoxia is coupled with increased respiratory load. Compensatory protective mechanisms are activated to oppose these impediments to respiration. However, hypoxia is associated with impaired neurocognitive function and recent studies have demonstrated that hypoxia suppresses respiratory load perception in healthy individuals and asthma patients. These recent findings raise the possibility that a variety of protective physiological reflex responses to increased respiratory load may be impaired during periods of hypoxia. The effects of hypoxia on several of these protective responses and possible mechanisms of respiratory sensory depression by hypoxia are explored in the experiments outlined in this thesis.

In the first study, the respiratory related evoked potential (RREP) was used to investigate the mechanisms underlying hypoxia-induced suppression of respiratory load sensation in healthy individuals. As a positive control the effects of hypoxia on respiratory load perception to inspiratory resistive loads were also measured. The amplitude of the first and second positive peaks (P1 and P2) of the RREP were significantly reduced during hypoxia. P1 is thought to reflect the arrival of the ascending respiratory signals to the somatosensory area of the cortex. The perceived magnitude of externally applied inspiratory resistive loads was also

reduced during hypoxia. These data provide further support that hypoxia suppresses respiratory load perception and suggest that this is mediated, at least in part, by suppression of respiratory afferent information prior to its arrival at the cortex.

In the second study, the effects of acute sustained hypoxia on the cough reflex threshold and cough tachyphylaxis to inhaled capsaicin were explored in healthy individuals. Acute sustained hypoxia suppressed cough reflex sensitivity to inhaled capsaicin. This finding raises the possibility that the cough reflex, important for protecting the lungs from inhalation or aspiration of potentially injurious substances and for clearing excess secretions, may be impaired during acute exacerbations of hypoxic-respiratory disease.

In the third study, reflex responses of the genioglossus and scalene muscles to brief pulses of negative airway pressure were compared between hypoxia and normoxia during wake and sleep in healthy males in the supine position. Cortical RREPs to the same stimuli were also examined under these conditions. The genioglossus is the largest upper airway (UA) dilator muscle and can be reflexively augmented in response to negative UA pressure. A diminished response of this muscle during sleep has been postulated to be a contributing mechanism to obstructive sleep apnea (OSA) in individuals with an anatomically narrow UA. Cortical activation (i.e. arousal) to sudden airway narrowing in OSA is an important protective response to help restore ventilation during an obstructive event. In this study, genioglossus reflex responses to negative pressure pulse stimuli were

maintained during mild overnight hypoxia. Conversely, reflex inhibition of the scalene muscle to the same stimuli was prolonged during hypoxia. In addition, a previously undescribed morphology of the genioglossus negative pressure reflex consisting of activation followed by suppression was observed with greater suppression during sleep than wake. The amplitude of the P2 component of the RREP was also significantly reduced during hypoxia.

In summary, the potential mechanisms underlying hypoxia-induced suppression of respiratory load sensation and the effects of hypoxia on several protective respiratory responses have been investigated in healthy subjects. The potential implications of these findings for patients with hypoxic-respiratory disease are discussed.

PUBLICATIONS

The following are publications that have arisen from work conducted towards this thesis:

Journal Articles:

Eckert DJ, Catcheside PG, McEvoy RD. Blunted sensation of dyspnoea and near fatal asthma. *Eur Respir J* (Invited Editorial) 2004;24:197-9.

Eckert DJ, Catcheside PG, McDonald R, Adams AM, Webster KE, Hlavac MC, McEvoy RD. Sustained hypoxia depresses sensory processing of respiratory resistive loads. *Am J Respir Crit Care Med* 2005;172:1047-54.

Eckert DJ, Catcheside PG, Stadler D, McDonald R, Hlavac MC, McEvoy RD. Acute sustained hypoxia suppresses the cough reflex in healthy subjects. *Am J Respir Crit Care Med* 2006;173:506-11.

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Eckert DJ, McDonald R, Catcheside PG, Webster KE, Hlavac MH, McEvoy RD. Targeted hyperventilation for matching respiratory related evoked potential stimuli during hypoxia and normoxia. *Respirology* 2004;9:A67.

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Eckert DJ, Catcheside PG, McDonald R, Adams AM, Hlavac MC, Webster KE, McEvoy RD. Amplitude Reductions in Early RREP Components and Blunted Perception of Respiratory Load with Hypoxia. *Proceedings of the American Thoracic Society* 2005;2:A6

Eckert DJ, Catcheside PG, Stadler DL, McDonald R, Hlavac MC, McEvoy RD. Acute Sustained Hypoxia Depresses Cough Reflex Sensitivity in Healthy Individuals. *Respirology* 2006;11:A17.

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Eckert DJ, Catcheside PG, George K, Thompson K, McEvoy RD. Evidence for Reflex Inhibition of the Genioglossus Muscle to Brief Pulses of Negative Upper Airway Pressure during Wake and Sleep. *Australian Society for Medical Research Annual Scientific Meeting*, *South Australian Branch*, Adelaide, Australia. 2006.

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

Danny Eckert

Date: Wednesday, 16 June 2006

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GLOSSARY OF ABBREVIATIONS

ARDS	Acute respiratory distress syndrome
BMI	Body mass index ($kg \cdot m^{-2}$)
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CSA	Central sleep apnea
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG_{DI}	Diaphragm electromyogram
EMG_{GG}	Genioglossus electromyogram
EMG_{IC}	Parasternal intercostal electromyogram
EMG_{SC}	Scalene electromyogram
EOG	Electrooculogram
ERP	Event related potential
GABA	γ-aminobutyric acid
IC	Inspiratory capacity (l)
F_B	Breathing frequency ($breath \cdot min^{-1}$)
F_ICO_2	Fraction of inspired carbon dioxide concentration (%)
F_IO_2	Fraction of inspired oxygen concentration (%)
FEV_1	Forced expiratory volume in 1 second (% predicted)
FRC	Functional residual capacity (l)
FVC	Forced vital capacity (% predicted)
LTF	Long-term facilitation
NREM	Non rapid eye movement sleep
N1 & N2	First and second negative peaks of the ERP respectively
Nf	Negative frontal peak of the RREP
NTS	Nucleus tractus solitarius

OSA	Obstructive sleep apnea
P1,P2 & P3	First, second and third positive peaks of the ERP respectively
P_{CHO}	Choanal pressure (cmH_2O)
$PaCO_2$	Partial pressure of arterial carbon dioxide (mmHg)
PaO_2	Partial pressure of arterial oxygen (mmHg)
P_{EPI}	Epiglottic pressure (cmH_2O)
$PETCO_2$	End-tidal partial pressure of carbon dioxide (mmHg)
PIF	Peak inspiratory flow ($l \cdot min^{-1}$)
PIP	Peak inspiratory pressure (cmH_2O)
P_{MASK}	Mask pressure (cmH_2O)
RDI	Respiratory disturbance index (events$\cdot hr^{-1}$ sleep)
REM	Rapid eye movement sleep
RREP	Respiratory related evoked potential
R	Resistance ($cmH_2O \cdot l^{-1} \cdot sec$)
RV	Residual volume (l)
SaO_2	Arterial oxygen saturation (%)
SEM	Standard error of the mean
SOL	Sleep onset latency (minutes)
TLC	Total lung capacity (l)
TST	Total sleep time (minutes)
UA	Upper Airway
VC	Vital capacity (l)
V_{TI}	Inspiratory tidal volume (l)
$\dot{V}_I$	Inspiratory minute ventilation ($l \cdot min^{-1}$)
ψ	Perceived magnitude of externally applied resistive loads

CHAPTER 1. GENERAL INTRODUCTION

1.1 Brief Overview of Hypoxia in Sleep and Respiratory Disease

Hypoxia (a low oxygen level) is a common feature of many respiratory disorders including acute episodes of asthma, chronic obstructive pulmonary disease (COPD) and pneumonia. Hypoxia also occurs during sleep-disordered breathing in conditions such as sleep hypoventilation syndrome and sleep apnea. Many of these hypoxic diseases are associated with significant morbidity.

Hypoxia in clinical disease can develop rapidly (eg acute bronchospasm to an allergen during acute life-threatening asthma) or more gradually (eg progressive worsening of lung function with disease progression in conditions such as COPD). During sleep the presence of hypoxia can be prolonged (eg sleep hypoventilation syndrome) or short and repetitive (eg obstructive sleep apnea).

Whatever the aetiology, the presence of hypoxia in respiratory disease arises due to a failure of the respiratory system to maintain homeostasis in gas exchange as a result of the disease pathology. In many instances hypoxia occurs concurrently with increased respiratory load. Several compensatory protective mechanisms are activated to oppose these impediments to respiration. The potential deleterious effects of hypoxia on several of these defensive processes during periods of

increased respiratory load and the possible underlying mechanisms are explored in the experiments outlined in this thesis.

1.2 Varied Physiological Effects and Consequences of Hypoxia

The physiological responses to hypoxia are diverse. Responses vary between species, throughout maturation, wake versus sleep and between sleep states, are dependent on the degree and duration of hypoxic exposure and are subject to plasticity. Even when experimental conditions have been replicated between studies discrepancies have remained in the study findings (1). This has led to much controversy and has fuelled a rapid growth of studies in this area. This section will briefly summarise the known physiological responses to hypoxia and highlight some of the controversies within the field. Particular emphasis will be placed upon reviewing literature that may assist in improving the understanding of human respiratory disease.

1.2.1 Oxygen Sensing and the Central Nervous System

Arterial chemoreceptors were first discovered by Haymans and Bouckaert in 1930 (2). Since this initial finding, our understanding of the ability of different organs to detect and respond to changes in oxygen levels has evolved dramatically. A variety of cells and organs are capable of varying their activity in response to changes in oxygen tension. However, the primary role of oxygen sensing mechanisms from a survival perspective, is to maintain appropriate function of the cardiovascular and

respiratory systems to ensure appropriate oxygen delivery to the tissues (3). Oxygen sensing and the subsequent responses vary according to the degree and duration of the hypoxic stimulus. As highlighted in a recent review of this topic by Weir and colleagues, all tissues are sensitive to severe hypoxia (4). However, in the adult human, there are several important specialised tissues that sense and respond to changes in oxygen tension within the physiological range (~40-100 mmHg). These include glomus cells of the carotid body and the small resistance pulmonary arteries (4). Decreases in blood oxygenation are sensed by the carotid body at the carotid-artery bifurcation and to lesser extent aortic chemoreceptors. This initiates an increase in action-potential frequency in the carotid-sinus nerve and reflex augmentation of ventilation to increase oxygen availability that serves to restore homeostasis. Vasoconstriction of the small resistance pulmonary arteries can also occur in response to acute hypoxia in order to optimise oxygen transfer in the lung (4).

The brain has limited oxygen reserves and is therefore crucially dependent on oxygen. There are populations of neurons within the brain that act as chemoreceptors and in times of limited oxygen supply modulate critical functions necessary for the overall survival of the whole organism (3). The thalamus, hypothalamus, pons, and medulla have each been implicated in modulating respiratory and sympathetic activity (either inhibitory or excitatory) and likely form a complex chemosensitive network (3, 5-11).

The central nervous system (CNS) displays both stimulatory and depressant effects in response to hypoxia. One of the most widely researched examples of this is the biphasic ventilatory response that occurs during mild to moderate acute sustained hypoxia (discussed in detail in section 1.2.3- “Hypoxia and Respiration”). In addition, there are several other well documented physiological changes that occur within the cardiovascular system. Hypoxia has a direct vasodilatory effect on peripheral arterioles. However, chemoreceptor mediated reflexes override these effects and induces peripheral vasoconstriction that redistributes cardiac output toward the myocardial and cerebral circulations with clear survival advantages (12). Other potential responses include reflex tachycardia. The characteristics of these responses may differ widely according to the duration and degree of hypoxia (13-15).

1.2.2 Effect of Hypoxia on Respiratory Mechanics and Airway Tone

There have been relatively few studies examining the effects of hypoxia on lung function, respiratory mechanics and airway tone and these have yielded varied and contradictory results. Early animal data suggested that hypoxia causes constriction of tracheobronchial smooth muscle (16-20). Sterling demonstrated a small but significant reduction in specific airway conductance in humans (21). Saunders and colleagues measured the effects of isocapnic hypoxia (PaO_2 ~40-50 mmHg) on a range of lung mechanics variables in healthy males (22). They reported changes in some of these measures (increased TLC, FRC, RV, decreased specific lung conductance and an upward shift in the static deflation pressure volume curve) but

not others (VC, dynamic compliance and end-expiratory lung recoil pressures, total respiratory resistance) during hypoxia compared with baseline. However, while this study did incorporate an isocapnic hyperventilation component (without hypoxia), it is likely that this arm of the study was underpowered (only n=4 subjects), making the effects of hypoxia *per se* difficult to discern. A subsequent study by Goldstein and colleagues used more sensitive measurement techniques in a larger group of subjects and found no change in TLC or measures of small airway function (maximum flow-volume curves and closing volumes) (23). Denjean and colleagues studied the effect of mild isocapnic hypoxia (F_IO_2 0.155) on lung mechanics (dynamic pulmonary compliance and pulmonary resistance) in patients with mild asthma and also reported no effect of hypoxia (24).

Brief progressive isocapnic hypoxia, using either the method of Rebuck and Campbell (25) or transient hypoxia (5 breaths of N_2), have been demonstrated to decrease pharyngeal and nasal resistance in healthy individuals (26-28). However, there were no control conditions in these experiments (i.e. isocapnic hyperventilation without hypoxia), raising the possibility that respiratory motor output rather than a direct effect of hypoxia may have caused the apparent decrease. Julia-Sarda and colleagues used an acoustic reflection technique to measure airway cross sectional area in healthy individuals and demonstrated dilation of the extrathoracic trachea, intrathoracic trachea and main bronchi and no change in the pharyngeal or glottic areas (29). These changes were not present during normoxic isocapnic hyperventilation. Conversely, Shea and colleagues reported an increase in nasal

resistance during isocapnic hypoxia (SaO_2 ~87%) and no significant change in pharyngeal resistance in healthy men and women (30).

To determine if hypoxia affects respiratory mechanics and modulates bronchomotor tone, Tam and colleagues studied the effects of isocapnic hypoxia (SaO_2 ~80%) on specific airway resistance and dry air bronchomotor challenge in asthmatic subjects (31). No hypoxia-induced changes were observed. In contrast, a subsequent study reported enhanced bronchial responsiveness to methacholine during mild isocapnic hypoxia (F_IO_2 0.155) in patients with asthma (24). Dagg and colleagues also reported enhanced bronchial responsiveness to methacholine in asthma patients (32). Our own recent study demonstrated a non significant trend towards less airway constriction for a given dose of methacholine immediately following acute sustained isocapnic hypoxia (SaO_2 ~80%, 30 min) in mild to moderate asthma patients (33).

In summary, the limited data available suggest that hypoxia does not play a major role in altering respiratory mechanics, particularly when changes in respiratory drive are taken into account.

1.2.3 Hypoxia and Respiration

The effects of hypoxia on respiratory drive and ventilation have been studied extensively. Compared to hypercapnia, hypoxia is a relatively weak respiratory stimulus. Oxygen has a high affinity to haemoglobin under usual ambient conditions (i.e. sea level, normal temperature, $PaCO_2$ and arterial pH). Under these conditions

PaO_2 must decrease substantially to approximately 60 mmHg before becoming a major stimulus to breathe (34). Thus, the degree of hypoxia and factors that modulate oxygen-haemoglobin affinity, such as disease state (changes in arterial pH and PCO_2) and altitude, are important determinants of hypoxia-induced ventilatory drive. The hypoxic ventilatory response is also attenuated during NREM sleep and declines further during REM sleep in healthy men and women compared to wakefulness (35, 36).

Experimentally induced hypoxia sufficient to increase ventilation is associated with an accompanying decrease in $PaCO_2$. Therefore, to study the effects of hypoxia *per se*, it is essential that $PaCO_2$ is controlled. The following review of the effects of hypoxia on respiration will therefore focus on the many available studies in which $PaCO_2$ has been controlled. In addition to the degree of hypoxia and sleep state, there are multiple factors that influence the ventilatory response to a hypoxic stimulus. Some of these factors will be discussed in the following section.

1.2.3.1 Acute Sustained Hypoxia

In the adult human acute sustained isocapnic hypoxia (SaO_2 ~80%) produces a biphasic ventilatory response characterised by a rapid augmentation (peak level 3-5 min) followed within minutes by depression of ventilation (to 40-60% of the initial peak over 15-30 min) which remains above baseline levels (37, 38). The underlying mechanisms mediating the excitatory and depressive components of this response have been the focus of a great deal of research effort. It is well established that the

initial stimulatory response is initiated via peripheral chemoreceptor reflex activation at the carotid body resulting in increased carotid sinus nerve activity to the respiratory motor neuron pool.

The subsequent depression of ventilation or “ventilatory roll-off” is believed to occur centrally as evidenced by continuous elevation of carotid sinus nerve activity during acute isocapnic hypoxia (39). Further, blocking of peripheral chemoreceptors using somatostatin abolishes the initial hyperpnea and unmasks a pure depression of ventilation (40). Respiratory depression predominantly causes a decrease in tidal volume rather than an alteration in respiratory timing (38). Several potential mechanisms for hypoxia-induced central depression of ventilation have been proposed. A shift in the balance of excitatory and inhibitory neurotransmitters within the CNS has long been believed to be important. Regional or whole brain direct biochemical or indirect pharmacological techniques have provided insight into the likely neuromodulators involved (38). Mild to moderate hypoxia reduces the synthesis or release of excitatory neurotransmitters (i.e. acetylcholine, aromatic monoamines and the amino acids aspartate and glutamate) and increases the synthesis or release of inhibitory neurotransmitters (i.e. γ-aminobutyric acid (GABA), adenosine, β-alanine, taurine, endogenous opioids and lactate) (38, 41-51). These inhibitory effects appear to persist for between 15 min to an hour following a 20 min period of acute sustained isocapnic hypoxia (SaO_2 ~80%), as demonstrated by a marked reduction in the initial excitatory ventilatory response to re-exposure to the same hypoxic stimulus during the recovery period (52).

The specific central pathways involved and the way each component interacts to coordinate the overall depression of respiration during acute sustained hypoxia are unclear. However, neurons within the pons and thalamus appear to be involved (3). It has been proposed that these neurons are actively recruited as part of a “hypoxic-induced inhibitory network” (3). The nucleus tractus solitarius (NTS) in the medulla also appears to be sensitive to the effects of hypoxia and may play an important role (53, 54).

1.2.3.2 Maturational Influences

Oxygen availability is less *in utero*. Accordingly, fetal oxygen sensors respond to lower absolute PaO_2 values (~20-40 mmHg). The fetus also has specialised oxygen sensors including; neuroepithelial bodies in the lungs, chromaffin cells of the fetal adrenal medulla, smooth muscle cells of the resistance pulmonary arteries, fetoplacental arteries, systemic arteries and the ductus arteriosus (4). The response of the fetus to hypoxia is characterised by an absence of respiratory facilitation. Instead, breathing movements are further reduced, often to complete silence and are accompanied by bradycardia (55, 56). This is believed to reflect central inhibition rather than an immature chemoreceptor response (57, 58). This trait may have an important survival advantage *in utero* when, during an asphyxial insult, respiratory movements are suppressed and energy conserved.

In the neonate a biphasic ventilatory response is clearly evident, consisting of an initial increase in ventilation followed within minutes by depression of ventilation to

below baseline levels (38, 59-66). With advancing age the initial augmentation increases and the subsequent depression declines (55). However, as highlighted in the previous section (1.2.3.1- “Acute Sustained Hypoxia”), hypoxia-induced ventilatory depression is still clearly evident in the adult. While hypoxia-induced depression of central autonomic function may be life saving in the fetus its function in adults is far from clear. It is possible that this is a vestigial function carried forward from foetal life, which in the adult can prove deleterious to cardiorespiratory function.

1.2.3.3 Intermittent Hypoxia, Differences between Animals and Humans

The biphasic ventilatory response associated with sustained hypoxia is observed in a wide range of mammalian species (38). The effects of intermittent hypoxia on the CNS and respiration are more variable. For example, long term facilitation (LTF) rather than depression of ventilation is observed in several mammalian species (67-72). LTF is characterised by a progressive rise in ventilation above baseline levels upon return to normoxia after repeated carotid body stimulation. Its presence may be beneficial by way of stabilising respiration and potentially upper airway (UA) patency by preventing periods of low respiratory drive. LTF has largely been attributed to centrally mediated mechanisms and varies in duration from several minutes to hours depending on the species and protocol (69, 70, 73). There is evidence to suggest that this is a serotonin dependent process (74). However, in addition to central mechanisms, recent evidence suggests that peripheral modulation may also be involved (75).

However, ventilation is not enhanced following intermittent hypoxia in adult humans during wakefulness (76-78). Instead, like sustained hypoxia, intermittent hypoxia causes ventilatory roll-off or depression rather than LTF of ventilation (76, 77). During sleep there is some evidence to suggest that LTF occurs in certain individuals in the presence of flow limitation (79-81). Nonetheless, the marked disparity in the ability to evoke LTF between humans and animals highlights one, of potentially many, species differences concerning hypoxia mediated effects.

1.2.3.4 Plasticity

The potential for a hypoxic stimulus to alter respiration well after the stimulus ceases appears to be dependent on various factors that influence the ventilatory response to hypoxia (i.e. degree, duration, maturation, species differences and the nature of the exposure) (73, 82). The existence and potential underlying mechanisms mediating respiratory neural plasticity have been studied extensively in animals (for example; LTF as discussed in the previous section). Fewer studies have been conducted in humans although literature reporting on altitude effects does provide some insight.

Animal data suggest that in the developing brain hypoxia can induce prolonged neural plasticity (both excitatory and inhibitory) in respiratory and non-respiratory neural networks (83). Further, there may be certain developmental windows in which plasticity may be most pronounced. For example, early postnatal chronic intermittent

hypoxia modifies hypoxic respiratory responses and long-term phrenic facilitation in adult rats (84).

Healthy individuals who experience chronic exposure to altitude-induced hypoxia demonstrate reduced hypercapnic and hypoxic ventilatory responses (85). There is some evidence to suggest that patients with conditions characterised by acute severe hypoxia may also have blunted ventilatory responses to hypoxic stimuli (86-88). However, it is not clear if the disease related deficits represent a pre-existing inherent trait or arise as a result of neural plasticity. While the evidence supporting respiratory neural plasticity in humans is relatively sparse at present, as our understanding of this phenomenon improves, it may be possible to capitalise on this to alter respiratory responses as a novel treatment for certain respiratory diseases (89). For example, there is recent evidence that sustained improvements in sensory and motor function can be induced by repeated sensory and motor cortical excitation (90, 91). It is conceivable that similar techniques could be used to modulate respiratory responses (92).

1.2.4 Hypoxia, Cognition and Neurophysiological Function

As early as the 1860s balloonists were some of the first to acknowledge the effects of altitude on visual, mood, mental and physical performance (93). In the 1930s and 1940s it was recognised that the performance of warplane pilots was importantly influenced by hypoxia (94). During this time military funded research documented the graded effects of hypoxia. A delay in the ability to adapt to darkness first occurs

when PO_2 drops by ~10% relative to sea level (ascent to ~4,000 feet) (95). At marginally lower oxygen tension, judgment is impaired. At PO_2 levels of approximately 75% of sea level (ascent to ~8,000 feet) the ability to conduct complex tasks is impaired. PO_2 levels of around 65% to 50% of sea level result in short term memory impairment and severe loss of judgement. Below 30-40% unconsciousness develops (94, 96, 97).

Since these initial reports the effects of hypoxia on a range of outcome measures have been explored including behaviour, mood, psychomotor performance and cognitive function. Hypoxia has long been associated with impairment of many of these factors. However, similar to hypoxia-related effects on respiration, experimental findings vary according to the extent and duration of hypoxia and the methodologies employed to measure neurobehavioral function. Many of the consequences of hypoxia appear to be subject to wide individual differences (98). Accordingly, studies that have examined the effects of altitude on cognitive and neurophysiological function have tended to produce mixed results. For example, decrements have been reported for psychomotor performance, mental skills, card sorting efficiency, learning of a new task, signal detection, reaction time, error rates, short term memory and changes in mood and personality across a range (~1000-9000m) of simulated or actual high altitude exposures in humans (96, 99-111). There is some evidence to suggest that a number of these decrements can persist for a prolonged period of time upon return to lower altitude (112-114). Conversely, other studies have reported an absence of hypoxia-induced decrements including psychological performance, learning of a novel task and general information retrieval

latency and accuracy (115-117). At the other extreme, a few studies have reported improvements in psychomotor performance (118, 119).

As highlighted in a recent review article by Virues-Ortega and colleagues, despite the altitude literature comprising the bulk of the available information regarding hypoxia-related effects, the vast majority of these studies were not designed to address the effect of hypoxia *per se* (120). Indeed, most studies did not control for important factors such as hypocapnia which, in itself has subsequently been shown to have deleterious effects (120, 121). To address these shortcomings Berry and colleagues performed a battery of neurophysiological tests in healthy males during isocapnic hypoxia (down to SaO_2 ~80%) (122). Results revealed impairment in overall performance on Digit Symbol and Finger Tapping tests during isocapnic hypoxia. Other test variables that had previously been shown to be impaired in the aviation literature, including immediate recall tasks, were not evident in this experiment highlighting the need to control $PaCO_2$ in studies examining hypoxia-mediated effects (122). In recent years, neurobehavioral impairment, largely pertaining to the pre-frontal cortex, such as attention, executive and intellectual function has been associated with sleep disordered-breathing (123-126). To gain mechanistic insight into the contributing role of hypoxia in mediating neurobehavioral impairment the effects of isocapnic intermittent hypoxia have been studied using animal models. These eloquent studies have demonstrated impairment in spatial learning and increased hyperactivity in rats and shown that behavioural deficits may be more pronounced at certain vulnerable periods of life, for example during neonatal development and with aging (127-129).

In summary, the potential for altitude-induced impairment of cognition and neurophysiological function has long been recognised. Predominantly driven by the increasing awareness of the consequences of sleep disordered-breathing, evidence is emerging to suggest that hypoxia *per se* may be an important modulator of these various deleterious effects.

1.2.5 Other Physiological Effects and Consequences of Hypoxia

While the effect of hypoxia on respiration has been studied extensively, far less is known regarding the effects of hypoxia on respiratory sensation and protective respiratory reflexes. The overall aim of this thesis is to use the knowledge that acute hypoxia can act centrally to suppress respiratory motor activity and impair cognition and investigate whether it may also affect the less well researched physiological responses of respiratory sensation and protective respiratory reflexes. The following section will provide a brief account of disease characteristics and the physiological processes that lead to acute hypoxia in a range of disease states.

1.3 Overview of Conditions in which Acute Hypoxia occurs in Human Disease

1.3.1 Acute Severe Asthma

Asthma is the most common chronic lung disease and is characterised by reversible airflow obstruction, inflammation and airway hyper-responsiveness (130). Asthmatic patients who experience an acute severe attack requiring hospitalization, intensive care admission or who die as a result of the attack make up a relatively small percentage of the total asthmatic population (131). However, these groups contribute disproportionately to the human and economic cost of this disease. It has long been held that it should be possible to identify such individuals ahead of time in order to focus therapeutic efforts in their direction to reduce morbidity and mortality. Despite substantial research effort, this goal remains elusive. The impediments to progress in this area are considerable. It appears from case control studies that multiple risk factors are involved. These include psychological and socioeconomic factors, poor asthma control, suppressed respiratory sensation and blunted chemoreceptor sensitivity (87, 131-137). Prior near-miss episodes are also predictive of subsequent near fatal asthma episodes and fatalities (138).

There are multiple triggers capable of precipitating exacerbations of asthma. Common triggers include allergen exposure, air pollutants, exercise, respiratory tract infections, weather conditions, certain foods and food additives and intense emotions (130). These various asthma triggers have the potential to evoke airway

inflammation or bronchospasm or both (130). The resultant airway narrowing that arises during an attack can occur rapidly in some instances but in most cases manifests progressively over days (131). The airway narrowing is coupled with increased flow resistance and altered lung mechanics resulting in increased work of breathing and progressive gas trapping and hyperinflation (130). As a consequence, varying degrees of gas exchange abnormalities arise. Mild to moderate hypoxia occurs during most acute exacerbations of asthma (139, 140). Hypocapnia and respiratory alkalosis are also common features (139, 140). If airflow obstruction is severe and remains uncorrected profound degrees of hypoxia, hypercapnia and acidosis can develop (130, 139, 140).

1.3.2 Chronic Obstructive Pulmonary Disease (COPD)

COPD is characterised by progressive irreversible intrapulmonary airflow obstruction and a relentless decline in exercise capacity and health status (141). Smoking is by far the major cause of COPD but rarely occupational aeroallergen exposure or alpha1 antitrypsin deficiency can be the cause (142). The dominant pathophysiologic abnormalities are bronchial obstruction and destruction of lung parenchyma (i.e. emphysema). The disease has widespread social and economic repercussions (143). It is the fourth most common cause of death in the world and is predicted to become the third leading cause of death by 2020 (144). Many predictive factors associated with increased COPD mortality have been reported including; FEV_1, age, blood gas disturbance, pulmonary hypertension, BMI, dyspnea, health related quality

of life and exercise tolerance (143). Further, mortality risk increases with increasing frequency of severe exacerbations (143).

Acute COPD exacerbations can arise from many differing factors (145). COPD exacerbations can range in severity from slight symptomatic deterioration to devastating life threatening episodes (146). Most COPD exacerbations are triggered by either bacterial or viral infections (147-149). Indeed, these account for 50-70% of exacerbations (150). A further 10% have been attributed to environmental pollution and up to 30% are of unknown aetiology (141, 151, 152). It has been estimated in a large cross-sectional observation study that COPD patients suffer on average two exacerbations per year (153). Exacerbations are characterised by an acute worsening of expiratory flow limitation and there is evidence to suggest that both increased airway inflammatory activity and worsening airway obstruction are contributing factors (154). During severe exacerbations hyperinflation develops leading to seriously compromised lung mechanics (146). Depending on the extent of disease progression, COPD may cause chronic hypoxia. In addition, COPD patients also experience periods of acute hypoxia with and without CO_2 retention during acute exacerbations (155). A study by Barbera and colleagues investigating the mechanics of abnormal gas exchange during acute exacerbations in 13 patients with severe COPD highlighted the extent to which gas exchange is compromised in these patients (156). Upon initial assessment during the exacerbation the degree of hypoxia present in these patients was severe with a mean PaO_2 of approximately 44 mmHg (SaO_2~ 65%). Indeed, progression to respiratory failure during exacerbations of COPD is a common occurrence (157).

The goal of predicting COPD exacerbations ahead of time remains elusive (146). There are a variety of symptoms that patients may experience during an exacerbation including increased cough, sputum production and sputum purulence (149). However, patients report that the most important symptom is an increase in sensations of dyspnea such as breathlessness (149, 158). In summary, COPD exacerbations are a common occurrence and associated with a variety of physiological consequences including further compromised lung mechanics, hypoxia and an increased risk of mortality.

1.3.3 Acute Respiratory Disease Syndrome (ARDS)

Ashbaugh and colleagues first reported the features of ARDS based on a series of case reports (159). Since this initial report the definition of ARDS has evolved and been refined (160, 161). ARDS is characterized by the acute onset of hypoxia and bilateral infiltrates on chest radiography in the absence of left atrial hypertension (161, 162). Several respiratory (i.e. pneumonia) and non-respiratory (i.e. pancreatitis) risk factors are associated with the development of ARDS (162). The most common causes include sepsis, severe pneumonia, peritonitis and multiple trauma (163). Mortality rates are high and vary from 26% to 74%, with the majority of deaths believed to occur as a result of other associated conditions, such as sepsis and multisystem organ failure, rather than hypoxia in isolation (161, 162, 164-167). Treatment options are primarily limited to mechanical ventilation and there is emerging evidence to suggest that ventilation in the prone position versus supine

may reduce mortality in patients with severe ARDS if initiated early and applied for a relatively long duration (168). In addition to the high mortality rates, a number of survivors of ARDS have reduced quality of life with physical, neurocognitive, and emotional morbidity (169-172).

1.3.4 Sleep-disordered Breathing

Sleep-disordered breathing refers to a wide spectrum of sleep-related breathing abnormalities. These include UA resistance syndrome, sleep apnea-hypopnea syndromes and obesity hypoventilation syndrome of which obstructive sleep apnea (OSA) is the most prevalent. A large epidemiologic study in individuals aged 30 to 60 years revealed that 24% of men and 9% of women had abnormal apnea/hypopnea indices (173). Of these 4% of males and 2% of females had associated hypersomnolence and met the definition of sleep apnea syndrome.

OSA is characterised by episodic collapse or narrowing of the UA during sleep (174). The pathophysiologic factors contributing to the development of OSA are complex and incompletely understood. In addition to male gender, there are several known risk factors for OSA including increased body mass, craniofacial skeletal dysmorphology and increased age (175, 176). A variety of other factors likely contribute and these may vary considerably between patients (177). Pathophysiological factors likely to be important include UA anatomy, UA muscle control, ventilatory control, lung volume and the sensitivity of the arousal response to

respiratory stimuli during sleep (177, 178). In addition, factors such as impaired UA sensory function may also contribute (179, 180).

During an obstructive apnea or hypopnea, hypoxia and hypercapnia develop and there is a progressive increase in breathing effort with continued compromised or absent flow through the UA. While there is marked variability within and between patients, significant arterial hypoxia occurs during each apneic event (181). Bradley and colleagues studied 44 OSA patients and reported that the overall mean arterial O_2 saturation was 88% during sleep and substantially less during apnea and hypopnea events (182). In most instances the physiological changes and increased breathing efforts during apnea or hypopnea culminate with arousal, re-opening of the UA and brief hyperventilation followed by return to sleep (176, 183-185). However, neurocompensatory mechanisms appear sufficient to restore ventilation in the absence of arousal from sleep in approximately 20% of events (184, 186).

Central sleep apnea (CSA) is characterised by a lack of drive to breath during sleep resulting in insufficient ventilation and compromised gas exchange. There are several types of central sleep apnea. These include high altitude-induced periodic breathing, the relatively uncommon disorder of idiopathic central sleep apnea and Cheyne-Stokes respiration, which generally occurs in patients with congestive heart failure. Patients with obesity hypoventilation syndrome may also experience central apneas during sleep (178). While the precipitating mechanisms involved in the various types of CSA may vary, CSA generally relates to an abnormality in loop gain and circulation time (178). It is also not uncommon for patients to experience mixed

central and obstructive events during sleep. Of the different types of CSA, idiopathic central sleep apnea and Cheyne-Stokes respiration are characterised by intermittent hypoxia during sleep. On the other hand obesity hypoventilation syndrome, which is commonly defined as a combination of obesity (BMI>30 $kg.m^{-2}$) and awake arterial hypercapnia ($PaCO_2$ >45mmHg), is typically characterised by prolonged periods of sustained hypoxia during sleep (187).

1.4 Respiratory Sensation and Respiratory Load Perception

1.4.1 Clinical Importance

Regardless of the origin and cause of acute periods of increased respiratory load in disease (i.e. bronchoconstriction in asthma or UA collapse in OSA) it is vital that protective mechanisms are in place to oppose these impediments to breathing. Indeed, the degree and extent of breathing impairment is dependent on the ability of these protective mechanisms to respond. There are numerous protective responses that can be activated in an attempt to maintain ventilation in response to respiratory load or airway obstruction. These responses require appropriate sensory signalling and activation of one or more respiratory reflexes. In some instances integrated alerting responses resulting in cognitive awareness of increased respiratory load are activated. The accurate recognition of asthma symptoms, for example, is crucial for prompting asthma patients experiencing an acute exacerbation to increase medication or seek medical assistance. Impairment of this process has the potential to increase asthma morbidity and mortality (87, 134). Since the initial report by

Rubinfeld and Pain, several studies have demonstrated that individuals with asthma, particularly those prone to severe or near fatal asthma, demonstrate blunted sensations to increased respiratory load (87, 134, 188-191). Any factor that impairs respiratory sensation, be it an inherent persistent blunting of sensation or one that is acquired acutely, could expose asthmatic patients to increased risk of morbidity or mortality (33, 92, 132, 192).

Similarly, it is crucial for patients experiencing an acute exacerbation of COPD to appropriately recognise that their condition is worsening in order to initiate an appropriate management response. While exacerbations in COPD are associated with numerous symptoms, patients report that the most important symptom is an increase in the respiratory sensation of breathlessness (149, 193). There can be however, a marked disparity between subjects' perception of disease severity and the reported intensity of breathlessness (193). This raises the possibility that inappropriate sensations of breathlessness are an important factor contributing to increased morbidity and mortality in COPD and highlights the importance of intact respiratory sensory mechanisms for patients experiencing an exacerbation of COPD.

In the case of sleep-disordered breathing an inability to appropriately sense periods of increased resistance to breathing may be a contributing factor to the disease pathology. Recent studies have demonstrated that OSA patients have reduced sensation in the pharyngeal airway (179, 180). Similar to other respiratory diseases it is not known if this effect occurs as a result of the disease or is a pre-existing inherent trait (194). Further, a blunted arousal response from sleep to increased

respiratory load or obstruction may contribute to increased disease severity (185, 195, 196). Thus, respiratory sensory processes may play an important role in disease progression and potentially pathogenesis across a range of respiratory disorders.

1.4.2 Psychophysics and Methodology used to Quantify Respiratory Sensations

There are many techniques used to quantify the relationship between sensation and the stimuli that provoke specific sensations (psychophysics). Studies using psychophysical principles have been utilized for over a century, particularly in the fields of vision and hearing (197). The adoption of these principles for the study of respiratory sensations has only occurred relatively recently (197-199).

1.4.2.1 Scaling Methods

Scaling entails the measurement of a magnitude. The scaling methods can be divided into nominal (determination of equality), ordinal (determination of greater or less), interval (determination of equality of intervals or differences) and ratio (determination of equality of ratios) (200). In the study of respiratory sensations subjects make judgements about the sensations experienced to supra-threshold stimuli. Each scaling technique carries with it various advantages and limitations (200, 201). To ensure that the most appropriate scaling tool is selected for a given study aim and population sample it is important to consider the relative

advantages/disadvantages of each. In general, interval and ratio scales tend to provide more information and are better suited to studying the mechanisms of respiratory sensation (200). The following section will provide a brief account of the most commonly used techniques to quantify supra-threshold respiratory sensations and will highlight some of the potential strengths and weaknesses of each.

Visual Analogue Scale

The visual analogue scale is a simple scale that can contain components of ordinal, and interval levels (200, 202). The scale consists of a straight line of known length representing the range of experienced intensities, the extremes of which can be bound by numbers, words or both. The intensity is judged by marking a line on the scale. The length of the mark is subsequently measured. Visual analogue scales have proved particularly popular in the study of the specific respiratory sensation of breathlessness (197, 203). The visual analogue scale is user-friendly and is particularly useful for examining the effects of an intervention within a subject. Its application becomes limited when attempting to compare sensations between subjects due to the lack of meaningful fixed reference points.

Modified Borg Scale

The modified Borg scale is a form of an interval scale (200). Its advantage over the visual analogue scale relates to its capability to quantify and compare respiratory sensations within and between subjects (87, 137, 204-206). This scale incorporates numbers (range ‘0-10’) with corresponding words (range ‘nothing at all’ to ‘maximal’) at the extremes and at various points in between. Subjects are able to make

judgements regarding the sensation in question at any point on this category scale (206).

Open Magnitude Scaling

Open magnitude scaling is a form of ratio scaling (200). The subject receives a reference stimulus and is required to scale all subsequent stimuli in relation to the reference. For example, if the initial stimulus is rated as 100 and the subsequent stimulus is perceived as double the original intensity the subject would assign it a value of 200. One of the advantages of this technique is that the subject is able to select whatever numerical scaling system that they feel most comfortable with (i.e. decimals, fractions, percentages or whole numbers). The method of grand modulus equalisation allows for comparison between subjects (207). However, the lack of fixed descriptive anchor points such as those included in the modified Borg scale is a limitation when attempting to compare absolute values between different study populations. Open magnitude scaling is however, highly reproducible and provides a particularly useful means for examining the effect of an intervention across a large range of perceptual sensitivities and stimulus magnitudes (200, 208, 209).

Cross Modality Matching

The most commonly used cross modality matching method in the study of respiratory sensation requires the participant to rate the magnitude of a respiratory stimulus using a handgrip dynamometer. The subsequent force generated is recorded and compared to the magnitude of the respiratory load. (210-212). This technique is particularly useful for study populations such as children due to its simplicity and the

lack of need for the participant to use scaling methods requiring numbers or words for which they may not have developed the appropriate skills (213). This technique also allows for investigation of the within-breath time course of respiratory sensations that is not possible with other techniques.

1.4.2.2 Defining Relationships between Respiratory Load and Sensory Perception

Once an appropriate scaling method has been selected quantification of the relationship between the scaling outcome measures and the experimental stimulus intensity is required. The stimulus for an experiment using auditory stimulation, for example, is relatively easy to define and quantify (i.e. signal intensity can be measured in dB) in order to establish perceptual relationships. However, for respiratory stimuli this is more problematic. Respiratory sensation is an integrated and incompletely understood neural process that arises from stimulation of potentially many receptor systems (see section 1.4.3- "Underlying Neurophysiology"). An important question therefore becomes; what is the most appropriate parameter to measure to quantify the magnitude of a respiratory stimulus? Choosing the best 'marker' of respiratory load magnitude in an attempt to define a complex and incompletely understood process is inherently difficult. Nevertheless, useful models have been adopted. To examine the relationship between respiratory sensation and experimentally induced bronchoconstriction for example, measures such as FEV_1 and IC have proved to be useful markers of load magnitude (214, 215). For the study of experimentally induced external resistive

loads, airway resistance and airway pressures have been demonstrated to be useful markers (216, 217). To select an appropriate 'marker' of respiratory load magnitude for a given experiment, objective statistical procedures can be employed. This includes 'best fit' procedures to compare the various potential 'markers' of a respiratory stimulus with the perceptual outcome measures (208).

Stevens' Power Function- only one of many potential models to describe respiratory sensation

Once a marker of respiratory stimulus magnitude is selected a perceptual relationship between the sensory perceptual response and the respiratory stimulus can be established. Stevens first described that the relationship between perceived magnitude and stimulus intensity can be described according to the following function $\psi=k\phi^n$ (where ψ is the perceived magnitude intensity, k is a constant, ϕ is the physical stimulus and n is the exponent) (207). As a result of this observation many psychophysiological studies of respiratory sensation have used the exponent of Stevens power function as the primary measure of perceptual sensitivity (218-220). While this function describes many stimulus-perceptual relationships well, several recently published studies have highlighted the importance of comparing other models in addition to the traditional Stevens approach. Indeed, recent studies that have compared linear versus power function models of respiratory load perception have found that the linear model statistically outperforms Stevens power function (33, 208, 221). These observations are further reinforced in recent reviews providing support that neural coding phenomena may largely behave linearly (222, 223).

1.4.2.3 Respiratory Related Evoked Potentials

Communication between neurons occurs continuously within the CNS and can be measured by way of surface electrical activity. Event related potentials (ERPs) are measured by recording electrical activity at the scalp in response to a particular stimulus (224). However, in order to establish the specific stimulus related electrical activation, which is generally relatively small compared to non-stimulus related electrophysiological activity occurring at the scalp, multiple trials are required to improve the signal to noise ratio using the averaged ERP waveform (for example see Figure 1). ERPs provide valuable information about the sensory and cortical processes that occur in response to a particular stimulus. Somatosensory evoked potentials have been utilised for many years to gain insight into the mechanisms and integrity of sensory processing pathways to many differing stimuli presented in a variety of patient populations (225-231). Compared to other sensory modalities, ERPs to respiratory stimuli have only been relatively recently investigated.

In a pioneering study by Davenport and colleagues brief occlusions to breathing were applied at the onset of inspiration in six healthy male subjects (232). Electroencephalography (EEG) activity to repeated stimuli was recorded at several electrode sites overlying somatosensory areas of the cortex (C3, Cz and C4) to generate the respiratory related evoked potential (RREP). Since this initial report RREPs have provided important insight into the sensory processes underlying respiratory load perception (220, 232-234). RREPs are characterised by positive (P) and negative (N) waveform components within time locked windows relative to

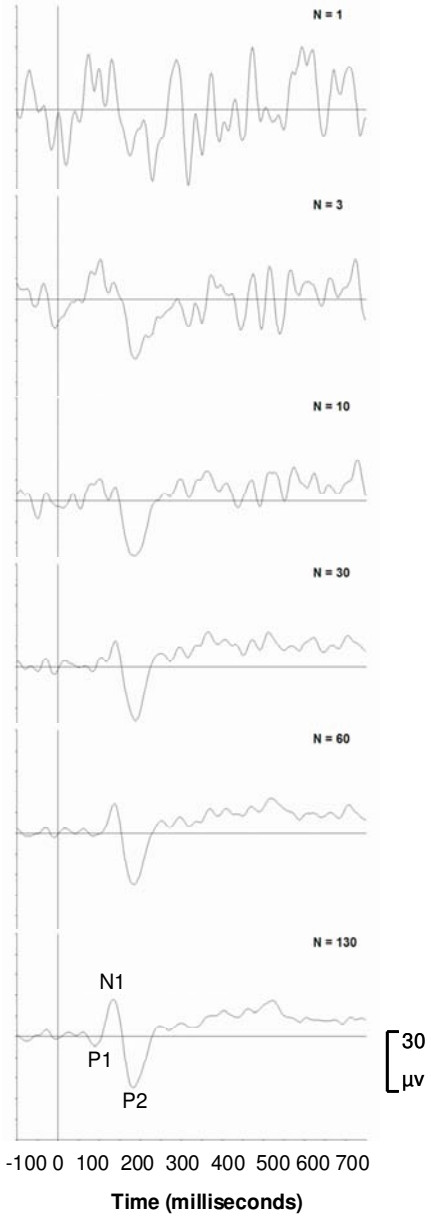
stimulus onset (see Figure 1). Respiratory load perception research has primarily focussed on the first (P1) and third (P3) positive peaks. P1 occurs 40-110 milliseconds after stimulus onset depending on the specific stimulus characteristics (220, 232, 235, 236) and is believed to reflect the arrival of ascending respiratory signals at the somatosensory cortex (232). P1 amplitude increases with increasing stimulus magnitude (234, 237, 238). The P3 component, occurring after 250 milliseconds, is thought to reflect cognitive and perceptual sensory processing, and unlike P1 is attention dependent (233). P3 is maximal more parietally than P1 overlying the secondary sensory association areas of the cortex (236). The amplitudes of both these RREP components strongly correlate with subjects' perception of the magnitude of respiratory resistive load (233, 234).

Far less is known regarding the neural origin and functional significance of the other predominant RREP waveform components (i.e. Nf, N1 and P2). Source localisation has modelled that the negative frontal (Nf) component is likely to be generated within the supplementary area of the cortex (239). However, the contribution of Nf to respiratory sensation remains unclear. Data using auditory ERPs suggest that the amplitude of the N1 component occurring at approximately 100 milliseconds is affected by both exogenous properties (i.e. stimulus intensity) and endogenous factors (i.e. attention) (240, 241). Similar to the auditory ERPs observations of N1, Block-Salisbury and colleagues utilised different magnitude resistive loads to produce RREPs and, although not quantitatively examined, their data suggest that N1 was present for the largest stimulus and the amplitude decreased thereafter (237). Further, Webster and colleagues demonstrated an increase in N1 amplitude

during an attend versus a non-attend condition (236). While N1 scalp topographic distribution shows a band of broad negativity overlying the scalp midline in most RREP studies its neural genesis and relationship to respiratory sensation and perception remains uncertain (235, 236, 242). Similarly, the functional significance and neural genesis of the second positive (P2) peak of the RREP remains poorly understood (243). However, its latency of approximately 200 milliseconds and preservation of the P2 peak in both stimulus attend and ignore conditions suggest that it also reflects a combination of exogenous and endogenous processes (236, 243).

During NREM sleep the RREP is dominated by the presence of K-complexes suggesting that sensory processing of respiratory afferent information is markedly altered during NREM sleep (242, 244, 245). There are some data to suggest that the early components remain relatively intact during NREM sleep (242). Thus, while RREPs have been a relatively recent addition to the vast array of ERP stimuli explored previously, RREPs provide an excellent objective means in which to explore the sensory and cortical processing characteristics of respiratory load perception.

Figure 1 Example of the Averaged Respiratory Related Evoked Potential (RREP) Overlying the Somatosensory Area of the Cortex at Cz



The effect of signal to noise enhancement by signal averaging. A respiratory stimulus was presented at time zero and with increasing replicate trial numbers (n=1-130) the non stimulus EEG is attenuated via averaging to reveal the stimulus related waveform. Note that the morphology and amplitude of the response varies only slightly beyond N=30 trials in this example.

1.4.3 Underlying Neurophysiology

The mechanisms subserving respiratory sensations are complex and incompletely understood. There are many neuro-anatomical locations that are capable of eliciting distinct respiratory sensations. Dyspnea (discomfort associated with the act of breathing) is the common term used to collectively describe many of these various sensations. Respiratory sensations may also be importantly influenced by multiple psychological, social and environmental factors (246). The following section will review the current understanding of the neurosensory mechanisms and their potential to influence specific respiratory sensations and the more global sensation of dyspnea.

1.4.3.1 Sensory Receptors Capable of Eliciting Respiratory Sensations

There are multiple sensory nerve endings located throughout the respiratory system that are capable of responding to increased respiratory load. Specific respiratory sensations rarely occur in isolation in respiratory disease. In most instances multiple sensations arise from different sensory networks and occur simultaneously. This makes attempts to isolate the neural origin of specific respiratory sensations extremely challenging. The precise sensory receptor networks that are activated are dependent on the characteristics of the respiratory stimulus. Respiratory *load detection* and respiratory *load magnitude perception* are two distinct perceptual processes and likely involve somewhat different neural mechanisms. The following

review of sensory receptors will encompass both phenomena to highlight the potential for different sensory networks to elicit perceptually distinct respiratory sensations.

Upper Airway

During periods of increased respiratory load there is an augmentation in the transluminal pressure gradient across the extrathoracic airways (247). Pressure sensitive mechanoreceptors located throughout the UA are capable of sensing these transient changes (224). Animal data suggest that inputs from the UA project to midbrain neurons and may contribute to sensory modulation of dyspnea (248). The precise contribution of UA receptors to respiratory sensations however remains unclear.

UA anaesthesia does not appear to affect the ability to detect added respiratory or elastic loads to breathing or magnitude estimation of these loads in healthy individuals (249-251). However, it remains possible that anaesthesia of the superficial mucosa occurred in the absence of anaesthesia to the deeper mechanoreceptors which may be particularly important modulators of respiratory sensation (224). Consistent with this hypothesis (although caution is warranted given the small sample size and potential for altered respiratory mechanics in the study population), bypass of the UA with tracheostomy diminished the ability to detect respiratory load (247, 252). Studies by Younes and colleagues comparing respiratory loads confined to the chest wall with loads applied to the upper airways suggested that UA mechanoreceptors are more sensitive than chest wall receptors in detecting

respiratory load (253, 254). More recently, Donzel-Raynaud and colleagues utilized respiratory related evoked potentials to demonstrate that UA afferents are particularly important in the generation of somatosensory cortical activation in patients with inspiratory pump dysfunction (255). Conversely, studying healthy individuals, Gandevia and colleagues demonstrated that changes in pressure applied to the UA when the glottis was closed were poorly detected when compared with an open glottis, suggesting downstream chest wall receptors are more sensitive than UA mechanoreceptors in detecting respiratory load (256). Thus, the precise role of UA receptors in respiratory load detection and magnitude estimation remains controversial and is likely dependent on the characteristics of the respiratory stimulus and the influence of accompanying activation from other receptor systems.

Lungs and Lower Airways

Afferent inputs from the lungs and lower airways project to the brain via the vagus nerve and vagal inputs have long been proposed to play an important role in regulating breathing pattern (257, 258). There are several receptor types throughout the lungs that may be independently capable of eliciting respiratory sensations. These include pulmonary stretch receptors that respond to lung inflation, irritant receptors in the airway epithelium that respond to a variety of chemical and mechanical stimuli and afferent C fibres within the alveolar wall and blood vessels that respond to interstitial congestion (259, 260).

Studying double-lung transplant recipients (lung denervation model) Zhao and colleagues reported an increase in the load detection threshold to externally applied

resistive loads (261). This suggests that load detection occurs in the absence of vagal afferent feedback from the lungs but that these afferents do contribute to the respiratory load detection threshold.

Peiffer and colleagues studied lung transplant patients and demonstrated a reduction in the slope of the relationship between mouth pressure versus Borg score to inspiratory resistive loads compared to healthy controls (262). The late P3 processing component of the RREP has also been shown to be impaired in double lung transplant recipients (263). These findings suggest that vagal afferents are important modulators of external respiratory load magnitude perception. Conversely, a recent study reported no significant difference in magnitude estimation responses to external resistive loading between double lung transplant recipients and controls (264). However, upon further examination of the data, the small sample size and the apparent tendency towards a difference, suggest that an apparent lack of between group differences may be explained by type II error.

In the absence of chest wall receptors, patients with high cervical cord transection are able to detect mechanical ventilator induced changes in tidal volume and report sensations of “air hunger” when tidal volume is restricted (265, 266). Vagal anaesthesia increases breath holding time in healthy individuals and may decrease breathlessness in some patients with cardiopulmonary disease (252, 267). In contrast to these findings, breath holding duration was found to be no different in heart-lung transplant recipients compared to controls (268). Together, these findings suggest that vagal afferents likely contribute to respiratory sensations arising from

limited chest expansion whereas the effect on breath holding duration remains unclear.

Respiratory sensation unique to bronchoconstriction would appear to be importantly influenced by vagal afferents. Taguchi and colleagues compared respiratory sensations during bronchoconstriction versus external respiratory loading of comparable total resistive load magnitudes and demonstrated qualitative and quantitative disparate sensations (269). Further, only bronchoconstriction induced sensations could be alleviated by airway anaesthesia (269). In a separate study "chest tightness" occurred in most instances during bronchoconstriction and rarely during external resistive loading (270). Binks and co-workers compared the respiratory sensations of "chest tightness" and "breathing effort" during methacholine bronchoconstriction in asthma with and without mechanical ventilation (271). The sense of "chest tightness" was unchanged between interventions whereas "breathing effort" was reduced during mechanical ventilation. This suggests that, although occurring simultaneously, these sensations are of differing neural origin and supports the hypothesis that pulmonary afferents contribute to "chest tightness". Laugheed and co-workers measured "breathing discomfort" using a modified Borg scale in spontaneously breathing low cervical quadriplegia patients (void of chest wall sensory inputs) during experimentally induced bronchoconstriction (272). Respiratory sensation to equivalent levels of bronchoconstriction (expressed as changes in IC) was quantitatively similar to an asthmatic control group. These results further highlight the importance of vagal afferents to bronchoconstriction induced respiratory sensations. Stimulation of pulmonary C fibres also appears capable of eliciting

distinct respiratory sensations (273). Together, these studies provide strong support that vagal afferents are important mediators in generating and modulating the intensity of specific respiratory sensations. The extent to which certain vagal receptor types are activated to a given stimulus is likely to be an important contributor to the overall perceptual outcome.

Chest Wall

Afferent information from joint mechanoreceptors, tendons and muscles of the chest appear capable of modulating various respiratory sensations (258). Intercostal muscle afferents project to the cortex (274). Muscle afferents likely play a key role in the perception of limb motion (224, 275) and in a similar fashion intercostal muscle spindles and rib joint receptors appear capable of reporting respiratory muscle tension and rib cage volume (199, 224, 276).

Previous studies in quadriplegia patients suggested that chest wall afferents were not required for the detection and magnitude estimation of respiratory loads (277, 278). However, Gottfried and co-workers subsequently reported impaired magnitude perception of added elastic and resistive respiratory loads in low cervical quadriplegia patients compared to controls (279).

Vibration of the parasternal intercostal muscles during combined hypercapnia and inspiratory resistive loading reduces breathlessness in healthy individuals (280). However, intercostal muscle vibration out of phase with respiration increases breathlessness in healthy individuals and asthmatics (281). Patients with chronic

respiratory disease report similar changes in dyspnea relative to the respiratory phase in which vibrations are applied to the intercostal muscles (282). While these studies suggest that chest wall afferents are important modulators of respiratory sensation, it is possible that chest wall vibration may also activate intrapulmonary receptors (283). Thus, the precise role of chest wall afferents in modulating respiratory sensation remains unclear and is likely dependent on a variety of factors including the timing of activation of these afferents with respect to respiration.

Diaphragm

Afferent information from both muscle spindles and golgi tendon organs is relayed via the phrenic nerve and may provide important information about thoracic displacement and muscle tension (278). Historically, the diaphragm has long been believed to possess relatively few muscle spindle afferents. Contrary to these earlier beliefs there is evidence to suggest that the diaphragm contains many afferents that behave as muscle spindles (284, 285). Regardless of their quantity, these afferents may still play an important role in eliciting respiratory sensations (224). Initial attempts to selectively block the phrenic nerve in healthy individuals by Noble and colleagues were incomplete as measured by fluoroscopy and hence, there was no evidence for altered load detection ability using this approach (252). A subsequent report by the same group assessed the role of the diaphragm in breath holding in healthy normal subjects (286). Again, diaphragmatic paralysis was not complete. However, cases that resulted in a decrease in IC of ≥ 20% were associated with increased breath hold time and decreased respiratory sensation compared to controls that received lignocaine to the neck without phrenic block. While this does

not exclude the possible contribution of other muscles it does suggest that the diaphragm contributes to breath hold sensation. Further, several other studies have supported the hypothesis that sensations of respiratory muscle force are generated by tension of the contracting respiratory muscles such as the diaphragm (287, 288). Zechman and colleagues measured the relationship between transdiaphramatic pressure, resistive and elastic loads to breathing and sensory detection of these loads in healthy individuals (289). A tight relationship was observed suggesting load-induced changes in diaphragmatic tension may play an important role in sensory detection of inspiratory loads. Thus, these data support the role of respiratory muscle force generation as a contributor to respiratory sensation.

Chemoreceptors

The ability of chemoreceptor stimulation to generate dyspnea and the neurosensory mechanisms mediating these effects are somewhat controversial. Using an animal model there is evidence for direct carotid chemoreceptor projection to the cortex (290). However, this does not establish whether or not increased chemoreceptor afferent information can be perceived as an abnormal respiratory sensation (224). Earlier studies suggested experimentally induced increases in inspired CO_2 resulted in dyspnea due to associated increases in respiratory muscle activity (270). Supporting evidence was based on observations that in the absence of respiratory muscle activity hypercapnia did not result in dyspnea (252, 291). However, these initial findings have since been authoritatively challenged and a body of evidence now exists demonstrating that hypercapnia and breath holding can elicit intense respiratory sensation in paralysed patients or healthy individuals experiencing

complete neuromuscular block, particularly the specific sensation “air hunger” (292-294). These findings suggest that under these conditions direct projections of chemoreceptor afferents and/or corollary discharge (see section 1.4.3.3- “Potential Mechanisms Underlying Respiratory Sensations”) contribute to respiratory sensation (224). The effect of chronic hypercapnia in naturally occurring disease and its effect on dyspnea is more difficult to discern due to the coexisting changes in respiratory mechanics in these patients and the observation that many have little dyspnea at rest (270).

The ability of hypoxia-induced chemoreceptor stimulation to elicit respiratory sensation is even more controversial than that of hypercapnia. Compared to hypercapnia, hypoxia is a weak respiratory stimulant and has historically been associated with an absence of dyspnea (295). Anecdotal reports state that “Unfortunately man is not endowed with any conscious sensory perception of hypoxia that might alert him to impending danger, such as the marked dyspnea caused by an excess of carbon dioxide” (295, 296). Individuals undergoing high-altitude decompression for training reasons typically express surprise at the lack of unpleasant breathing sensations; i.e. “Breathing feels perfectly normal, with no gasping or shortness of breath. That’s what makes hypoxia so dangerous: It sneaks up on you” (295, 297). “Air hunger” or “breathlessness” is not induced by ascent to altitude” (295, 298). Despite these anecdotal observations there is a surprising lack of formal studies investigating the ability of hypoxia *per se* to elicit respiratory sensations. Many of the earlier high altitude studies were performed during free breathing trials not controlled for associated changes in CO_2. Thus, hypocapnia likely

developed which may alleviate respiratory sensation and confound interpretation (295). Further, as discussed previously (see section 1.2.4- “Hypoxia, Cognition and Neurophysiological Function”), hypoxia is associated with cognitive impairment which may mask the ability of subjects to recognise respiratory sensations arising from hypoxia.

Studies during exercise have revealed varied effects regarding the ability of hypoxia to elicit breathlessness and dyspnea. Chronos and colleagues used a visual analog scale to measure breathlessness in normal healthy subjects during a constant workload cycle ergometer task while subjects breathed a) normal air b) hyperoxic (F_IO_2 1) and c) hypoxic (F_IO_2 0.15) gas mixtures (299). This study revealed higher ratings of breathlessness during hypoxia and reduced breathlessness during 100% O_2 trials, suggesting hypoxia may increase breathlessness. However, neither $PETCO_2$ nor ventilation was controlled in this experiment making interpretation of the origin of heightened sensation difficult to ascertain. Ward and Whipp performed a similar exercise experiment but attempted to control ventilation and compared respiratory sensation during a) isocapnic hypoxia (F_IO_2 0.14; F_ICO_2 0.03) b) isocapnic hyperoxia (F_IO_2 1) and c) hypercapnic hyperoxia (F_IO_2 0.95; F_ICO_2 0.05) (300). Respiratory sensation was found to be disproportionably greater during the isocapnic hypoxia condition compared to the hypercapnic hyperoxia condition. The authors concluded that this observation supports the ability of carotid body stimulation in the absence of changes in ventilation to elicit dyspnea. Conversely, Lane and colleagues used a carefully matched ventilation exercise protocol and compared breathlessness scores during a) exercise alone b) isocapnic hypoxia (F_IO_2

0.15; F_ICO_2 adjusted as necessary to maintain isocapnia) and c) hypercapnia (F_ICO_2 adjusted as necessary to match the target ventilation level in part a). These investigators found breathlessness scores to be lower during hypoxia compared to the other two experimental conditions (301). Thus, these data do not support the specific role of chemoreceptor activation in the genesis of dyspnea. Similarly, after 20 mins of isocapnia hypoxia (SaO_2 ~80%), Chonan and colleagues demonstrated a reduced perception of dyspnea during a low-intensity exercise task compared with dyspnea measured during the early phase of hypoxia under resting conditions (302). This was despite ventilation during exercise being greater than during early hypoxia. These authors acknowledged that respiratory sensory attenuation may have occurred due to hypoxia-induced CNS depression. In addition to some of the specific highlighted limitations, all of the above mentioned studies assessed respiratory sensation during a background of exercise which in itself stimulates various proprioceptive and mechanoreceptor afferents that are capable of eliciting distinct sensations. Thus, the observed effects of hypoxia-induced chemoreceptor stimulation during exercise may be quite different from those induced by hypoxia at rest.

Masuda and colleagues used a modified rebreathing test under 4 different inspired O_2 free-breathing conditions (F_IO_2 range 0.11-0.93; F_ICO_2 0.07 under each condition) to examine the relationship between ventilation and respiratory sensation (303). The slope of the ventilation-respiratory sensation relationship was depressed with advancing hypoxia. Conversely, Moosavi and colleagues compared the specific sensation of “air hunger” using a visual analog scale during matched ventilation free

breathing and restricted ventilation trials using a mechanical ventilator under conditions of isocapnic hypoxia (SaO_2 range 75-90%) and hypercapnia ($PETCO_2$ range 40-50 mmHg) (295). During free-breathing trials there was an absence of air hunger during both gas conditions. However, during constrained mechanical ventilation when SaO_2 reached a level sufficient to generate ventilatory drive (SaO_2~ 80%) the intensity of air hunger during hypoxia was comparable to hypercapnia. The authors concluded that the perception of air hunger arises as a result of reflex respiratory drive. A recent study using the constrained mechanical ventilation technique by the same group reported a similar observation during hypoxia whereby the intensity of air hunger followed a similar pattern to ventilatory drive albeit slightly delayed in time (304). Together these data support that under conditions of limited ventilation, hypoxia is capable of eliciting air hunger. However, the ability of hypoxia to elicit other respiratory sensations and the precise underlying sensory pathways remain unclear.

1.4.3.2 Central Integration of Respiratory Sensations

The cortical structures responsible for processing respiratory sensory information involved in dyspnea production has received relatively little attention compared to the number of studies investigating the sensory origins for respiratory sensation. RREP studies have demonstrated activation of the primary somatosensory cortex (afferent arrival) followed by activation in the secondary sensory association areas (signal processing) of the cortex (233, 234, 305) (also see section 1.4.2.3- “Respiratory Related Evoked Potentials”). Interestingly, RREP techniques have revealed

perceptual processing deficits in conditions characterized by intermittent periods of increased respiratory load such as asthma and OSA (235, 306-309). However, it is not clear if these deficits are inherent or arise as a result of the disease pathology (92, 194). Further, while source dipole techniques provide insight into the potential neural origin of RREP components, the technique is limited in its ability to identify the distinct cortical sites involved in respiratory load perception (239).

In recent years neuroimaging techniques have allowed for the investigation of the cortical structures activated during volitional breathing tasks or experimentally induced load compensation challenges (310). Cortical sites that have been consistently implicated include; bilateral activation of the primary motor cortex, the right premotor area, the supplementary motor area, the cerebellum, and the thalamus (310). In addition, some studies have reported further activation in regions of the pontomedullary respiratory oscillator, sensorimotor areas, anterior cingulate structures, prefrontal cortex and the parietal cortex (310-319). Integrated activation of many of the cortical and subcortical structures mentioned above during voluntary control of respiration is consistent with other non-respiratory voluntary tasks (314). Other studies that investigated the effect of increased inspired CO_2 on brain activation similarly demonstrated activity in the cerebellar, frontal and occipital regions (310, 320, 321). In addition, unlike the volitional tasks, hypercapnic breathing was associated with activations of the limbic system and an absence of activation in the motor cortex areas. These data support the involvement of cortical regions caudal to the pontomedullary respiratory oscillator in the processing of respiratory sensory information (310). However, while some of the above mentioned studies

utilized dyspnea provoking stimuli, none of these studies were designed to explore the cortical structures involved in dyspnea *per se* and did not incorporate scaling methodology to assess dyspnea.

Thus far, there are only 4 separate studies (from which there have been 6 reports) that have specifically explored the potential cortical sites that are activated during dyspnea using imaging techniques (310, 322-327). Each has studied normal healthy individuals and measured the cortical response to dyspnea producing stimuli such as resistive loading or increased inspired CO_2. Despite the varied interventions three of the four studies revealed predominant common activations in several brain regions including the insula, insular agranular extensions, anterior and posterior cingulate cortices, cerebellum, thalamus and the amygdale. Some of the studies have revealed activations in the primary motor cortex, premotor area, supplementary motor area and somatosensory areas, all of which have been implicated in volitional breathing tasks. Further, neural activation to experimental dyspnea has been observed in the pons, putamen, hypothalamus, hippocampus and in the frontoparietal network. Of all the structures highlighted the multifunctional sensorimotor integration area, the anterior insula, displayed strong activations in all four studies. This suggests that this component of the limbic system may be crucially important in the overall larger cortical network underlying dyspnea (326).

However, while these initial studies have provided some insights into which distinct brain regions might be activated during experimentally-induced dyspnea many unanswered questions remain. Current time and resolution constraints of

neuroimaging technology have not allowed the elucidation of all the cortical sites involved, nor is the technology currently capable of separating activation of afferent versus efferent components involved in respiratory sensory processing. Thus, while hypothesized models based on the current understanding of the subcortical and cortical structures likely to be involved are helpful, the precise sequence of activation and the neuronal pathways involved remain uncertain. In addition, like pain, respiratory sensations are influenced by multiple psychological, social and environmental factors (258). Thus, neuroimaging studies that are able to carefully control and separate the affective components from the sensory elements needed for further insights into the neural processing mechanisms that arise from differing stimuli contributing to dyspnea. There is also a need to study brain activation during naturally occurring dyspnea in patients (310).

1.4.3.3 Potential Mechanisms Underlying Respiratory Sensations

Campbell and colleagues first hypothesized that “length-tension inappropriateness” was the cause of breathlessness (328). This theory in its original form proposed that breathlessness occurs as a result of an imbalance between the force or tension generated by the respiratory muscles and the resultant change in respiratory muscle length and lung volume. Subsequently, many studies have highlighted the important role that muscle afferents play in contributing to conscious perception of joint movement, position and muscle tension (275, 329, 330). The term “length-tension inappropriateness” has since been adapted and used widely to describe many

respiratory sensation processes. Although some adaptations of this model are more difficult to discern, the most widely accepted extension to this original model relates to a mismatch between efferent respiratory motor command and afferent feedback from respiratory afferents (270, 331, 332).

Distinct from sensations directly related to muscle length or tension, the mechanism mediating the sense of outgoing respiratory motor command has been hypothesized to arise as a result of “corollary discharge” (333, 334). This theory is not unique to respiratory sensations but rather encompasses all motor commands and their perceptual consequences. Indeed, the term “corollary discharge” was first used by Sperry to describe the potential mechanism underlying sensorimotor predication (335). In this model an efferent copy of motor command from eye movements is compared to other vestibular and sensorimotor signals to form an ‘expected’ perceptual response. Further, a recently published study by Gandevia and colleagues convincingly demonstrated that the ability to sense efferent motor command is an important mechanism by which healthy individuals sense limb position (336).

In the context of respiratory sensations the theory suggests that copies of outgoing respiratory motor command are relayed to the sensory areas of the cortex where they are perceived as respiratory effort (224, 258). The efferent respiratory motor commands are then compared to the ‘expected’ magnitude of the other respiratory afferent signals occurring at that time. If there is an imbalance between these inputs dyspnea may arise. Factors that give rise to increased motor command such as

decreasing muscle length, muscle fatigue or respiratory muscle weakness have been associated with augmented sensations of respiratory effort (219, 285, 333, 337). These findings provide functional support for the corollary discharge hypothesis rather than a precise knowledge of the sensory pathways involved (224, 258). However, identification of rostral projections from brainstem respiratory motor neurons to the midbrain and the thalamus in the cat, provide neuroanatomical support for the corollary discharge hypothesis (338, 339). Like dyspnea, the presence of corollary discharge would appear to be a threshold dependent phenomenon (304, 339).

In addition to the generalised sensation of respiratory effort there are several carefully executed studies that support the role of corollary discharge as an important mechanism underlying the genesis of the chemoreceptor activated sensation of “air hunger” (292-295). The sensation of air hunger to increased CO_2 would appear to be a time dependent phenomenon whereby sensations arise rapidly (within minutes) and subside with time (340). This suggests that if corollary discharge mediates this sensation the pathways involved may be prone to plasticity. Further, the potential contribution of direct projections from chemoreceptor afferents to sensory areas of the cortex can not be dismissed as a potential mechanism contributing to air hunger (290, 295).

Further, it is clear that not all dyspneic sensations arise as a result of respiratory motor command corollary discharge. Binks and colleagues performed methacholine challenge testing in asthmatic patients during free breathing trials and under

conditions in which active respiratory muscle contraction was minimized via mechanical ventilation (271). In accordance with the corollary discharge hypothesis, the sensation of breathing effort was substantially reduced under the mechanical ventilation condition. However, the intensity of the sensation of chest tightness did not differ between experimental conditions, suggesting that this sensation arises via a different neurosensory mechanism.

Based on a summary of the current understanding of the sensory and cortical processing structures implicated in eliciting dyspnea, Von Leupoldt and Dahme have constructed a preliminary cortical scheme consisting of two major pathways (310). Briefly, the first pathway has been hypothesized to predominantly arise from respiratory muscle afferents relayed via the medulla to the ventroposterior thalamus. Thalamocortical projections then ascend to the primary and secondary somatosensory areas of the cortex (305, 310). Similar to other sensory modalities these cortical regions may be involved with the processing of sensory intensity components of dyspnea (310, 341). The second hypothesized pathway consists mainly of vagal afferents from the lungs and airways which are relayed in the medulla. Brainstem projections then ascend to the amygdala, medial dorsal areas of the thalamus, the insular and cingulate cortex. This predominantly limbic pathway may involve connections to the hippocampus, operculum, putamen and other prefrontal areas and may be involved with the affective components of dyspnea (305, 310, 342). Both these pathways may include projections from respiratory motor commands originating in the brainstem to the higher motor cortex (310).

1.4.4 Effect of Hypoxia on Respiratory Load Perception

Unlike the well researched effects of hypoxia on respiration, the effects of hypoxia on respiratory sensation and perception have received very little attention. There is some evidence to suggest however, that similar to the ventilatory response to sustained hypoxia, perception of respiratory sensations such as “difficulty breathing” and “air hunger” may also be biphasic (304, 343). As highlighted previously (see section 1.4.3.1- “Sensory Receptors Capable of Eliciting Respiratory Sensations”), the ability of hypoxia to elicit respiratory sensations has been controversial. It does seem clear however, that when ventilation is constrained, hypoxia can elicit “air hunger”, possibly via corollary discharge (see section 1.4.3.3- “Potential Mechanisms Underlying Respiratory Sensations”). The precise mechanisms however, remain uncertain (295, 304).

Separate but interrelated to the question concerning the ability of chemoreceptor activation to elicit dyspnea, our group has been particularly interested in the ability of individuals to perceive increased respiratory load during acute periods of hypoxia. As previously highlighted, appropriate load perception may be crucially important in respiratory disorders where increased respiratory load and acute hypoxia occur concurrently (see section 1.4.1- “Clinical Importance”). To explore the relationship between respiratory load perception and hypoxia, Orr and colleagues measured the ability of healthy individuals to perceive externally applied inspiratory resistive loads to breathing during 30 min free breathing trials of isocapnic hypoxia (SaO_2~ 80% F_ICO_2 adjusted as necessary to maintain isocapnia) (208). Compared to normoxia,

during progressive sustained hypoxia, subjects demonstrated a progressive reduction in inspiratory external load magnitude perception compared to normoxia. It was hypothesised that the suppression of respiratory load perception occurred as a result of CNS accumulation of neuroinhibitory modulators that are known to be involved in hypoxia-induced respiratory depression (3, 38, 208). Eckert and colleagues repeated this study in stable asthma patients and observed decrements in external load perception during hypoxia of a similar magnitude in this clinically relevant population (33). However, the asthmatic patients demonstrated more rapid suppression of respiratory load sensation. Further, sensations of dyspnea (i.e. chest tightness, difficult breathing and breathlessness) to methacholine induced-bronchoconstriction were reduced by ~30% immediately following hypoxia but not following hypercapnia. While these observations support the role of hypoxia-induced suppression of respiratory load perception during acute hypoxia, the underlying mechanisms remain unclear. This issue is addressed in experiment 1 (CHAPTER 2).

1.5 Protective Respiratory Reflexes

There are numerous protective reflexes that are activated in an attempt to maintain ventilation in the face of respiratory load or airway obstruction. The following section will focus on two such reflexes, namely the cough reflex and the genioglossus negative pressure reflex. The clinical importance of these reflexes will be highlighted. Current understanding of the underlying physiology, the measurement techniques required and the potential role of hypoxia in modulating these reflexes will be

discussed. The reflex response to brief respiratory loads to breathing for several inspiratory muscles will also be discussed.

1.5.1 Cough Reflex

1.5.1.1 Clinical Implications

Chronic cough can be a problematic symptom of respiratory disease and is one of the most common reasons for patients seeking medical attention (344). Several studies have demonstrated that cough sensitivity is heightened during periods of acute respiratory infection, an effect which appears to be reversed upon resolution of infection (345-347).

The cough reflex does however, serve as a fundamentally important protective mechanism. It is one of several defensive respiratory reflexes needed to protect the lungs from inhalation or aspiration of potentially injurious substances and for clearing excess secretions. A blunted cough reflex can be harmful (i.e. lead to further gas exchange abnormalities, atelectasis and infection), or even fatal in the presence of severe respiratory disease (345, 348, 349). Indeed, there are many disease states whereby an absent or blunted cough reflex may render patients vulnerable to increased morbidity and mortality (349-351). Thus, any factor that impairs the function of this vital protective response has the potential to increase disease severity.

1.5.1.2 Physiology of the Cough Reflex

The cough reflex can be elicited in response to a variety of chemical stimuli and via direct mechanical stimulation of the larynx or tracheobronchial airway. Differing stimuli likely elicit the cough reflex via independent but potentially interrelated neurosensory mechanisms. Investigation into the underlying physiology of the cough reflex has largely been derived from animal studies (352). Interestingly, some species such as the mouse and the rat lack a typical cough response (353). Thus, species differences must be considered when extrapolating to the human. Nonetheless animal models, particularly the guinea pig model, have proved insightful in furthering the understanding of this important reflex and form the basis for much of the following description of cough reflex physiology.

While substantial advances have been made in understanding the physiology of the sensory and central mechanisms underpinning the cough reflex, considerable uncertainty remains (354-357). Myelinated, rapidly adapting receptors and nonmyelinated C-fibers located throughout the laryngeal and tracheobronchial tree have long been proposed as important sensory afferent nerve fibers capable of mediating cough (358). However, the role of C-fiber activation in the production of cough is controversial and in some circumstances these inputs may be inhibitory (355, 359, 360). More recently, a distinct subset of myelinated mechanoreceptors originating in the nodose ganglia and termed "cough receptors" have been identified in guinea pigs (354). Similarly, identification and characterisation of the functional properties of the capsaicin sensitive type 1 vanilloid receptors suggests that these

sensory nerve endings may also act as cough receptors (357). Supra-threshold stimulation of one or more of these sensory nerve endings, which is dependant on the properties of cough-provoking agent/s, initiates the cough reflex. Slowly adapting receptors may also play a key role in regulating the cough reflex (355, 359). Sensory information from the various nerve endings arrives at the NTS via the nodose or jugular ganglia and respective branches of the vagus nerve. Afferent information may be gated prior to its arrival at the NTS to regulate cough and ensure appropriate timing of cough genesis with respiration (356). The generation of the efferent component of the reflex responsible for the sequence of respiratory muscle contractions required to produce cough is believed to originate in the medulla (361). There are likely many interaction pathways between the NTS, the medulla and other brainstem structures capable of modulating cough (362). Humans can also voluntarily suppress cough, highlighting the role of inhibitory cortical projections to the cough neural network (363).

1.5.1.3 Measurement Techniques

The most commonly utilized method for quantifying cough reflex sensitivity in humans is via cough challenge testing. The methodology is similar to that developed for bronchoprovocation testing in that a provocant (a tussive agent) is delivered to the airway via a jet or ultrasonic nebulizer. Cough challenge testing however, has not reached the same level of standardization between laboratories as bronchoprovocation testing (364). Tussive agent delivery circuits, dosages, duration of exposure for a given dose, time between dosages, nebulization types and flow

rates used have all varied between laboratories making between laboratory comparisons difficult (364, 365). Most protocols do however, utilize a doubling dose regime of the tussive agent whereby the primary outcome measures are the concentration of tussive agent that causes two (C2) or five (C5) coughs as calculated via linear interpolation of the log-dose response curve (364). Many tussive agents have been employed including, ammonia, capsaicin, cigarette smoke, citric acid, distilled water, sulphur dioxide and tartaric acid (364). However, capsaicin (the pungent extract of pepper) has emerged as the most commonly used tussive agent in human cough reflex studies. Capsaicin has been used extensively to examine the efficacy of antitussive agents and to explore physiological differences in cough reflex sensitivity in healthy subjects and patient groups (345, 366, 367). This is likely a reflection of its favourable reproducibly characteristics and ability to produce cough in a dose dependent fashion with few side effects (365, 368-370).

The cough reflex sensitivity is subject to tachyphylaxis (a time dependent decrease in frequency). Adaptation appears to be less pronounced for capsaicin compared to other tussive agents such as citric acid (371). Cough reflex sensitivity is also subject to diurnal variation (372). Changes in inspiratory flow may strongly influence cough reflex characteristics, both in terms of aerosol deposition throughout the airway and the potential role of increased activation of slowly adapting pulmonary stretch receptors via lung inflation at higher inspiratory flows (373-375). Thus, these various physiological characteristics need to be carefully considered when designing experimental cough reflex studies.

1.5.1.4 Effect of Blood Gas Changes on the Cough Reflex

Despite recognition of the physiological and potential clinical importance of this issue (356, 373), there have been few studies conducted to investigate the effects of hypoxia and hypercapnia on cough reflex sensitivity. Of the limited animal data available, the effect of chemoreceptor stimulation on cough would appear to be variable and somewhat inconsistent (359). Expiratory abdominal and laryngeal neural activity is dramatically suppressed and in many cases abolished during moderate to severe hypoxia which would act to depress the cough reflex (376, 377). Tatar and colleagues examined the effects of prolonged hypocapnic hypoxia (30 hours, F_IO_2 0.11) on cough, the expiration reflex and sneezing elicited via mechanical stimulation in 11 awake cats (378). In the acute phase of hypoxia exposure (<15 mins) the authors reported an increase in the intensity of these respiratory defence reflexes. However, prolonged hypoxia (>15 mins) resulted in no significant change from baseline. Conversely, in an anaesthetized cat model these authors demonstrated marked suppression of laryngeal and tracheobronchial cough to mechanical stimulation during poikilocapnic hypoxia (7 mins, F_IO_2 range 0.03-0.11) (379). Similarly, in a separate study by the same group, again utilizing the anaesthetized cat model, hypercapnic-hypoxia (5 mins, F_IO_2 0.11 + F_ICO_2 0.07) was also found to depress mechanically induced cough (380). Hypercapnia alone also appears to suppress the cough reflex in anesthetised humans (381).

Two studies examined the effects of prolonged high altitude exposure (up to 1 month) on cough reflex sensitivity to citric acid in humans, one under simulated

altitude (382, 383). Both studies reported a small decrease in citric acid cough threshold at extreme altitude. The authors postulated that sub-clinical pulmonary edema and/or airway drying effects secondary to an altitude-induced increase in ventilation may have contributed to this effect (382, 383). While not specifically designed to examine the effect of hypoxia, and caution is warranted given the small sample sizes, the degree of hypoxia appeared to have no effect on cough threshold when examined using a linear regression model. However, the effect of normobaric hypoxia on cough reflex sensitivity in the absence of cough provoking effects of hypobaric hypoxia remains unclear. Thus, there have been no studies conducted to specifically examine the effect of hypoxia on cough reflex sensitivity. This issue has been addressed in experiment 2 (CHAPTER 3).

1.5.2 Genioglossus Upper Airway Negative Pressure Reflex

1.5.2.1 Clinical Implications

Activation of UA dilator muscles is believed to be an important mechanism for opposing collapsing negative pressure forces that occur during inspiration (384-386). Of the 20 or more muscles surrounding the UA the genioglossus is the largest UA dilator muscle (387). Activation of the genioglossus causes the tongue to protrude, which acts to open the pharyngeal portion of the airway. Its size, accessibility and the tendency for the nearby pharyngeal airspace to collapse in OSA has fuelled research into the properties of this multifunctional muscle.

Consistent with its role in stabilising the UA, the genioglossus muscle demonstrates phasic activation during inspiration (384, 388-390). Initial studies reported marked reflex activation of the genioglossus muscle in response to rapid changes in UA negative pressure, present in animals and in humans (391-393). Mezzanotte and colleagues postulated 1) that OSA patients would display augmented genioglossus activation during wakefulness as a compensatory mechanism for a narrow UA and 2) sleep related decrements in muscle reflex excitability would subsequently render the UA prone to collapse during sleep (394). Consistent with part 1 of this hypothesis, these investigators demonstrated greater baseline activation of the genioglossus muscle in OSA patients compared to controls during wakefulness (394). This observation was confirmed in a subsequent investigation (395). In addition, Berry and colleagues recently demonstrated greater reflex activation of the genioglossus in response to brief negative pressure pulse stimuli of modest magnitude (~10-13 cmH_2O) in OSA patients compared to controls during wakefulness (396). However, larger stimuli (~20 cmH_2O) revealed no significant between group differences in reflex activation (396).

To examine part 2 of the above mentioned hypothesis, Wheatley and colleagues measured the genioglossus negative pressure reflex in healthy individuals in the lateral position during sleep versus wake (397). The UA was found to be more collapsible to negative pressure pulse stimuli and the reflex was markedly reduced during sleep compared to wake (397). Horner and colleagues also reported attenuation of the genioglossus negative pressure reflex during sleep in healthy subjects (398). In addition, Malhotra and colleagues studied healthy individuals and

demonstrated a strong relationship between pharyngeal pressure and genioglossus activity during wakefulness that was reduced during sleep (389). Thus, these data support the hypothesis that the genioglossus muscle is less able to reflexively respond to negative pressure during sleep. Sleep-related depression of genioglossus reflex responsiveness is thus likely to be a contributing mechanism to pharyngeal collapse in OSA.

In further support of genioglossal muscle inactivity in sleep contributing to UA obstruction, the increased baseline activity of the genioglossus muscle observed in OSA patients while awake was lost in most patients at sleep onset, a time when UA obstruction first occurs (399). Shea and colleagues studied the genioglossus negative pressure reflex during sleep onset in healthy individuals (400). Despite no significant difference from wakefulness, there was wide variation in reflex amplitude responses with many subjects displaying marked attenuation. In the same study, there was evidence for genioglossus reflex inhibition rather than excitation to negative pressure pulse stimuli during REM sleep, a time when OSA severity tends to be most severe (400). More recently however, studying healthy individuals in the supine position, Malhotra and colleagues demonstrated preservation of the genioglossus negative pressure reflex in NREM sleep, and in many cases augmented activation during NREM sleep versus wake in healthy individuals (401). Thus, this recent evidence suggests that this important protective reflex mechanism may be position dependent. The effects of sleep in the supine posture on genioglossus reflex responsiveness in OSA patients remains unknown. Nonetheless, the majority of these data support the notion that the genioglossus muscle may play

an important role in the maintenance of UA patency in OSA during wakefulness and reduced muscle activity during sleep may predispose the UA to collapse.

1.5.2.2 Physiology of the Genioglossus Negative Pressure Reflex

The genioglossus muscle is innervated by the hypoglossal nerve and has respiratory related activity (178, 384, 388). This is believed to occur partly due to a reflex response to the negative pressure generated during inspiration and partly due to central activation from respiratory premotorneurons (178, 385, 402). Hypoglossal motor activity to the genioglossus muscle can also be reflexively modulated by non respiratory afferents at the level of the NTS such as pharyngoesophageal stimulation (403). As highlighted in the previous section (1.5.2.1- “Clinical Implications”), state dependent neuromodulators may also modulate muscle activity (177, 404, 405). Recent studies recording human genioglossus single motor unit activity have further highlighted the heterogeneity of this muscle and demonstrated that even when single motor units are located within very close proximity, the neural inputs and their functionality can be quite diverse (406). For example, different units recorded at the same electrode site may show respiratory phasic, tonic or phasic and tonic activity.

The precise neural pathways involved in the genioglossus negative pressure reflex are yet to be defined. Our current understanding of the basic reflex arc derived from multiple unit recordings suggests that pressure sensitive nerve endings in the pharyngeal airway respond to rapid changes in negative UA pressure. Sensory

information is relayed via the superior laryngeal nerve and arrives at the NTS. The motor output to the muscle originates at the nearby hypoglossal motor nucleus. The efferent response is relayed to the muscle via the hypoglossal nerve (178, 407-409).

1.5.2.3 Measurement and Analysis Techniques

The majority of studies examining genioglossus EMG recordings in humans have been concerned with elucidating the overall response of the muscle under varying conditions and stimuli with reference to the clinically driven questions concerning sleep-disordered breathing, rather than from a neurophysiological perspective. Initial measurements of genioglossus muscle activation in humans were performed using intra-oral bipolar surface electrodes (410). Quantification of the negative pressure reflex was also first performed using surface recordings and was expressed as a 10 millisecond moving time average of the rectified ensemble averaged raw EMG (393). Ensemble averaged rectified EMG is the typical approach for characterising reflex properties (411, 412). However, in Horner and colleagues' initial studies, rectified ensemble averages were generated from relatively few replicate trails (n=6). Surface EMG recordings are also known to have a relatively poor signal to noise ratio when compared to intramuscular recordings (413, 414). In addition, the use of averaging windows in these studies would to some extent blunt or distort the true underlying reflex morphology. For the reasons listed above, elucidation of the precise morphology of the genioglossus negative pressure reflex is probably not possible from these early studies.

To improve the signal to noise ratio, fine wire electrodes inserted directly into the muscle per orally or percutaneously have been utilized to measure genioglossus EMG characteristics (394, 415). Following Horner and colleagues initial report, the majority of reflex studies did indeed use intramuscular electrodes and increased the number of replicate trials to improve the signal to noise ratio (30, 397, 400, 401). However, these subsequent reflex studies expressed reflex characteristics as a moving time average of the rectified EMG (averaging time window 50-100 ms). While this approach allows for examination of the generalised muscle response for the relatively slow time constants of respiration, it acts to blunt and distort the more rapidly occurring true underlying reflex morphology and peak latencies. Accordingly, from a neurophysiological perspective, transient excitatory and inhibitory reflex components may have been overlooked. Thus, the need remains to more fully characterise the genioglossus negative pressure reflex using more sensitive neurophysiological techniques and analysis methods in light of the limitations of these earlier studies. This issue has been addressed in experiment 3 (CHAPTER 4).

1.5.2.4 Effect of Hypoxia on Genioglossus EMG

Similar to respiration, the effects of hypoxia on genioglossus EMG (EMGgg) activity appear highly dependent on factors such as hypoxia severity and duration, species and level of maturation. Studying chronically instrumented adult goats, Parisi and colleagues demonstrated that EMGgg was not activated beyond baseline levels until SaO_2 dropped below 70% during wake and NREM sleep (416). Conversely, Martin and colleagues reported increased EMGgg activation during a 10 min period of

sustained hypoxia (F_IO_2 0.12) in piglets (417). During moderately severe hypoxia (PaO_2 ~50 mmHg) the contractile properties of the genioglossus muscle appear vulnerable to suppression in rats (418). Following long-term intermittent hypoxia (episodic hypoxia achieved by varying F_IO_2 from 0.21-0.09 every 90 sec during the sleep cycle for 3 weeks), Vaesey and co-workers recently reported a reduction in hypoglossal nerve output in rats (419). Conversely, McKay and colleagues reported the presence of long term facilitation of respiratory-modulated EMGgg activity following severe, brief repetitive hypoxia (3 x 5 min periods of F_IO_2 0.05 remainder N_2), but not after sustained hypoxia in rats (15 min F_IO_2 0.05 remainder N_2) (420). A recent review by Bradford and colleagues highlighted the many potential deleterious effects that episodic hypoxia may have on UA dilator muscle function and speculated that these effects are likely to contribute to the pathophysiology of sleep-disordered breathing (421).

However, given the known species differences in ventilatory responses and consequences of hypoxia, EMGgg may also be markedly different between animals and humans. For example, studying healthy men during wakefulness, McEvoy and colleagues demonstrated reductions in EMGgg below baseline activity during repetitive intermittent hypoxia (10 x 2 min periods of isocapnic hypoxia each episode separated by 2 min normoxia; during hypoxia F_IO_2 0.11 reminder N_2 and CO_2 titrated as necessary to maintain isocapnia) rather than facilitation (76). In a subsequent report using similar procedures this observation was replicated for healthy women but was not observed in men (77). Conversely, Harris and colleagues recently reported the presence of LTF of peak EMGgg in awake humans following moderately

severe repetitive intermittent hypoxia (8 x 4 min periods of hypoxia each separated by 5 min; during hypoxia F_IO_2 0.08 remainder N_2) albeit only in the presence of hypercapnia (~5 mmHg above baseline) (422). Despite the suggestion that LTF of EMGgg to hypoxia may exist during sleep, there are currently no formal studies that have investigated the existence of this phenomenon (422, 423).

The effects of sustained hypoxia on baseline EMGgg are also varied. During progressive isocapnic hypoxia lasting only several minutes (according to the Rebuk and Campbell method (25)), peak phasic EMGgg increased linearly within the SaO_2 range of 90-60% in healthy males during wakefulness in the supine posture (424). Using similar methodology, there is evidence to suggest that this relationship may be impaired in OSA patients and with increasing age (425, 426). Okabe and colleagues measured EMGgg during 20 min of sustained isocapnic hypoxia (SaO_2 ~80%) in OSA patients and controls during wakefulness (427). These investigators reported a biphasic pattern of activation consisting of an initial increase in phasic EMGgg (~150% baseline at 3 min) which subsequently declined to below baseline levels by the end of the 20 min period (~90% baseline). EMGgg responses were not different between patients and controls (427). Conversely, studying healthy individuals, McEvoy and colleagues reported sustained elevation of EMGgg (~120% of baseline) during a comparable degree and duration of isocapnic-hypoxia (20 min, SaO_2 ~80%) (76). Kimura and colleagues studied the effects of sustained hypercapnic-hypoxia (20 min, SaO_2 ~80%, $PETCO_2$ ~5 mmHg above eucapnia) on EMGgg in OSA patients and controls during wakefulness (428). In this report the relative decline in EMGgg from peak activation levels was found to be greater in patients than controls.

Studying healthy individuals during NREM sleep in the lateral position, Stanchina and colleagues reported no change in EMGgg activity during brief periods of isocapnic hypoxia alone (3 min, SaO_2 ~80-85%) or when combined with inspiratory resistive loading (~5-15 $cmH_2O.l^{-1}.sec$) (429).

Gauda and colleagues reported increased EMGgg activation in response to tracheal occlusion during hypoxia (F_IO_2 0.1) compared to normoxia in anaesthetised cats (430). There has been one study investigating the effects of background chemoreceptor stimulation on the EMGgg reflex response to negative pressure in humans (30). In this study, EMGgg reflex latency and peak amplitude responses were not significantly different between sustained isocapnic hypoxia (SaO_2 ~80%, 15-20 min) compared to normoxia in healthy individuals during wakefulness in the supine posture. The effect of hypoxia on EMGgg reflex properties during sleep in humans has not been previously investigated. This issue has been addressed in experiment 3 (CHAPTER 4).

1.5.3 Inspiratory Muscle Reflexes to Brief Respiratory Occlusion Stimuli

Following the initial report by Newsom-Davis and Sears (431), there have been several studies investigating inspiratory muscle reflex properties and the underlying mechanisms in humans across a range of disease states (285, 432-436). Unlike the stretch or loading responses in limb muscles, which consist of reflex excitation without inhibition (437-439), the response of several human inspiratory muscles to a

sudden increase in respiratory load is an initial inhibition (onset ~35-40 ms) followed by an apparent subsequent excitation (onset ~80-100 ms) (431-433). This response has been demonstrated to be present in muscles such as scalene, parasternal intercostal and diaphragm and is likely to be evident in all active inspiratory muscles (433). While the functional role of inspiratory muscle reflex inhibition is unclear it has been proposed that it may have a protective role preventing further inhalation of an object causing airway obstruction and decreasing the likelihood of UA collapse to increased negative airway pressure (433).

Anesthesia of upper and lower airway receptors and bypass of the UA via an endotracheal tube does not appear to influence these reflex responses (431, 433). These findings suggest that these reflexes are mediated by either intramuscular receptors and/or intrathoracic receptors. To definitively exclude the role of intrapulmonary receptors in the genesis of the short latency inhibition, Butler and colleagues studied reflex responses to brief (250 ms) occlusive loading to respiration in patients with recent bilateral lung transplantation (complete pulmonary denervation model) (440). Using this model, the inhibitory component of the reflex was maintained, supporting the role of intramuscular (muscle spindles and tendon organs) in its genesis. Interestingly, in conditions characterised by periods of increased respiratory load such as asthma and OSA, the duration of reflex inhibition appears prolonged (434, 436). Further, the peak inhibition latency and the duration of the inhibition were correlated with the RDI in OSA patients (436). The effect of sleep and chemoreceptor stimulation on these reflex responses has not been examined. These issues have been addressed in experiment 3 (CHAPTER 4).

1.6 Summary and Aims of Thesis

In summary, there are several common respiratory diseases that are characterised by acute periods of increased respiratory load accompanied by hypoxia. Hypoxia has long been associated with impaired cognitive function and recent evidence suggests that respiratory load perception may be similarly affected. While there have been many animal and human studies investigating the effect of hypoxia on respiration, the effect of hypoxia on respiratory sensation and protective respiratory reflexes in humans is limited. The aim of this doctoral thesis is therefore to examine the effects of acute hypoxia on respiratory load sensation and protective respiratory reflexes in humans and to highlight the potential implications of these findings for patients with hypoxic-respiratory disease.

CHAPTER 2. SUSTAINED HYPOXIA DEPRESSES SENSORY PROCESSING OF RESPIRATORY RESISTIVE LOADS

2.1 Introduction

Acute hypoxia coupled with increased respiratory load is a feature of life-threatening asthma, acute exacerbations of chronic obstructive lung disease and sleep-disordered breathing. Dyspnea, a manifestation of load sensory processing, is a common feature of breathing impairment during wakefulness (258). There is also somatosensory cortical activation (K-complexes, evoked potentials and arousal) to increased respiratory load during sleep (196, 242, 245). Transient exposure to brief episodes of hypoxia may produce specific sensations of dyspnea such as “air hunger” (295). However, hypoxia has also been shown to inhibit neurocognitive functioning and sensory processing in non-respiratory related tasks (122, 441, 442). Furthermore, moderate isocapnic hypoxia (SaO_2 ~80%) lasting greater than 5 minutes, reduces the perception of externally applied inspiratory resistive loads in healthy individuals and stable asthmatics (33, 208). Following hypoxia, asthmatics reported 25-30% reductions in symptoms of dyspnea to methacholine bronchoconstriction (33). Thus, hypoxia has the potential to suppress respiratory sensory processing and respiratory reflexes, and to increase disease severity in a range of hypoxic respiratory disorders. The underlying mechanisms mediating suppression of respiratory load sensation with hypoxia have yet to be elucidated.

Respiratory related evoked potentials (RREP) provide insight into the sensory processes underlying respiratory load perception (220, 232-234). RREPs are characterised by positive (P) and negative (N) waveform components within time locked windows relative to stimulus onset. Respiratory load perception research has primarily focussed on the first (P1) and third (P3) positive peaks. P1 occurs 40-110 milliseconds after stimulus onset depending on the specific stimulus characteristics (220, 232, 235, 236) and is believed to reflect the arrival of ascending respiratory signals at the somatosensory cortex (232). P1 amplitude increases with increasing stimulus magnitude (234, 237, 238). The P3 component, occurring after 250 milliseconds, is thought to reflect cognitive and perceptual sensory processing and unlike P1 is attention dependent (233). The amplitudes of both these RREP components strongly correlate with subjects' perception of the magnitude of respiratory resistive load (233, 234). RREP techniques have revealed perceptual processing deficits in patients with asthma and OSA (235, 306-309).

The purpose of this study was to investigate which components of the RREP elicited by brief resistive loads are influenced by acute isocapnic hypoxia. To avoid the confounding influence of greater resistive load stimulus magnitude during hypoxia (increased respiratory drive) compared to normoxia (baseline respiratory drive), experiments were conducted during matched targeted hyperventilation. Measurements were also made in the recovery period immediately following gas inhalation during normal tidal breathing. It was hypothesized that hypoxia would blunt

resistive load sensation and reduce the amplitude of the early and late RREP components.

2.2 Methods

2.2.1 Subject Selection

14 healthy non-smokers with normal lung function gave informed written consent to participate. The study was approved by the Daw Park Repatriation General Hospital and Adelaide University Human Research and Ethics Committees.

2.2.2 Preliminary Visit

Subjects attended a preliminary visit for familiarization with the respiratory equipment and protocols and to practice the method for matching a predetermined target level of ventilation ($\dot{V}_I$). Initially, pulmonary function testing including spirometry and whole body plethysmography (JLab software version 4.53; Compactlab, Jaeger™, Wuerzburg, Germany) was performed to ensure FEV_1 and FVC were >80% predicted.

2.2.2.1 Target Ventilation Method

Following a 10 minute baseline period, the ventilatory response to 15 minutes of isocapnic hypoxia (SaO_2 ~80%) was recorded (208). From this a target level of minute ventilation ($\dot{V}_I$) to be used in the main visits was determined as follows:

Target $\dot{V}_I$ = 10% (peak hypoxic $\dot{V}_I$ – average of baseline $\dot{V}_I$) + peak hypoxic $\dot{V}_I$.

Subjects practiced the targeted $\dot{V}_I$ task by breathing, mouth closed, through a nasal mask (Profile™ Lite Gel, Respironics, Murrysville, PA, USA), and unidirectional breathing valve (2600 Series, Hans Rudolph Inc., Kansas City, MO, USA) attached to a 2-litre collapsible bag filled at the target flow rate with compressed air (Figure 2). Subjects were required to maintain a constant end-inspiratory bag volume by increasing tidal volume without changing breathing frequency. Specifically, they were instructed to take deeper breaths while maintaining the same rate of breathing. After an initial coaching period in which adjustments to breathing frequency (measured online) were verbally relayed to the subject as necessary, most subjects readily adopted the targeted breathing pattern without requiring further feedback. However, during subsequent visits if the target breathing frequency deviated greater than ±3 breaths per minute, subjects were instructed to adjust breathing rate as required. A manual inspiratory bleed of CO_2 maintained isocapnia.

2.2.3 Study Design, Techniques and Measurements

The effects of hypoxia on respiratory load perception and RREP were examined in two separate experiments approximately one week apart, in random order. Prior to both experiments, subjects abstained from alcohol, caffeine and other drugs for at least 12-hours. On each day, isocapnic hypoxia and isocapnic normoxia were administered, in random order, while subjects maintained voluntary hyperventilation at the target level. One hour separated each gas condition. Subjects were studied seated upright in a comfortable chair.

During hypoxia trials the inspired O_2 fraction (F_IO_2 0.09 in N_2, BOC gases, Torrensville, South Australia) was adjusted as necessary by bleeding small amounts of medical air or N_2 into the inspirate to maintain SaO_2 ~80%. To test for residual effects after hypoxia, load perception and RREP measurements were repeated during room air breathing commencing 2 minutes after terminating each experimental gas. The electroencephalogram (EEG) at C3 and electrooculograms were monitored throughout to ensure wakefulness. The electrocardiogram and heart rate were monitored continuously.

2.2.4 Magnitude Perception of Externally Applied Inspiratory Resistive Loads (ψ)

Following 5 minutes of room air breathing, subjects were switched to the target $\dot{V}_I$ arm of the circuit (Figure 2), through which the experimental gas (hypoxic mixture or medical air) was introduced at the required target $\dot{V}_I$ for 30 minutes. Five resistive loads (8.6± 0.1, 13.4± 0.2, 22.3± 0.4, 30.4± 0.6 and 43.7± 1.1 $cmH_2O \cdot l^{-1} \cdot sec$), were presented for a single breath, 12-times each in random order as described previously (208). Subjects rated their perception of load intensity using open magnitude scaling (208, 209). Following this, subjects resumed room air tidal breathing and measurements continued over 30 minutes (Figure 3A). After a 1-hour break, these procedures were repeated for the remaining test gas condition.

2.2.5 Respiratory Related Evoked Potentials

RREPs were measured from EEG recordings overlying the central somatosensory (C3, Cz, C4) and parietal secondary sensory association (P3, Pz, P4) areas of the cortex. Electrodes were reapplied as necessary until impedance at each site was less than 5 kOhms. Subjects listened to music through earphones throughout the protocol. Following 5 minutes of room air breathing, subjects were switched to the target $\dot{V}_I$ arm of the circuit (Figure 2). One of the experimental gases was introduced at the required target $\dot{V}_I$ for three 20 minute periods, each separated by 15 minutes of room air tidal breathing (Figure 3B). This design was chosen to maximize the likelihood of detecting post-hypoxia effects that previous studies have shown to persist for at least 10-15 minutes after returning to room air breathing (33, 52).

RREPs were elicited by mid-inspiratory, 500-millisecond external resistive load stimuli presented in random order every 2-6 breaths via a fast actuating computer controlled solenoid (SXE9575-A70-00, Iso star, Norgren, Switzerland). To minimize noise, the solenoid and the breathing circuit were located in a room adjacent to the subject separated by a thick masonary wall (Figure 2). In order to facilitate P3 responses, subjects were asked to mentally count (i.e. attend to the stimulus) and later report the number of loads presented. This procedure was repeated for the remaining test gas condition.

2.2.6 Data Analysis and Recording Methods

2.2.6.1 Respiratory Variables

Non-event related respiratory parameters including inspiratory flow, tidal volume, end tidal CO_2 ($PETCO_2$) and SaO_2 were recorded on a WINDAQ recording system (DATAQ Instruments Inc, OH, USA) at a sampling rate of 200 Hz per channel to ensure ample recording resolution. $\dot{V}_I$ and inspiratory timing were calculated breath-by-breath using custom designed software and averaged at 30 second intervals.

2.2.6.2 Magnitude Perception of Externally Applied Inspiratory Resistive Loads (ψ)

ψ during gas inhalation were transformed by modulus equalization (207). Relationships between peak inspiratory pressure (PIP) versus ψ, and resistance (R) versus ψ were assessed using linear and Steven's power function analyses described previously (208, 209). PIP was calculated as the minimum pressure attained during inspiration and R was calculated as the slope of the flow versus pressure relationship throughout inspiration. The primary analysis to explore between gas differences was performed using mixed model analyses (443) to compare ψ for a given level of PIP or R based on the model providing the best fit according to the lowest residual sum of squares in untransformed (i.e. comparable) units. To examine main gas effects for curvi-linear ψ relationships using the mixed model analyses approach, data were first log-log transformed. Example tracings of

one individual's inspiratory flow and mask pressure response to a normal and loaded breath during target ventilation is displayed in Figure 4.

2.2.6.3 Respiratory Related Evoked Potentials (RREPs)

In order to capture fast frequency reflex components and synchronise key stimulus magnitude parameters for event related analysis, EEG and mask pressure channels were recorded on a WINDAQ recording system (DATAQ Instruments Inc, Akron, OH, USA) at a sampling rate of 1000 Hz per channel. EEG was amplified and band pass filtered at 0.3-30 Hz (Model 15LT, Grass Telefactor Inc, West Warwick, RI, USA). Load sequence and mid inspiratory delivery was controlled by custom written software. RREP stimulus onset (time zero) for each individual RREP trial was determined using custom designed software to identify the last flow point preceding the sudden decrement in inspiratory flow following solenoid activation Figure 5. RREP trials free from movement artefact were grouped for the three target $\dot{V}_I$ and three recovery periods respectively and averaged on a per subject basis. RREP waveforms were constructed on a per subject basis for target and recovery periods at each of the measured scalp electrode sites and visually inspected to identify the presence, timing and amplitude of each positive and negative RREP component. Example tracings of one individual's inspiratory flow and mask pressure to a normal breath and in response to a brief mid inspiratory resistive load during target ventilation are displayed in Figure 5.

2.2.7 Statistical Procedures

Mixed model analyses were used to compare perception scores between gas treatments (SPSS version 12.1, SPSS Inc., Chicago, IL, USA). ANOVAs for repeated measures were used to examine central (C3, Cz and C4) and parietal (P3, Pz and P4) gas and site effects on RREP component amplitudes and latencies. ANOVA for repeated measures was also used to examine gas effects on ventilatory parameters across study periods (baseline, target $\dot{V}_I$, recovery). Where main ANOVA effects were observed, post-hoc comparisons were performed using Dunn-Sidak adjusted Student's paired t-tests (444). Statistical significance was inferred when $p<0.05$. All data are reported as means ± SEM.

2.3 Results

2.3.1 Anthropometric Data

Three subjects exhibited substantial variability in breath volume and timing during targeted ventilation such that stimulus magnitude matching was not achieved (difference in minimum mask pressure between gas conditions exceeded ±20%). Therefore, 11 subjects successfully completed all study requirements. The 11 subjects who completed the study (6 males) were young (24.9± 1.1 years) and had normal body mass index (22.2± 0.6 $kg.m^{-2}$) and lung function (forced expiratory volume in one second and total lung capacity 109.3± 3.6 and 104.8± 9.2 % predicted respectively).

2.3.2 Ventilatory Parameters during the Preliminary Visit

During the 15 minute hypoxic ventilatory assessment, SaO_2 dropped rapidly within the first 3 minutes to 82.4± 0.7 % and was maintained thereafter at 81.5± 0.3 %. $\dot{V}_I$ prior to hypoxia was 8.0± 0.3 $l.min^{-1}$ and reached a peak level of 15.1± 1.2 $l.min^{-1}$ during hypoxia, 3.7± 0.7 minutes after the initial decline in SaO_2 before rolling off to 10.8±1.0 $l.min^{-1}$ just prior to the end of the 15 minute trial. Isocapnia was maintained throughout the trial ($PETCO_2$= 39.1± 0.3 during baseline versus 39.9± 0.2 mmHg during hypoxia, p=0.16). Increases in $\dot{V}_I$ occurred as a result of increased tidal volume (0.90± 0.1 versus 0.64± 0.1 l, p<0.01). Breathing frequency remained unchanged from baseline levels (13.4± 0.3 versus 13.6± 0.2 breaths per minute, p=0.63).

2.3.3 Ventilatory Parameters during the Main Experimental Visits

Figure 3 displays $\dot{V}_I$ during RREP and load perception protocols. $\dot{V}_I$ was well matched between gas conditions during target $\dot{V}_I$ and was not different during recovery periods. Table 1 and Table 2 show other ventilatory characteristics during load perception and RREP experiments respectively. By design SaO_2 was lower during hypoxia target $\dot{V}_I$ trials compared to normoxia, baseline and recovery periods. SaO_2 was marginally lower during the normoxic recovery period compared to baseline during the RREP protocol. As instructed, subjects achieved target $\dot{V}_I$ by increasing peak inspiratory flow and tidal volume without changing breathing frequency. These ventilatory parameters were not different between normoxia and

hypoxia. End tidal carbon dioxide levels were not different between gas conditions or between baseline, target $\dot{V}_I$ and recovery periods.

2.3.4 Magnitude Perception of Externally Applied Inspiratory Resistive Loads (ψ)

2.3.4.1 Target Ventilation

ψ increased nearly linearly with peak inspiratory pressure (PIP) (Figure 6A) and a linear model tended to provide a better fit compared to Stevens' power function (ψ=aPIP+b overall r^2=0.97± 0.01, residual sum of squares= 5.9± 2.1 versus $\psi=kPIP^n$ r^2=0.96± 0.01, residual sum of squares= 9.2± 3.4, p=0.06). This accords with several recently published studies that have compared linear versus power function models of respiratory load perception (33, 208, 221) and recent reviews supporting that neural coding phenomena may behave linearly (222, 223). Mixed model analyses showed a main gas effect with lower ψ during hypoxia compared to normoxia (Figure 6A). In addition, there was a trend for a lower slope of the PIP versus ψ relationship during hypoxia compared to normoxia (1.71± 0.29 versus 2.06± 0.36 $\psi.cmH_2O^{-1}$, p=0.07).

In contrast, ψ tended to increase in a curvi-linear manner with resistance (R) (Figure 7A). Stevens' power function provided a superior fit compared to a linear model ($\psi=kR^n$ overall r^2=0.94± 0.02, residual sum of squares= 18.0± 6.3 versus ψ=aPIP+b r^2=0.90± 0.02, residual sum of squares= 20.4 ± 6.1, p=0.02), although this was

poorer than the fit of a linear model of PIP ($\psi=kR^n$ versus $\psi=aPIP+b$, p=0.03). Mixed model analyses of the log-transformed data also revealed a main gas effect with lower ψ for a given R during hypoxia than normoxia (Figure 7A). There were no differences in the slope of log(R) versus log(ψ) relationships between hypoxia and normoxia (0.57± 0.07 versus 0.59± 0.08 log(ψ).log($cmH_2O \cdot l^{-1} \cdot sec)^{-1}$, p=0.47).

2.3.4.2 Recovery

ψ for a given level of PIP tended to be lower during the post-hypoxia compared to post-normoxia period using mixed model analyses, although this was not statistically significant (Figure 6B, p=0.12). In addition, there was a trend for the slope of the PIP versus ψ relationship to be lower post-hypoxia than post-normoxia (2.06± 0.31 versus 2.42± 0.40 $\psi.cmH_2O^{-1}$, p=0.06). There was no mixed model analyses main effect (Figure 7B, P=0.51) or difference in the slope of Steven's power function of R versus ψ post-hypoxia compared to post-normoxia (0.79± 0.12 versus 0.91± 0.14 $\psi.(cmH_2O \cdot l^{-1} \cdot sec)^{-1}$, p=0.18).

2.3.5 Respiratory Related Evoked Potentials

2.3.5.1 Target Ventilation

119.7± 12.9 brief resistive loads per subject during hypoxia and 131.2± 10.5 during normoxia (p=0.15) were averaged to construct group average RREP waveform and stimulus characteristics. RREP stimulus magnitude was well matched between

hypoxia and normoxia (minimum mask pressure -9.7± 0.6 versus -9.2± 0.4 cmH_2O, p=0.18, Figure 8A).

ANOVA revealed a significant main gas effect for P1 and P2 amplitudes at scalp electrode sites overlying the central primary somatosensory (C3, Cz and C4) and the parietal secondary sensory association (P3, Pz and P4) areas of the cortex. Compared with normoxia, overall P1 amplitude at central and parietal sites was lower during hypoxia (2.1± 1.0 μv versus 3.1± 1.0 μv, p=0.02 and 2.8± 0.6 μv versus 3.9± 0.7 μv, p=0.02, respectively). Similarly, overall P2 amplitude at the central and parietal sites was lower during hypoxia compared to normoxia (7.1± 1.7 μv versus 8.9± 1.6 μv, p<0.01 and 4.6± 1.1 μv versus 5.7± 1.1 μv, p=0.02, respectively). An example of one individual's evoked potential response across each of the measured scalp electrode sites is displayed in Figure 9.

ANOVA site effects for each RREP component consistently revealed maximal activation at the central electrode site (Cz for Nf, P1, N1, P2 peaks and Pz for the P3 peak). Group grand mean RREP waveforms at Cz and Pz are presented in Figure 8A. The grand mean amplitudes and latencies at the site where each component was maximal are presented in Table 3. P1 and P2 amplitudes were significantly reduced during hypoxia compared to normoxia. Although N1 amplitude appeared to be greater during hypoxia compared to normoxia, this difference was not statistically significant (Table 3, p=0.12). When P2 was expressed as a N1-P2 difference at the maximal Cz site there were no differences between hypoxia and normoxia (17.4± 3.6

versus 17.5± 2.3 μv, p=0.94). There were no further between-gas differences in RREP amplitude or latency.

2.3.5.2 Recovery

90.0± 7.6 brief resistive loads per subject post-hypoxia and 90.5± 7.5 post-normoxia (p=0.91) were averaged to construct group average RREP waveforms. RREP stimulus magnitude was not different between recovery periods (minimum mask pressure -6.3± 0.4 versus -6.0± 0.2 cmH_2O following hypoxia versus normoxia, p=0.45, Figure 8B). Similar to the target $\dot{V}_I$ periods, P1 and P2 amplitudes at the central sites tended to be lower post-hypoxia compared to post-normoxia, although these differences were not statistically significant (P1; hypoxia 2.4± 1.0 μv versus normoxia 3.4± 0.9 μv, p=0.19, P2; hypoxia 7.2± 2.0 μv versus normoxia 8.7± 1.5 μv, p=0.17). There were no amplitude or latency differences in any other RREP components between recovery periods at the maximal sites of activation (Table 3 and Figure 8B).

Table 1 Group Average Ventilatory Characteristics during Inspiratory Resistive Load Perception Protocol

	Normoxic Experiments			Hypoxic Experiments		
	Baseline	Target	Recovery	Baseline	Target	Recovery
SaO_2	99.2 ± 0.2	99.2 ± 0.2	99.0 ± 0.2	99.2 ± 0.2†	82.4 ± 0.3*	99.0 ± 0.2‡
$PETCO_2$	39.3 ± 0.6	40.2 ± 0.6	38.9 ± 0.5	39.6 ± 0.6	40.4 ± 0.6	39.7 ± 0.6
Fb	14.1 ± 0.4	14.2 ± 0.4	14.6 ± 0.5	13.6 ± 0.5	13.9 ± 0.4	14.5 ± 0.6
V_{TI}	0.6 ± 0.1†	1.2 ± 0.1	0.7 ± 0.1‡	0.7 ± 0.1†	1.2 ± 0.1	0.6 ± 0.1‡
PIF	29.9 ± 0.7†	44.3 ± 0.7	30.8 ± 0.7‡	29.9 ± 0.7†	45.3 ± 0.7	30.3 ± 0.7‡

Arterial blood oxygen saturation (SaO_2), end-tidal carbon dioxide levels measured at the mask ($PETCO_2$), breathing frequency (Fb), inspiratory tidal volume (V_{TI}) and peak inspiratory flow (PIF) during baseline, isocapnic targeted ventilation gas trials and recovery periods for the load perception protocol. Data are group averages ± SEM calculated at 30-second intervals from the commencement of the first loaded breath (2 minutes after each respective transition). * indicates a significant difference between normoxia and hypoxia within a trial period ($p<0.01$). † indicates a significant difference within a gas condition between baseline and target ventilation periods ($p<0.05$). ‡ indicates a significant difference within a gas condition between target ventilation and recovery periods ($p<0.05$). § indicates a significant difference within a gas condition between baseline and recovery periods ($p<0.05$). N=11.

Table 2 Group Average Ventilatory Characteristics during RREP Protocol

	Normoxic Experiments			Hypoxic Experiments		
	Baseline	Target	Recovery	Baseline	Target	Recovery
SaO_2	99.5 ± 0.2	99.5 ± 0.1	99.1 ± 0.1§	99.1 ± 0.3†	82.2 ± 0.3*	98.8 ± 0.2‡
$PETCO_2$	39.0 ± 0.5	39.1 ± 0.6	38.5 ± 0.6	39.2 ± 0.6	39.4 ± 0.5	39.3 ± 0.6
Fb	13.6 ± 0.5	14.2 ± 0.4	14.1 ± 0.4	13.9 ± 0.4	14.3 ± 0.4	14.2 ± 0.4
V_{TI}	0.7 ± 0.1†	1.1 ± 0.2	0.6 ± 0.1‡	0.6 ± 0.1†	1.1 ± 0.2	0.6 ± 0.1‡
PIF	30.1 ± 0.8†	42.5 ± 0.8	29.5 ± 0.7‡	28.0 ± 0.6†	42.8 ± 0.8	29.0 ± 0.6‡

Arterial blood oxygen saturation (SaO_2), end-tidal carbon dioxide levels measured at the mask ($PETCO_2$), breathing frequency (Fb), inspiratory tidal volume (V_{TI}) and peak inspiratory flow (PIF) during baseline, isocapnic targeted ventilation gas trials and recovery periods for the RREP protocol. Data are group averages ± SEM calculated at 30-second intervals from the commencement of the first loaded breath (2 minutes after each respective transition). Target ventilation and recovery data represent overall averages for 3 separate target ventilation and recovery periods combined. * indicates a significant difference between normoxia and hypoxia within a trial period ($p<0.01$). † indicates a significant difference within a gas condition between baseline and target ventilation periods ($p<0.05$). ‡ indicates a significant difference within a gas condition between target ventilation and recovery periods ($p<0.05$). § indicates a significant difference within a gas condition between baseline and recovery periods ($p<0.05$). N=11.

Table 3 Effect of Hypoxia on RREP Mean Amplitude and Latency Data at Maximal Sites of Activation during Target Ventilation and Recovery Periods

	Nf Maximal at Cz		P1 Maximal at Cz		N1 Maximal at Cz		P2 Maximal at Cz		P3 Maximal at Pz	
	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia
Target Ventilation										
amplitude (μv)	-2.6 ± 0.5	-3.1 ± 0.6	3.9 ± 1.2	2.5 ± 1.1*	-4.4 ± 0.5	-6.7 ± 1.6	12.4 ± 2.1	10.0 ± 2.2†	8.5 ± 1.0	8.6 ± 1.3
latency (ms)	66.5 ± 2.3	65.7 ± 2.4	105.1 ± 3.8	106.1 ± 4.0	151.0 ± 4.8	152.2 ± 5.1	222.3 ± 6.9	222.9 ± 7.7	328.2 ± 9.3	324.2 ± 6.4
Recovery										
amplitude (μv)	-2.5 ± 0.5	-2.4 ± 0.8	4.5 ± 1.3	3.6 ± 1.3	-6.3 ± 1.4	-6.2 ± 1.0	12.3 ± 1.8	10.8 ± 2.5	9.6 ± 1.1	9.0 ± 1.0
latency (ms)	61.7 ± 2.8	60.5 ± 2.7	97.6 ± 4.1	96.7 ± 3.1	139.1 ± 7.0	141.9 ± 6.4	215.6 ± 7.1	220.6 ± 6.8	316.5 ± 8.8	315.4 ± 7.5

Respiratory related evoked potential mean amplitude and latency data at sites of maximal activation during isocapnic hypoxia and normoxia targeted ventilation and respective recovery periods. Values are means ± SEM. N=11 subjects for all peaks except N1 which was present in 10 of the 11 subjects. Difference between normoxia and hypoxia; * $p<0.05$, † $p<0.01$

Figure 2 Schematic of the Breathing Circuit for Study 1 (Chapter 2) and an Example of a Subject Performing the Target Ventilation Task

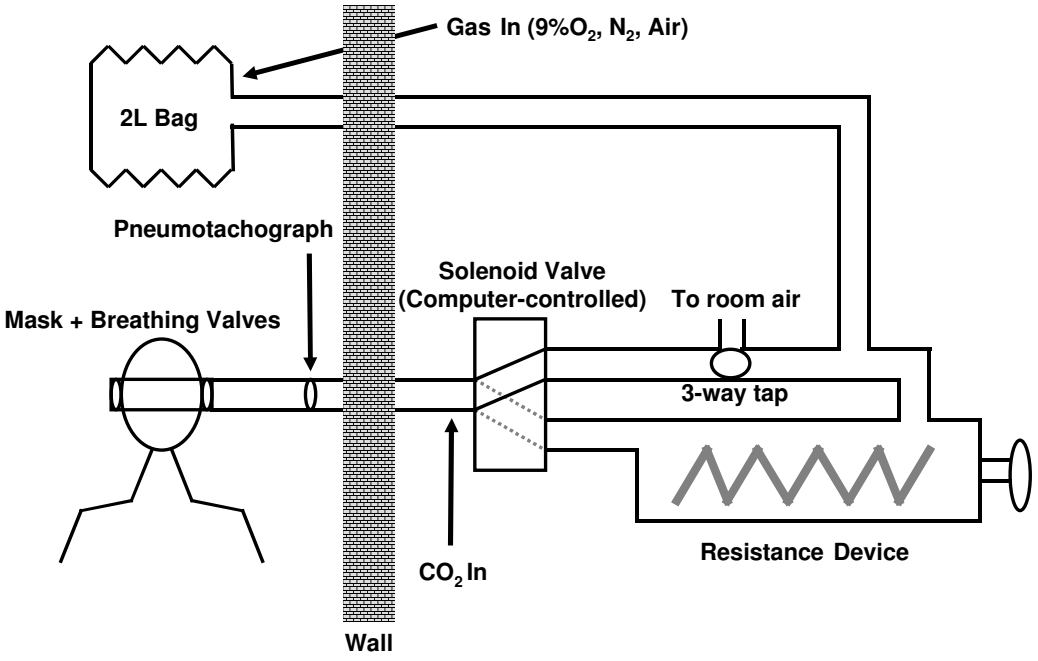
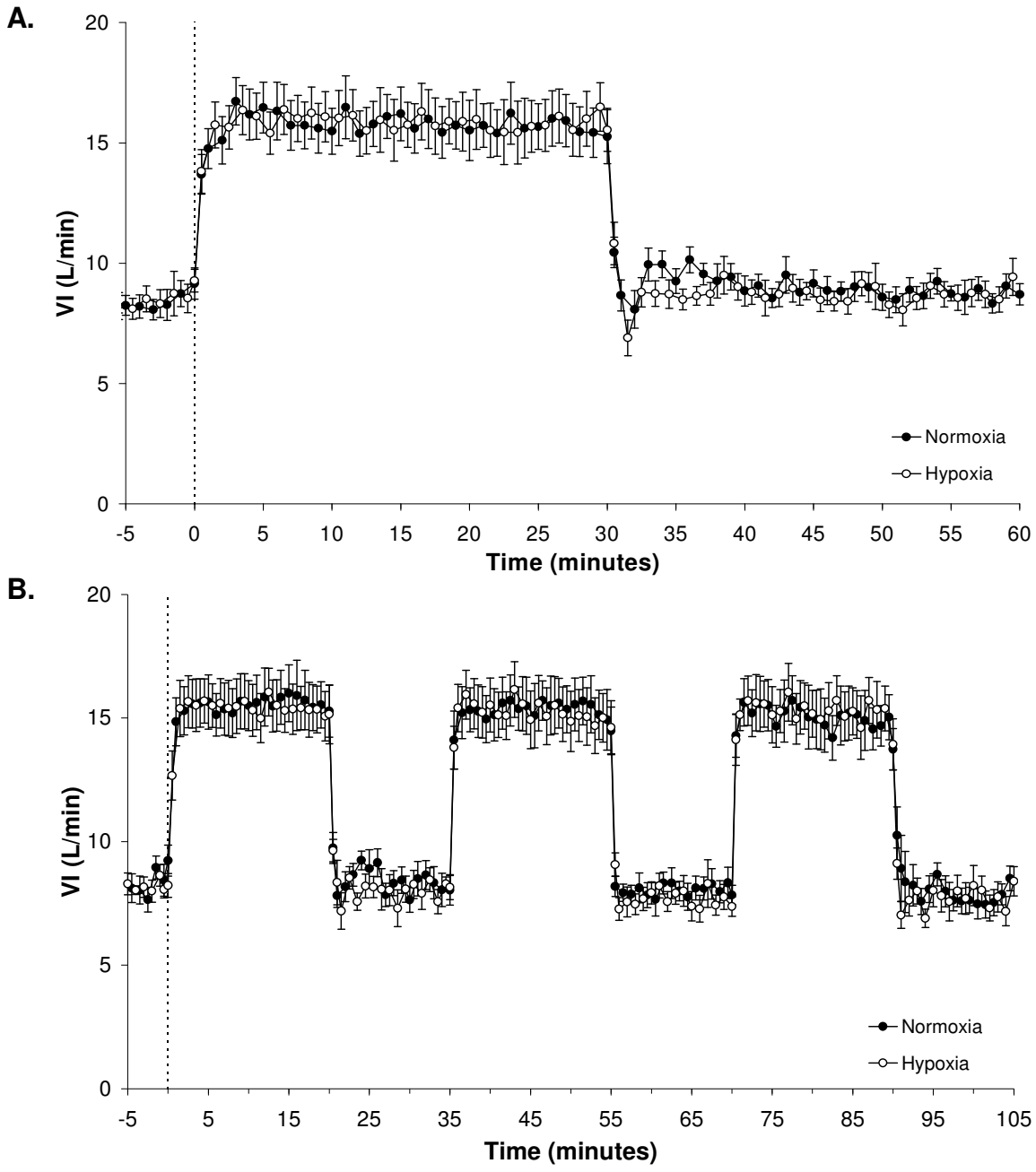
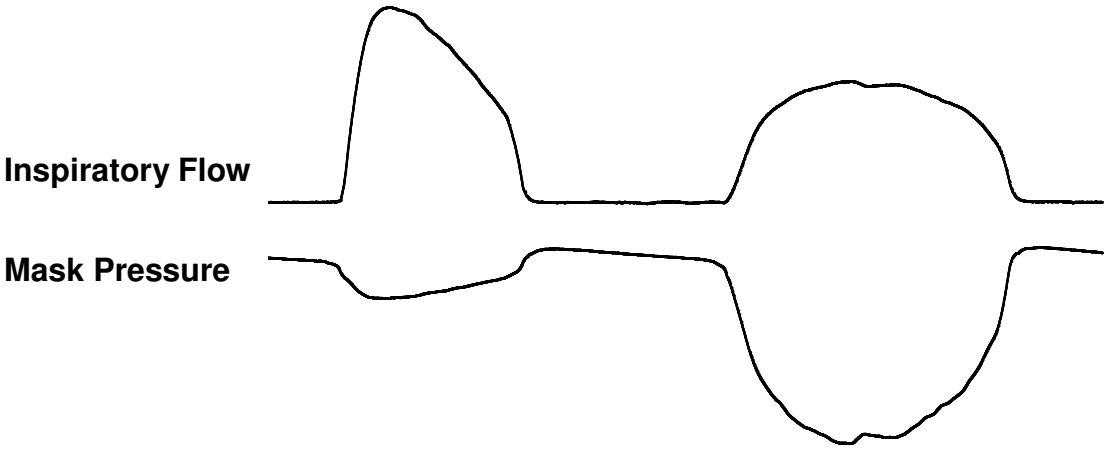


Figure 3 Group Average Minute Ventilation Data during Load Perception and RREP Protocols



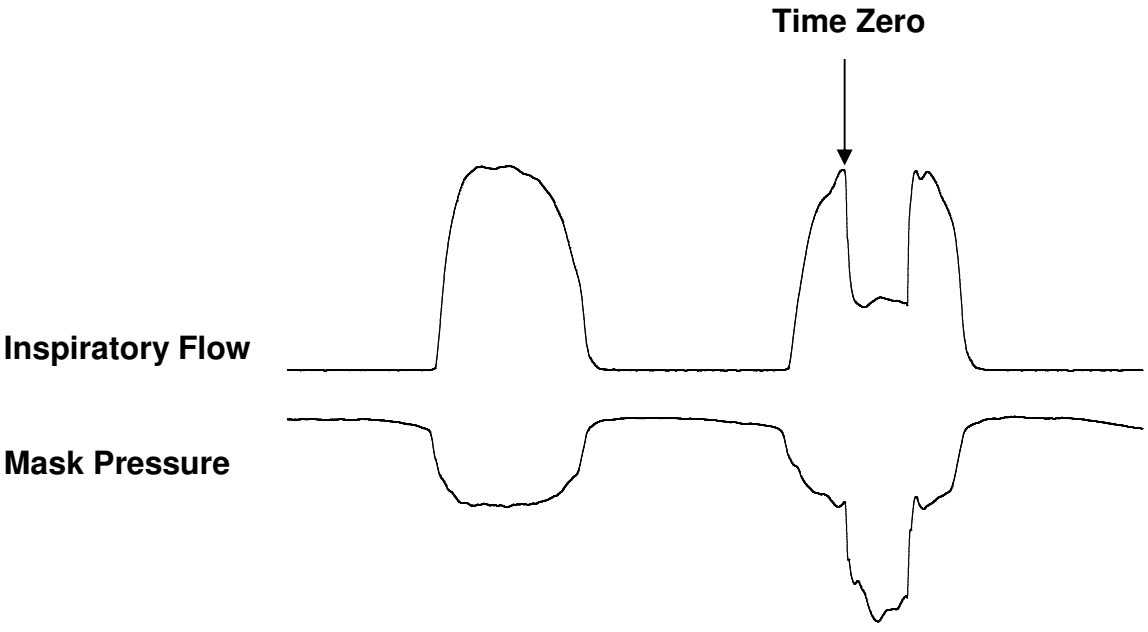
Minute ventilation during the load perception (A) and evoked potential protocols (B). Data are expressed as 30-second averages plotted at alternate intervals between isocapnic normoxia and isocapnic hypoxia. Values are means ± SEM, N=11.

Figure 4 Example of a Loaded Breath during Load Perception Protocol



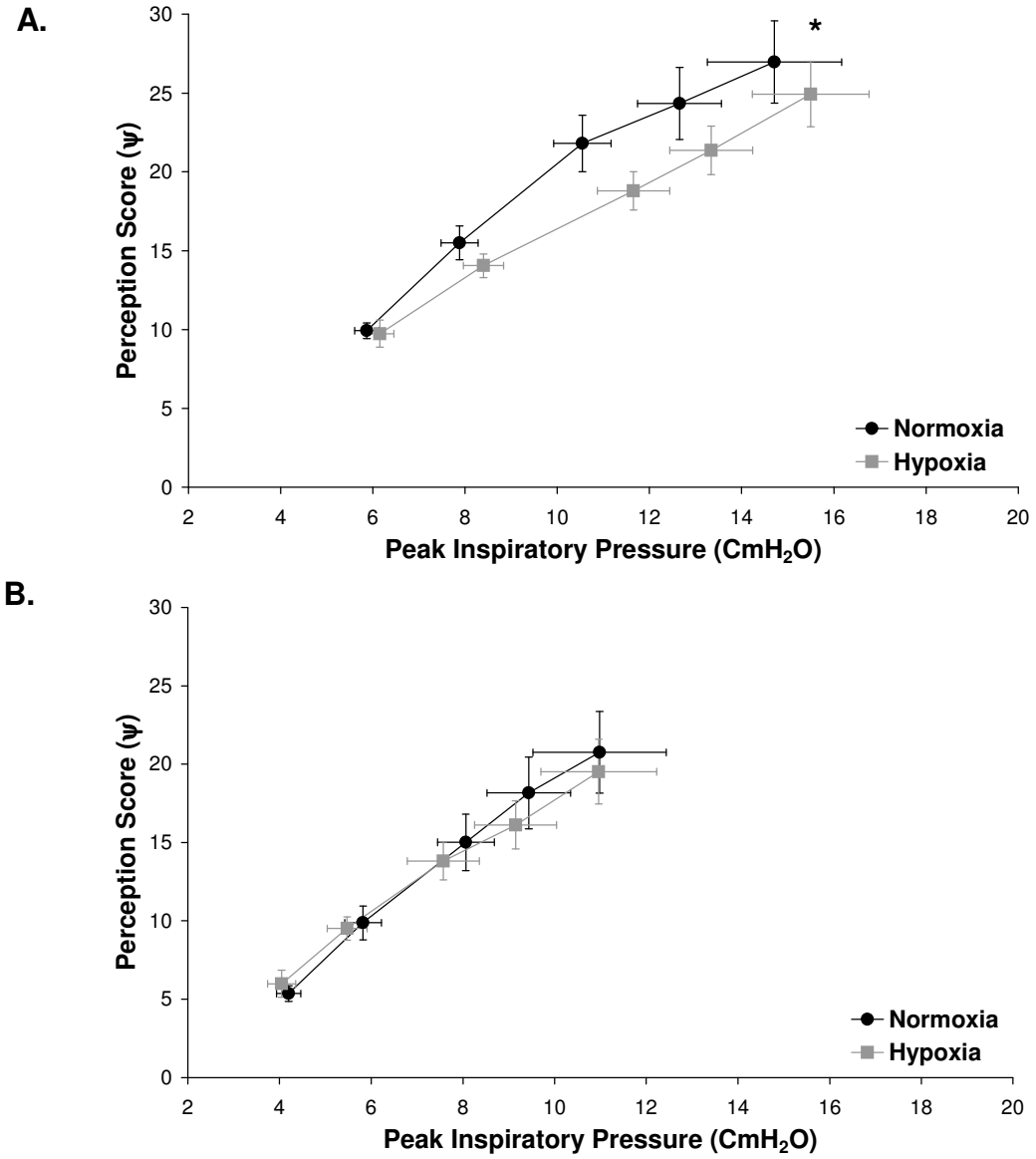
Inspiratory flow and mask pressure tracings without (1st breath) and with (2nd breath) resistive load application during target ventilation in one individual subject.

Figure 5 Example of a Mid Inspiratory Load during RREP Protocol



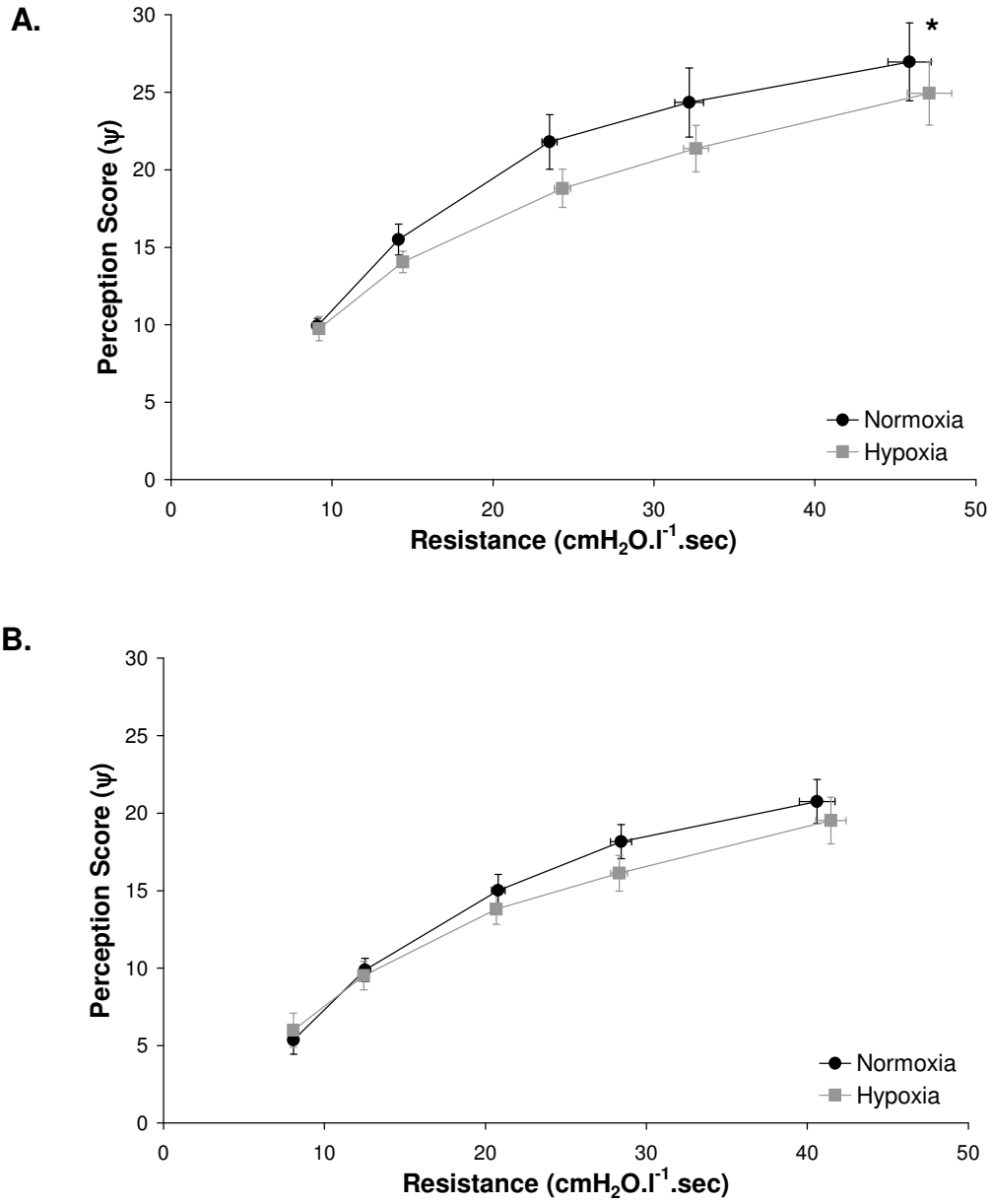
Inspiratory flow and mask pressure tracings without (1st breath) and with (2nd breath) a brief mid inspiratory resistive load application during target ventilation and the point at which stimulus onset was ascertained in one individual subject.

Figure 6 Load Perception versus Peak Inspiratory Pressure during Target Ventilation Gas Inhalation and Recovery Room Air Breathing



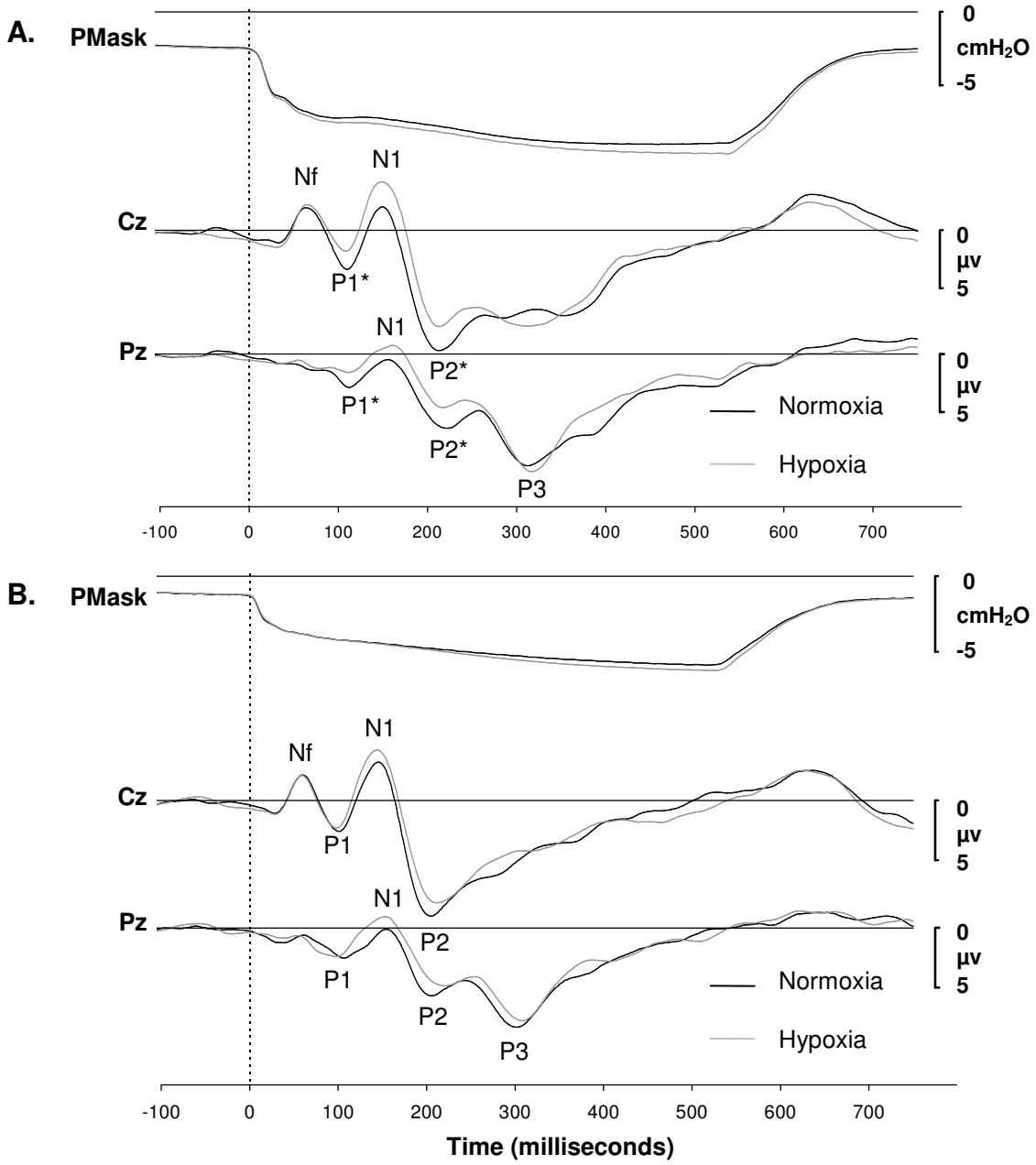
Peak inspiratory pressure (PIP) versus the perceived magnitude of externally applied resistive loads (ψ) during 30 minutes of targeted ventilation with isocapnic normoxia and isocapnic hypoxia (A) and recovery periods (B). * represents a significant decrease in ψ during hypoxia compared to normoxia ($P<0.01$). Values are means ± SEM, N=11.

Figure 7 Load Perception versus Resistance during Target Ventilation Gas Inhalation and Recovery Room Air Breathing



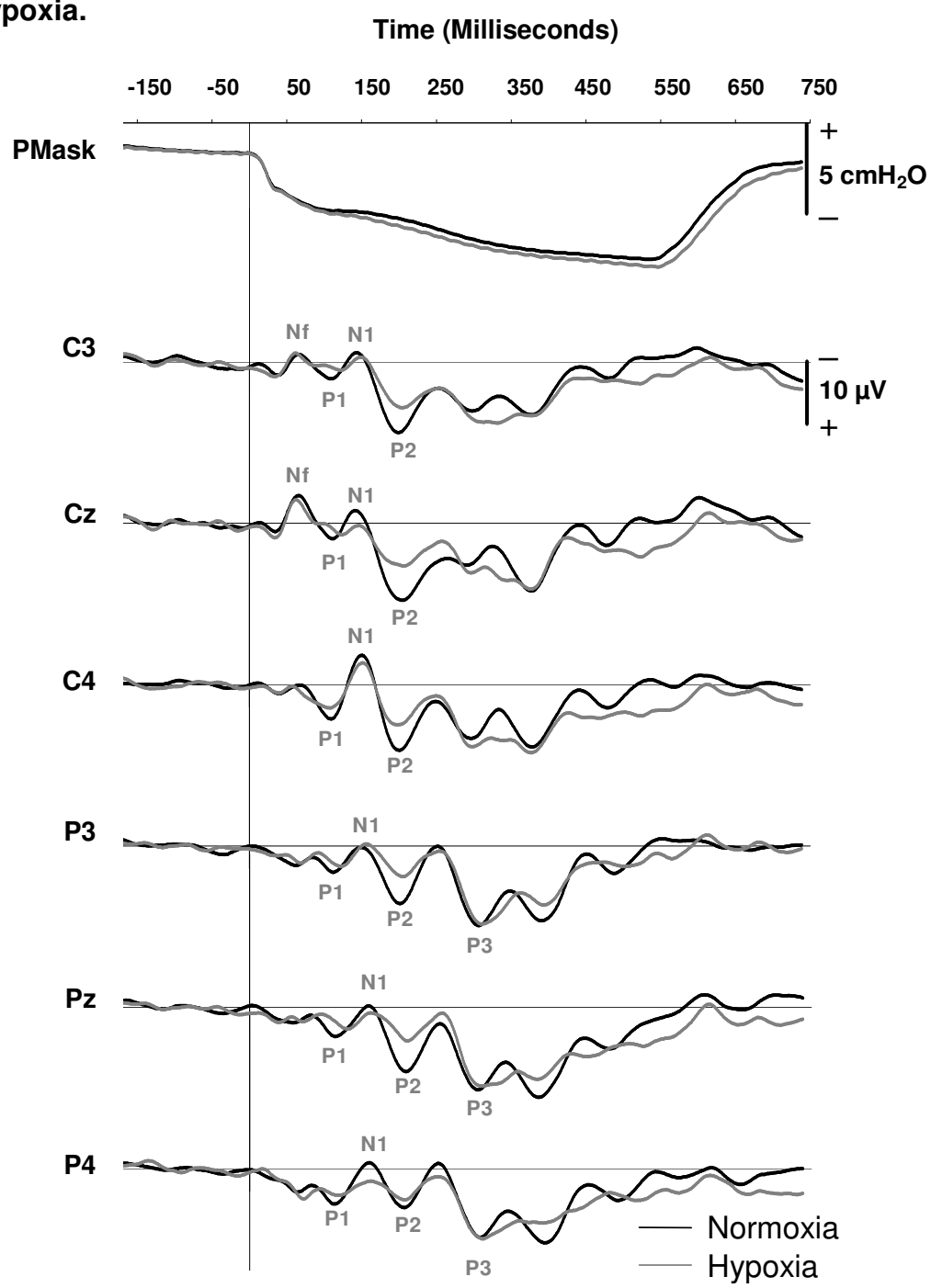
Resistance versus the perceived magnitude of externally applied resistive loads (ψ) during 30 minutes of targeted ventilation with isocapnic normoxia and isocapnic hypoxia (C) and recovery periods (D). * represents a significant decrease in ψ during hypoxia compared to normoxia ($P<0.01$). Values are means ± SEM, N=11.

Figure 8 RREP Group Average Waveforms during Target Ventilation and Recovery



Group mean average respiratory related evoked potential waveforms at Cz and Pz and stimulus magnitude during target ventilation periods (3x 20 min periods combined) during isocapnic normoxia and isocapnic hypoxia (A) and during recovery periods (3x 15 min periods combined) (B). Differences between normoxia and hypoxia; * $p<0.05$, † $p<0.01$. N=11.

Figure 9 Example Tracing in One Individual Subject at Each of the Measured Scalp Electrode Sites during Target Ventilation with Normoxia and Hypoxia.



Average stimulus intensity (Pmask) and RREP waveform responses at each of the measured scalp electrode sites in one individual subject during target ventilation with normoxia and hypoxia

2.4 Discussion

The main finding of this study was that in healthy subjects hypoxia resulted in decreased amplitudes of the early P1 and P2 components of the RREP to resistive load stimuli during conditions confirmed to produce a reduction in the perceived magnitude of respiratory resistive loads. These comparisons during hypoxia and normoxia were obtained during carefully matched conditions of ventilatory output and consequently respiratory stimulus magnitude. The findings provide further evidence that sustained hypoxia leads to impaired sensations of respiratory load and importantly, show that these effects are not mediated solely by higher centre cortical cognitive depression. The suppression of the early P1 component of the RREP suggests that acute hypoxia likely depresses respiratory afferent neural transmission low in the neuraxis.

These data extend earlier observations during free breathing trials in healthy individuals and asthmatic subjects (33, 208). The decrements in respiratory load perception in the current study were comparable to those observed in previous experiments providing strong support that the background pattern of ventilation *per se* is not responsible for hypoxia-induced sensory impairment. While this agrees with other reports of suppressed respiratory sensation to a variety of sensory stimuli during sustained hypoxia (301-303), it contrasts with the recent work of Moosavi and colleagues (295). These investigators demonstrated sensations of “air hunger” during 20 minutes of isocapnic hypoxia in healthy individuals when ventilation was constrained using a mechanical ventilator (295). Visual analog

scores of air hunger assessed at 30-second intervals were found to remain proportional to ventilatory drive (measured on another occasion during unrestrained breathing) throughout hypoxic ventilatory roll off. Thus, although sustained hypoxia caused ventilatory depression, presumably due to hypoxia-induced CNS inhibitory effects (3), this was not reflected in a disproportionate depression of “air hunger”. While the reasons for these seemingly disparate results are unclear, we speculate it may relate to different susceptibilities of various respiratory afferent pathways to hypoxia. Sensations of “air hunger” are likely mediated by a mismatch between respiratory afferent and efferent signals, perhaps reflecting corollary discharge from the respiratory pattern generator itself (293, 334). Corollary discharge neural pathways may remain relatively intact during hypoxia, potentially serving as a last line of defence during asphyxial insults. Other respiratory sensations such as load perception mediated by different afferent pathways (e.g. airway, lung or chest wall receptors) may be more vulnerable to suppression by hypoxia.

2.4.1 Respiratory Afferent Neural Transmission and Respiratory Load Perception

Dyspnea and the perception of increased respiratory load are highly integrated neural responses arising from the stimulation of a variety of respiratory sensory receptors. Upon presentation of an external resistive load to breathing, as in this study, breathing effort is reflexively augmented to compensate, respiratory muscle tension increases and more negative inspiratory pressures are generated in the pleural space and airways. Together these changes have the potential to stimulate

UA mechanoreceptors and a variety of respiratory pump receptors (258, 270). Activated respiratory sensory receptors relay electrical impulses via various myelinated and unmyelinated afferents (including the vagus, phrenic, hypoglossal and laryngeal nerves) to the central nervous system. While direct cortical projections may exist, numerous brain stem structures likely receive impulses from many of these primary sensory afferents, including structures such as the NTS. Activated brainstem structures project to the primary somatosensory cortex, either directly, or via the thalamus (445). The higher centre processing of summated afferent information relative to motor output and previous experience is believed to underlie what subjects cognitively perceive as “increased load”.

2.4.2 Potential Mechanisms for Hypoxia-Induced Suppression of Respiratory Afferent Transmission and Load Perception

There are two main possible explanations for our findings. In response to a sustained reduction in oxygen availability there is a central accumulation of neuroinhibitory modulators including adenosine, γ-aminobutyric acid (GABA) and endogenous opioids (38). The presence of these modulators may act to suppress respiratory sensory synaptic transmission and therefore decrease awareness of intensifying respiratory load. An alternate explanation is that acute hypoxia actively triggers a “central inhibitory network” within the midbrain and brain stem. This mechanism has recently been proposed to contribute to hypoxic ventilatory depression (3). In the same way that specific neuronal networks may inhibit

ventilatory motor output they could potentially inhibit respiratory sensory afferent pathways and contribute to impaired sensations of respiratory load.

A reduction in P1 amplitude during hypoxia suggests that there is a reduction in neural transmission of respiratory afferent information to the cortex. Should inhibitory neuromodulators accumulate widely throughout the nervous system in response to sustained hypoxia, respiratory sensory depression could occur at multiple levels. Primary muscle spindle function has been shown to be impaired during hypoxia in cats (446). Additionally, the dorsal central column of primary afferent fibres within the spinal cord appears susceptible to the inhibitory effects of hypoxia in rats (447). Thus, sensory information processing could be impaired as low in the neurosensory axis as the level of the primary receptor.

However, the CNS is known to exhibit a rostral to caudal vulnerability to hypoxia and rostral structures may be more prominent in mediating these effects. Neurons within the pons and thalamus, which may be actively recruited as part of a "hypoxic-induced inhibitory network", may be involved (3). Indeed, various hypoxic sensitive neurons may actively inhibit caudal sensory projections at one or at multiple levels. The NTS in the medulla also appears to be sensitive to the effects of hypoxia and may play an important role (53, 54). GABA, which appears in part to mediate hypoxic respiratory depression, is augmented within the NTS during sustained hypoxia (53). The NTS receives afferent fibres from the vagus and glossopharyngeal nerves by way of the tractus solitarius, and the caudal two-thirds of the nucleus processes afferent neural activity originating in the pharynx and

larynx (445). GABA and potentially other neuroinhibitory modulators and transmitters within the NTS may not only suppress respiratory motor output but also a wide range of respiratory afferent pathways projecting to this nucleus. Axons from the NTS project to the thalamus (445) which acts as a relay to direct sensory traffic for a variety of sensory stimuli to higher centres (primary somatosensory cortex). During sustained hypoxia increased thalamic gating may decrease respiratory sensory transmission to higher centers. In support of this hypothesis, the red nucleus within the mid brain, recently identified as an anatomical location activated during inspiratory resistive loading (313), is highly sensitive to hypoxic inhibition (3).

The functional significance and neural genesis of the second positive (P2) peak of the RREP remains poorly understood (243). The latency and preservation of the P2 peak in both stimulus attend and ignore conditions suggest that it reflects a combination of exogenous and endogenous processes. Earlier work in non-respiratory sensory modalities incorporated the P2 as part of a N1-P2 complex. However, this approach has recently been challenged in a review on the subject by Crowley and Colrain (243). These authors suggest P2 has an independent sensory role and highlight the need for future studies to elucidate its genesis and functional significance. Given the current level of understanding, interpretation of the P2 findings in this study must be treated with caution. While there were no between gas differences in N1-P2 amplitude, hypoxia-induced decreases in P2 amplitude in the current study may be indicative of suppression of early perceptual processing. An alternate possibility is that reduced P2 amplitude during hypoxia may have been a carry over effect from the earlier reduction in P1 amplitude rather than a separate

independent effect. In support of this possibility there was a tendency toward an overall negative shift in the RREP during hypoxia from ~100 to 250 msec.

It was somewhat unexpected that P3 amplitude was not also depressed in the current study. While it is possible that the later processing components of respiratory sensory information are not affected by hypoxia, several other methodological factors may explain this finding (see below).

2.4.3 Methodological Considerations

In this study subjects were required to voluntarily target their ventilation. While this was essential in order to match respiratory stimuli between gas conditions it unavoidably introduced differences in volitional versus chemoreceptor drive. Even though these differences were likely minimal, particularly after the first five minutes when hypoxic ventilatory drive declines, behavioural and attention dependent responses such as P3 may have been affected. While these differences do not likely affect earlier exogenous components (236), later components such as P3 during the target ventilation arm of this study should be treated with caution.

During the RREP arm of this study, subjects were asked to attend to and mentally count the number of pulses presented in order to facilitate P3 responses. P3 amplitude appears to be a sensory threshold dependent phenomenon. In the current study, RREP stimuli were well in excess of sensory threshold and were easily discernable with little attention. Had subjects been presented with a range of

resistive load magnitudes during the RREP protocol and been asked to differentiate load magnitude between presentations, gas related differences in P3 may have been detected. Hypoxic-induced P3 amplitude reductions and increased P3 latency in non-respiratory sensory modalities (hearing and vision) support this hypothesis (448, 449). These experiments did not incur the need to volitionally match stimuli between gas conditions as in this experiment.

In this study we did not observe statistically significant deficits in external load perception or reductions in RREP responses in the post-hypoxia period. In contrast, in a previous study we found reductions in symptoms of bronchoconstriction in asthmatics in the 10 minute post-hypoxia period (33). There was however, a tendency for reduced load magnitude perception and to a lesser extend P1 and P2 amplitudes to be decreased post-hypoxia in the current study. Given the longer recovery time frame necessary to collect sufficient trials for meaningful RREP analysis combined with uncertainty regarding the time-course of recovery from hypoxic effects, it is possible that shorter-term post-hypoxia effects were missed due to type II error combined with averaging effects over a longer recovery time.

Alternatively, while bronchoconstriction and external resistive loading may share some common mechanisms there may be important differences in afferent stimulation with differing susceptibilities to hypoxia. Finally, asthmatics show respiratory sensory processing deficits (309) which may render them more susceptible to hypoxia-induced sensory impairment.

While we hypothesize that the deficits in early RREP components and load perception during hypoxia are caused by suppression of respiratory sensory pathways, it is possible that hypoxia may have altered respiratory mechanics and the nature of the respiratory stimulus during brief respiratory loads. However, we believe that this is an unlikely explanation of our findings. The level of hypoxia administered in our experiments appears unlikely to alter respiratory mechanics (i.e. airway caliber or respiratory function) (22, 23) and certainly we did not find differences in the magnitude or pattern of inspiratory mouth pressure generated during brief resistive loads.

2.4.4 Summary and Clinical Implications

In summary, the main findings of this study were that acute sustained hypoxia reduced P1 and P2 amplitudes of the RREP and decreased the sensation of respiratory loading. Mechanistically, in addition to a potential role for impaired cognitive processing, the reduced amplitude of P1 in the RREP suggests that primary respiratory afferent neural transmission may be impaired by hypoxia.

Should hypoxia disrupt respiratory afferent neural transmission (and/or higher cortical processing) in hypoxic respiratory disease, as the findings in this study suggest, several vital protective respiratory responses could potentially be adversely affected. These might include arousal and neuromuscular compensation to increased respiratory load during sleep and important reflexes such as cough. We recently found, for example, that hypoxia caused an increase in arousal

threshold in sleep to inspiratory resistive loads (450). Hypoxia-induced depression of respiratory load sensation could lead to treatment delays and adverse outcomes in conditions such acute exacerbations of chronic lung disease and acute life threatening asthma.

CHAPTER 3. ACUTE SUSTAINED HYPOXIA SUPPRESSES THE COUGH REFLEX IN HEALTHY SUBJECTS

3.1 Introduction

The cough reflex is one of several defensive respiratory reflexes that are important for protecting the lungs from inhalation or aspiration of potentially injurious substances and for clearing excess secretions. A blunted cough reflex can be harmful or even fatal in the presence of severe respiratory disease (345, 349). Any factor that impairs the function of this vital protective response has the potential to increase disease severity.

Acute hypoxia is a common feature of conditions such as pneumonia and exacerbations of chronic obstructive lung disease in which cough is an important protective reflex. Recent studies have demonstrated that acute hypoxia suppresses the perception of respiratory load, which may contribute to treatment delay (33, 208). Impairment of respiratory afferent neural transmission below the level of the cortex appears to contribute to this depression (451). This finding raises the possibility that acute hypoxia may impair a range of protective respiratory reflexes that are subserved by subcortical structures e.g. brainstem nuclei involved in respiratory reflex integration. Respiratory brainstem neural networks and those responsible for modulating cough share common neuronal pathways such as the nucleus tractus solitarii (NTS) (362). In the same way that subcortical structures

mediate hypoxia-induced respiratory depression (3, 38) and potentially depression of respiratory load sensation (451), cough may be similarly depressed during hypoxia. In support of this hypothesis, animal studies suggest that the cough reflex is impaired during acute hypoxia (373, 379). Should this also be the case in humans, it may contribute to a progressive clinical worsening in hypoxic-respiratory diseases.

This study aimed to test the hypothesis that acute sustained hypoxia in healthy subjects impairs the cough threshold and results in more rapid adaptation (tachyphylaxis) of the cough reflex. Inhaled capsaicin was chosen as the provocative agent as it reproducibly produces cough in a dose dependent fashion, and has been used extensively to examine the efficacy of antitussive agents and to explore physiological differences in cough reflex sensitivity in healthy subjects and patient groups (345, 366, 367, 370). Changes in inspiratory flow may strongly influence cough reflex characteristics via flow related changes in aerosol deposition throughout the airway and/or the potential role of increased activation of slowly adapting pulmonary stretch receptors via lung inflation at higher inspiratory flows (373-375). Consequently, to avoid confounding influences of hypoxic ventilatory stimulation on cough reflex sensitivity, cough reflex characteristics were compared during normoxia (baseline drive) and hypoxia (increased drive) at matched levels of mild voluntary hyperventilation.

3.2 Methods

3.2.1 Subject Selection

18 young healthy non-smokers, with no history of respiratory disease, current or recent (less than 2 months) upper respiratory tract infection and with baseline FEV_1 >80% predicted gave informed written consent to participate in the study. The study was approved by the Daw Park Repatriation General Hospital and Adelaide University Human Research and Ethics Committees.

3.2.2 Preliminary Visit

Pulmonary function testing, including spirometry and whole body plethysmography was performed to ensure normal lung function. Following lung function testing, subjects were fitted with a full face mask (ComfortFull™, Respironics, Murrysville, PA, USA) fitted with a pressure transducer (MP45, Validyne Engineering, Northridge, CA, USA) and connected to the breathing circuit (Figure 10). The nose was taped closed to ensure mouth breathing (Sleek, Smith and Nephew, London, UK). After ten minutes of room air baseline breathing each individual's ventilatory response to 15 minutes of isocapnic hypoxia (SaO_2 ~80%, POET II model 602-3, Criticare Systems, Waukesha, WI, USA) was recorded (451).

3.2.2.1 Target Ventilation Method

A target minute ventilation ($\dot{V}_I$) level was selected according to each individuals hypoxic ventilatory response (~190% above baseline) as described previously (451). Subjects practiced the targeted $\dot{V}_I$ task during the preliminary visit for 10-15 minutes by breathing via a reservoir filled at the target flow rate with humidified compressed air (Figure 10). Subjects were instructed to maintain a constant breathing frequency and tidal volume according to the predetermined levels via real-time feedback of the inspiratory volume trace on a computer monitor (Figure 10).

3.2.2.2 Assessment of Cough Reflex Threshold and Acute Tachyphylaxis

Cough challenge testing was performed at the preliminary visit for familiarization and to minimize "learning effects" in subsequent experiments (364, 371). Measurements were performed while subjects breathed at the target $\dot{V}_I$. A piezo electric sensor attached to the subjects neck (Sleepmate, Sleepmate Technologies, Midlothian, VA, USA) and a dictaphone recorded cough vibration and sound. Following an initial challenge with normal saline doubling doses of capsaicin aerosol (range 0.49-500 µM) were administered for the first 15-seconds of every minute until 5 or more coughs were elicited prior to the next dose as described previously (452). Once 5 or more coughs occurred during a 60 second period, the next incremental doubling dose of capsaicin was introduced continuously for one

minute to assess acute tachyphylaxis (371). A single observer, blinded to the gas condition, defined the presence of coughs as any brief expulsive event in mask pressure associated with excursion in the vibration channel accompanied by sound recordings indicative of cough.

3.2.3 Main Experimental Visits

Subjects attended the laboratory on 2 separate occasions, at the same time of day, approximately one week apart. Subjects abstained from alcohol and caffeine for at least 12-hours prior to each visit. The order of the two main visits (hypoxia or normoxia) was randomised between subjects via a coin toss, and subjects remained blinded to the experimental gas.

On each occasion, after 5 minutes of room air breathing, subjects were switched to the target $\dot{V}_I$ arm of the circuit (Figure 10) through which the experimental gas (compressed dry 9% O_2 in N_2, or medical air) was introduced. A manual inspiratory bleed of CO_2 was employed to ensure isocapnia. During hypoxia trials the inspired O_2 fraction was adjusted as necessary to maintain SaO_2 ~80% (451). After 30 minutes, cough threshold and acute tachyphylaxis were assessed as per the preliminary visit while subjects continued breathing at the targeted $\dot{V}_I$. During each experiment subjects listened to music through earphones. All measurements were performed while subjects were seated upright in a comfortable chair.

3.2.4 Data Analysis

The concentration of capsaicin required to elicit 2 (C2) and 5 (C5) coughs was determined using linear interpolation of log concentration-response curves for each test. Trials in which C2 could not be accurately determined because the subject coughed more than twice during the first capsaicin dose were excluded from between-gas comparisons of C2. To further characterize the cough dose-response curve, the linear regression slope (cough sensitivity) and ordinate intercept of the log dose versus number of coughs was calculated from all available data points between the first and last threshold doses. Trials in which C5 occurred on the first capsaicin dose were excluded from cough sensitivity analysis, as insufficient data were available to perform linear regression. Coughs during the final one minute of continuous capsaicin nebulization were counted and grouped in 10-sec bins to assess acute tachyphylaxis (371). Tachyphylaxis was defined as a reduction over time in the mean number of coughs evoked by capsaicin during the 60-sec of continuous capsaicin inhalation.

3.2.5 Statistical Procedures

Given that cough threshold challenge testing yields non-normally distributed data (366), related samples non-parametric tests (Mann-Whitney) were used to compare cough threshold measurements between gas treatments (SPSS version 12.1, SPSS Inc., Chicago, IL, USA). Repeated measures ANOVA was used to examine normally distributed data, including acute tachyphylaxis time, gas and gas-by-time

interaction effects, and to compare between-gas ventilatory parameters across study periods (baseline, target $\dot{V}_I$, cough threshold and tachyphylaxis) and gas-by-period interaction effects. Statistical significance was inferred when $p<0.05$. Data are reported as means ± SEM unless otherwise stated.

3.3 Results

3.3.1 Anthropometric Data

16 subjects (9 males) successfully completed all of the study requirements. The mean age and body mass index of the 16 subjects were 24.5± 1.0 years and 22.8± 0.6 $kg.m^{-2}$ respectively. Subjects had normal lung function (Mean FEV_1 109.2± 2.9, forced vital capacity 100.2± 3.2 and total lung capacity 104.9± 3.4 % predicted).

3.3.2 Ventilatory Data

Figure 11 displays $\dot{V}_I$, SaO_2 and $PETCO_2$ during baseline, targeted ventilation, cough threshold and cough tachyphylaxis periods in normoxia and hypoxia. $\dot{V}_I$ and $PETCO_2$ were well matched between gas conditions across each study period (Figure 11A and Figure 11C). There were no between gas differences in any ventilatory parameters except, by design, SaO_2 which was lower during hypoxia following the baseline period compared to normoxia (Figure 11B). With repeated coughing during the acute tachyphylaxis protocol there was a marginal decrease in $PETCO_2$ below baseline levels, but this occurred to the same extent under both gas

conditions (Figure 11C). $PETCO_2$ was slightly higher during the target $\dot{V}_I$ period compared to baseline but was not different from baseline during cough threshold testing (Figure 11C). As instructed, subjects achieved target $\dot{V}_I$ across the three targeted $\dot{V}_I$ periods by increasing peak inspiratory flow (36.5± 1.5 vs. 56.3± 2.9 $l \cdot min^{-1}$ $p<0.001$) and tidal volume (0.72± 0.04 vs. 1.1± 0.06 litres $p<0.001$) without changing breathing frequency (14.2± 0.5 vs.15.3± 0.6 breaths per minute $p=0.066$). There were no gas or gas by period interaction effects for these ventilatory parameters ($p \geq 0.315$).

3.3.3 Cough Threshold

The initial saline dose did not evoke coughing in any subjects. In contrast, capsaicin challenge evoked a dose-dependent increase in the number of coughs. The capsaicin dose required to elicit 5 coughs was significantly greater during hypoxia compared to normoxia (Figure 12A). Three subjects coughed 5 times on the first capsaicin dose during the normoxia protocol leaving 13 subjects available for paired cough sensitivity comparisons. Similar to C5 cough threshold, there were significantly fewer coughs per log increment dose of capsaicin during hypoxia versus normoxia (Figure 12B). The ordinate-intercept of the log-capsaicin dose versus the number of coughs was also significantly lower during hypoxia compared to normoxia (1.86± 0.68 vs. 3.28± 0.80, $p=0.004$). 8 of the 16 subjects coughed more than 2 times on the first capsaicin dose during at least one of the tests (3 during normoxia, 3 during hypoxia and 2 during both) leaving 8 subjects for paired

C2 comparison. C2 in the remaining 8 subjects was not different between hypoxia and normoxia (18.76± 6.87 vs. 14.15± 7.94μM, p=0.263).

3.3.4 Cough Tachyphylaxis

Given the increased C5 cough threshold during hypoxia, a significantly higher dose of capsaicin was administered for the 1 minute acute tachyphylaxis challenge during hypoxia compared to normoxia (68.9± 33.5 vs. 50.7± 31.7μM, p=0.018). However, the mean number of coughs elicited at the capsaicin dose producing 5 or more coughs (immediately prior to acute tachyphylaxis assessment) was not different between hypoxia and normoxia (6.6± 0.4 vs. 6.2± 0.3, p=0.535). During acute tachyphylaxis assessment the mean number of coughs elicited was maximal during the second 10-sec period of continuous capsaicin inhalation and decreased thereafter (Figure 13). Acute tachyphylaxis was evident by a significant analysis of variance time effect (p<0.001) and a reduction in the mean number of coughs in the last compared to first 10-seconds (1.3± 0.3 vs. 2.3± 0.3, p<0.001). However, the mean number of coughs elicited throughout the 60-seconds was not different between hypoxia and normoxia (12.5± 2.1 vs. 12.6± 1.5, p=0.938) and there were no gas by time interaction effects (p=0.821).

Figure 10 Schematic of the Breathing Circuit for Study 2 (Chapter 3)

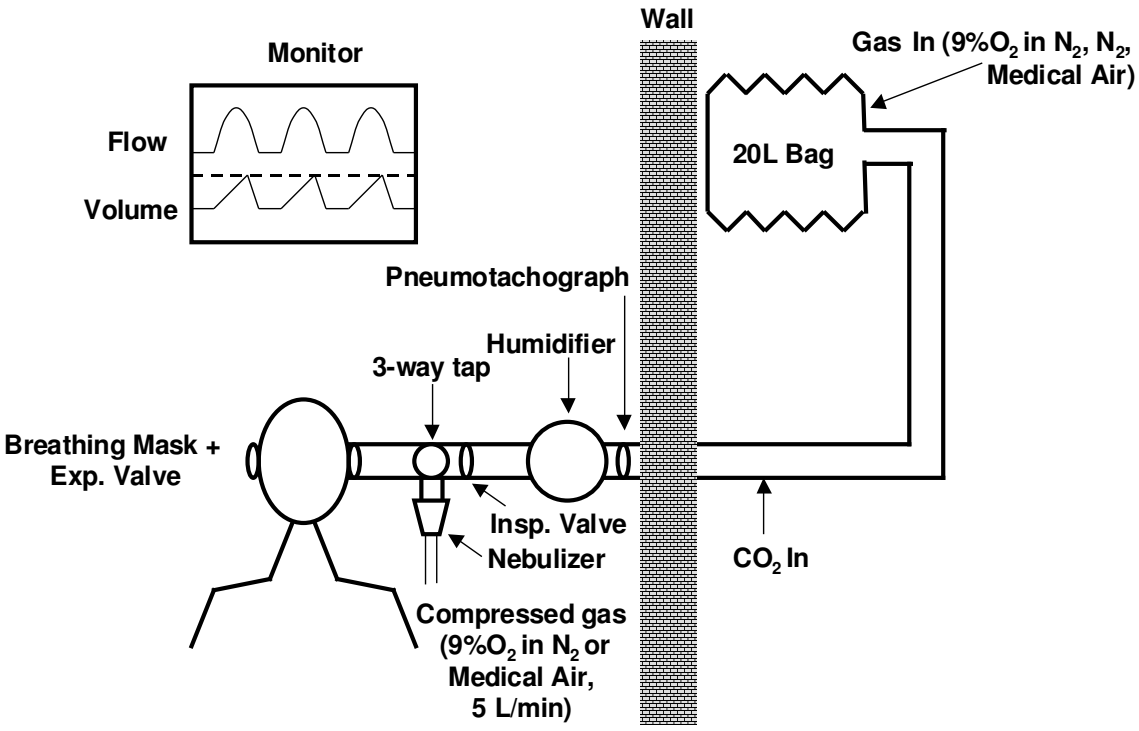
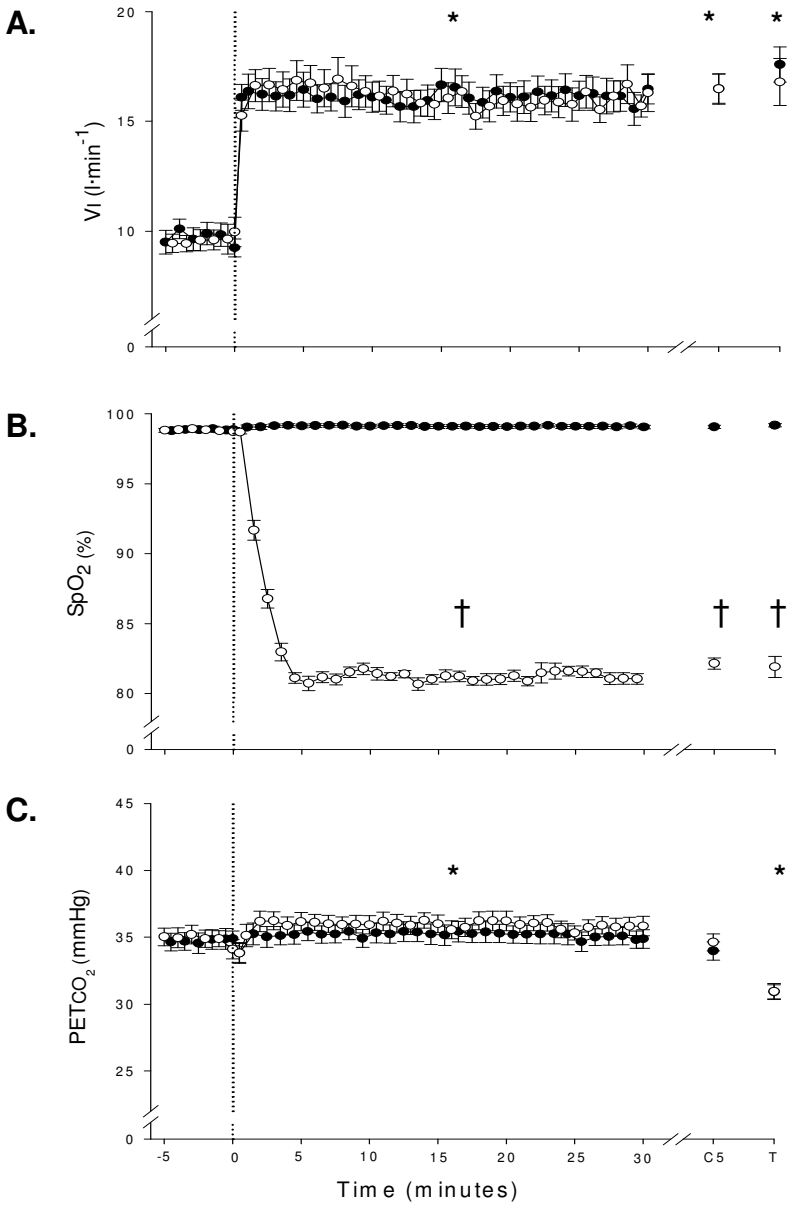
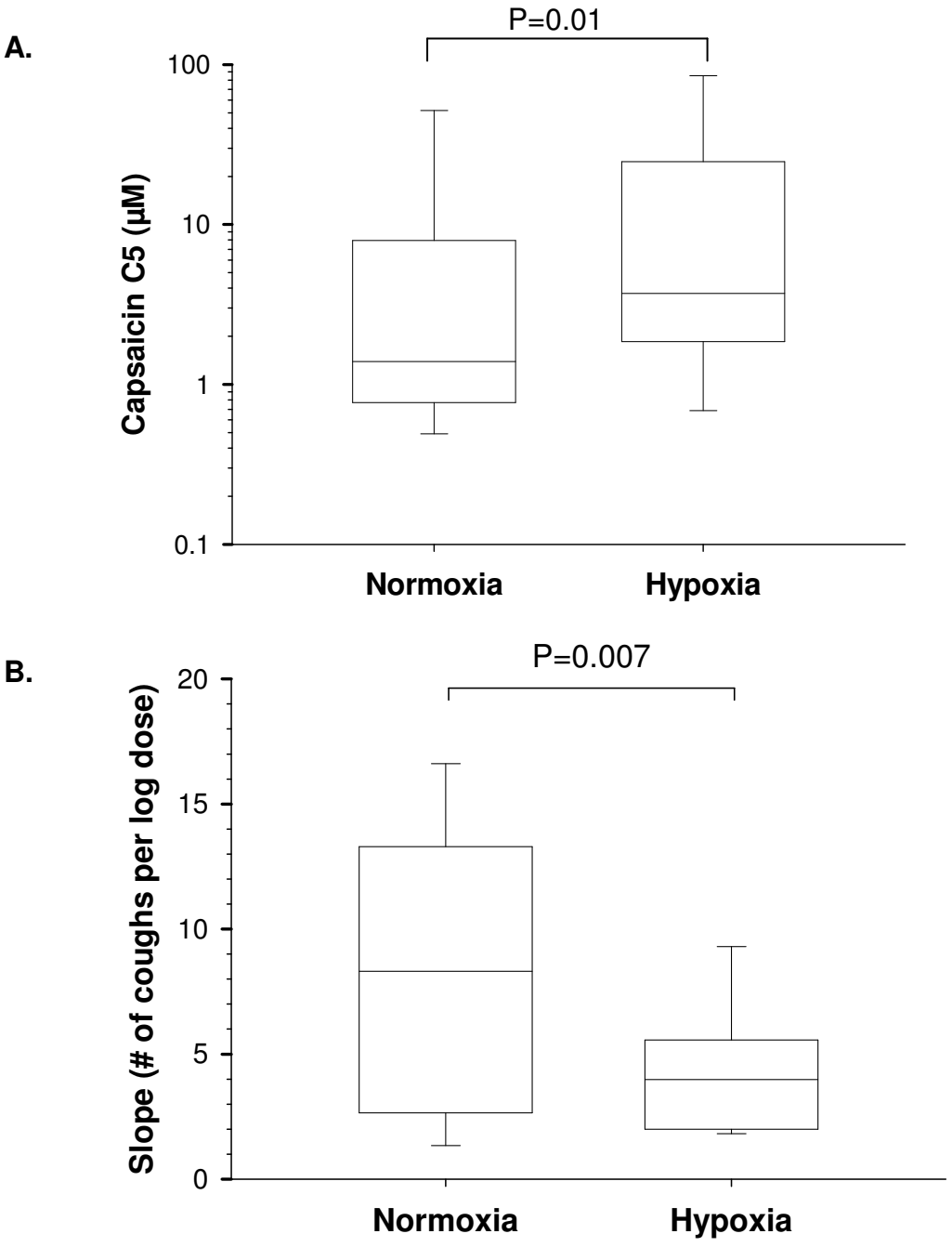


Figure 11 Group Average Ventilatory Parameters



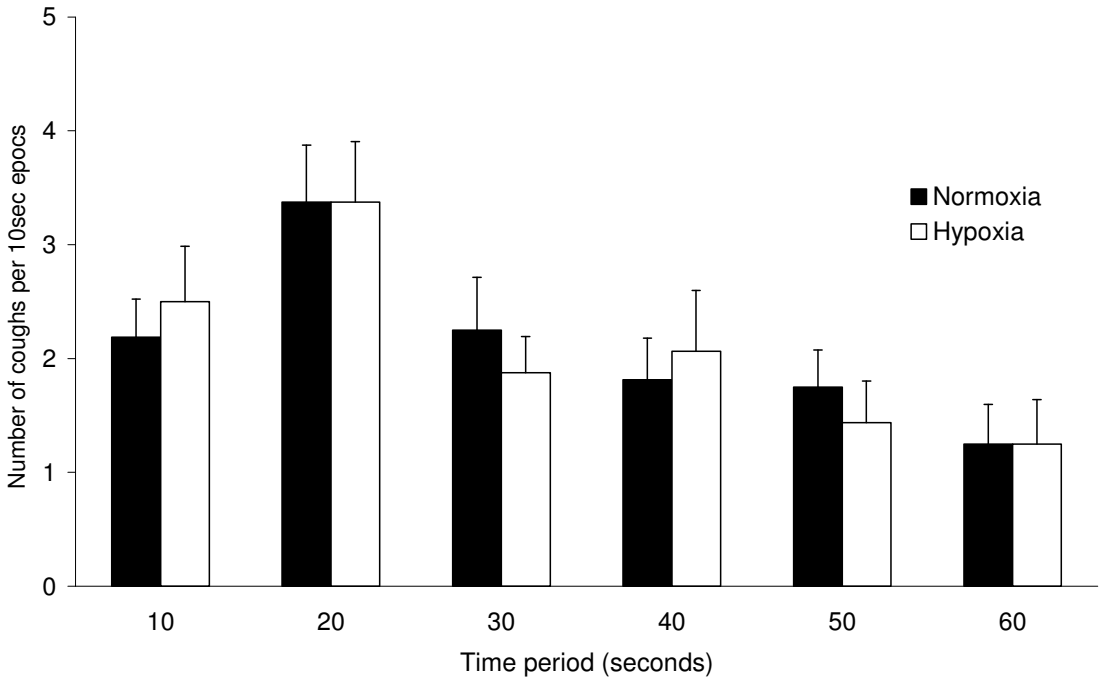
(A) Group minute ventilation, (B) arterial blood O_2 saturation and (C) end tidal carbon dioxide levels during the two main experimental visits. Data points are 30-second averages presented at alternating intervals between normoxia and hypoxia with the exception of C5 and T data points, which represent the mean value over the duration of cough threshold and acute tachyphylaxis periods respectively. * denotes a significant difference compared to baseline, † denotes a significant difference compared to normoxia. Values are means ± SEM, N=16.

Figure 12 Cough Threshold and Cough Sensitivity



Box plots (A) concentration of capsaicin required to elicit 5 coughs (C5) N=16 and (B) slope of log dose versus number of coughs relationship N=13. Values are medians, interquartile range and whiskers represent the 10th and 90th centiles.

Figure 13 Acute Tachyphylaxis



Number of coughs in each 10 sec interval during 1 min of continuous capsaicin inhalation during normoxia and hypoxia. Acute tachyphylaxis was confirmed by a significant ANOVA time effect, $p<0.001$. Values are means ± SEM. N=16.

3.4 Discussion

The main finding of this study was that acute sustained isocapnic hypoxia increased the C5 cough threshold to inhaled capsaicin and decreased the slope of the cough sensitivity relationship in healthy individuals. Cough tachyphylaxis to 1-min of continuous capsaicin inhalation was present but not different between gas conditions.

Despite recognition of the physiological and potential clinical importance of this issue (356, 373), there have been few studies conducted to investigate the effects of hypoxia on cough sensitivity. Tatar and colleagues demonstrated suppression of laryngeal and tracheobronchial cough to mechanical stimulation in cats during poikilocapnic hypoxia (379). Hypoxia-induced impairment was noted at both anatomical locations, however tracheobronchial cough appeared particularly vulnerable to suppression. While it is not possible to determine if tracheobronchial cough was preferentially down-regulated during hypoxia and if the effect of hypoxia to capsaicin-induced cough differs to mechanically-induced cough, the findings of decreased cough reflex sensitivity during hypoxia in the present study are in agreement with Tatar and colleagues. Two studies examined the effects of prolonged high altitude exposure (up to 1 month) on cough reflex sensitivity to citric acid, the latter under simulated altitude (382, 383). Both studies reported a small decrease in citric acid cough threshold at extreme altitude. The authors postulated that sub-clinical pulmonary edema and/or airway drying effects secondary to an altitude-induced increase in ventilation may have contributed to this effect (382,

383). While not specifically designed to examine the effect of hypoxia, and caution is warranted given the small sample size, the degree of hypoxia appeared to have no effect on cough threshold when examined using a linear regression model. However, potentially cough provoking effects of hypobaric hypoxia may have masked hypoxic cough suppression evident in the current study where ventilation and inspiratory flow were carefully controlled 30 minutes prior to and during normobaric hypoxia and normoxia provocation testing. However, it is also possible that capsaicin but not citric acid-induced cough is suppressed by hypoxia. Finally, the cough reflex exhibits plasticity (453, 454) and may be importantly influenced by the duration of hypoxia (acute versus chronic).

There has been some uncertainty as to the presence of cough tachyphylaxis to inhaled capsaicin. While not specifically designed to test tachyphylaxis, several studies have reported the absence of cough adaptation to capsaicin in adults (374, 455-457). Chang and colleagues (375) performed two repeated capsaicin cough challenges separated by 10 minutes in children, and noted a tendency towards an increase in cough threshold during the second test, although the difference was not statistically significant (375). In a systematic examination of the long and short term adaptation characteristics of the cough reflex to capsaicin and citric acid, Morice and colleagues observed marked tachyphylaxis to both cough-provoking stimuli, although adaptation appeared more prominent for citric acid than capsaicin (371). Using an acute tachyphylaxis protocol similar to that of Morice and colleagues (371), we observed a similar pattern of cough adaptation during capsaicin inhalation. However, the peak number of coughs occurred slightly later in the

current study. This variation is likely explained by differences in cough provocation delivery systems and inspiratory flow rates. Together, these studies suggest that acute tachyphylaxis of the cough reflex occurs during capsaicin inhalation, an effect which is likely to be dose dependent. The current study also suggests that acute sustained hypoxia does not influence the extent and time course of acute cough tachyphylaxis.

3.4.1 Possible Mechanisms contributing to Blunted Cough Reflex Sensitivity during Hypoxia

The underlying physiology of the sensory and central mechanisms responsible for activating the cough reflex to various cough provoking stimuli remains under investigation (354-357). Briefly, sensory information from stimulation of the afferent nerve endings capable of producing cough is relayed to the NTS via the vagus nerve. The neural origin of the various respiratory-muscle contractions producing cough is believed to originate in the medulla (361). Down-regulation of the cough reflex during hypoxia could be the result of impairment at one or more levels along the cough reflex arc.

The CNS is believed to have rostral to caudal sensitivity to the depressant effects of hypoxia (38, 447). This supports a centrally located origin for hypoxia-induced depression of cough e.g. brainstem. In support of this hypothesis, the NTS appears particularly sensitive to hypoxia and has been proposed to be a key mediator of hypoxic ventilatory depression via a γ-aminobutyric acid (GABA) mediated pathway

(53). All vagal respiratory sensory afferent neurons mediating cough relay through the NTS. Thus, this is potentially a primary site for hypoxia-induced down-regulation of cough, perhaps via elaboration of GABA at this site. The GABA agonist baclofen has been shown to decrease cough sensitivity to capsaicin in healthy individuals (458). This action is believed to be largely centrally mediated, although a peripheral depressant action of GABA is also possible (459). Hypoxia has also been shown to increase CNS levels of endogenous opioids (38), and some opiate receptor agonists have been shown to have antitussive properties via inhibition of the central component of cough (460). Cough can also be voluntarily suppressed, highlighting the role of inhibitory cortical projections to the cough neural network (363). Central activation of inhibitory pathways or down-regulation of facilitatory pathways to the cough neuronal network may contribute to impaired cough reflex sensitivity in what may be a part of a hypoxia-sensitive "central inhibitory network" (3).

While central depressant effects may play a key role in mediating down-regulation of cough during hypoxia, a role for peripheral depression cannot be excluded. Respiratory afferent neural transmission appears to be suppressed below the level of the cortex during acute hypoxia in healthy individuals (451). This finding raises the possibility that respiratory sensory depression may occur as low down in the neurosensory axis as the primary sensory nerve ending. Indeed, primary receptor function has been shown to be impaired during hypoxia in other receptor systems (446).

3.4.2 Methodological Considerations

Unlike C5 cough threshold, the C2 cough threshold was not different between gas conditions. This most likely reflects a type II error given the reduced sample size for this comparison and that the linear regression slope (cough sensitivity) derived across all measured capsaicin doses was significantly reduced during hypoxia. Similarly, a lack of between gas difference in cough tachyphylaxis could reflect type II error. Indeed, to detect a significant between gas difference of the magnitude observed in this study with 80% power would have required in the order of 42 subjects.

$PETCO_2$ was not precisely controlled at eucapnic levels during targeted ventilation and tachyphylaxis periods. However, this is unlikely to effect the main conclusions given that $PETCO_2$ was not different from baseline during cough threshold testing and modest differences during targeted ventilation and tachyphylaxis periods were not different between gas conditions. In addition, although the presence of capsaicin-induced cough was defined in a manner consistent with the literature (364), it is possible that some of the expiratory events identified as cough may have been other expiratory reflexes.

Finally, whist we hypothesize that down-regulation of the cough reflex during hypoxia occurs due to sensory and/or central depression, it remains possible that provocant deposition and/or respiratory and airway mechanics were affected by hypoxia. However, these would appear to be unlikely explanations for our findings.

Firstly, by design, inspiratory flow, breath timing and volume were matched between gas conditions. Secondly, while one study reported a small dilatory effect of hypoxia on airway caliber, attributed to changes in ventilatory pattern from normoxic conditions (29), most studies have shown no effect of hypoxia on respiratory function or airway mechanics (22, 23).

3.4.3 Summary and Clinical Implications

This study has demonstrated that acute sustained hypoxia depresses cough reflex sensitivity to inhaled capsaicin in healthy individuals. This finding raises the possibility that vital protective respiratory defensive mechanisms may be impaired during acute exacerbations of hypoxic-respiratory disease, such as pneumonia, bronchiectasis and COPD. Several studies have emphasized how an absent or blunted cough reflex may render patients vulnerable to increased morbidity and mortality (349-351).

Although acute cough serves as a fundamental protective mechanism, chronic cough can be a problematic symptom and is one of the most common reasons for patients to seek medical attention (344). Several studies have demonstrated that cough sensitivity is heightened during periods of respiratory disease, which may be reversed upon recovery (345, 346). To date there have been no studies conducted in acutely hypoxic patients. Although cough is likely further influenced by disease, the results of this study in healthy individuals suggest that acute hypoxia may impair the cough reflex.

CHAPTER 4. THE EFFECTS OF HYPOXIA ON RESPIRATORY REFLEXES AND RREPs DURING SLEEP

4.1 Introduction

There are numerous protective respiratory reflexes that are activated during acute periods of increased respiratory load or airway occlusion. The genioglossus (EMGgg) is the largest UA dilator muscle and it is reflexively augmented in response to negative UA pressure to oppose UA collapse. Previous studies suggest that this response is largely attenuated during sleep thereby contributing to the development of sleep-disordered breathing in individuals with an anatomically narrow airway (397, 398, 400). However, recent data demonstrating maintenance, and in many individuals increased reflex activity to negative pressure pulse stimuli in the supine position during NREM sleep, suggest that EMGgg reflex attenuation is position dependent (401).

Unlike the stretch or loading responses in limb muscles, which consist of reflex excitation without inhibition (437-439), the response of several human inspiratory muscles (i.e. scalene, parasternal intercostal and diaphragm) to a sudden increase in respiratory load is an initial inhibition followed by an apparent subsequent excitation (431-433). Inspiratory muscle reflex inhibition has been proposed to play a protective role in preventing further inhalation of an object causing airway

obstruction and decreasing the likelihood of UA collapse to increased negative airway pressure (433). In conditions characterised by periods of increased respiratory load such as asthma and OSA the duration of sudden, load induced reflex inhibition is increased (434, 436). Increased peak inhibition latency and the duration of inhibition measured during wakefulness correlate with the severity of sleep disordered breathing in OSA patients (436).

Prolonged intermittent hypoxia is a feature of both obstructive and central sleep apnea. Sustained hypoxia also occurs during sleep hypoventilation syndrome. Hypoxia has long been known to have depressant effects on respiration and cognition (38, 122). More recently, several studies have demonstrated that hypoxia can also lead to impairment of a range of other vital protective responses including respiratory load sensation, arousal from sleep to respiratory stimuli and the cough reflex (33, 208, 450, 461). As measured by a reduction in P1 amplitude of the RREP, depression of respiratory afferent transmission below the level of the cortex appears, at least in part, to mediate hypoxia-induced decrements in respiratory load sensation (451). Together, these findings suggest that many protective respiratory reflexes that are subserved by subcortical structures, e.g. brainstem nuclei involved in respiratory reflex integration, may be vulnerable to suppression during hypoxia.

By applying brief pulses of negative airway pressure during wakefulness and sleep in healthy individuals in the supine posture this study aimed to test the following hypotheses: 1) hypoxia suppresses the EMGgg UA negative pressure reflex 2) hypoxia impairs inspiratory muscle reflex responses and 3) hypoxia reduces the

early components of the RREP. Most previous human studies of the EMGgg negative pressure reflex have employed a moving time averaging technique to quantify the rectified EMGgg that could potentially obscure the true nature of the reflex EMG response. Thus, a secondary aim of this study was to describe in detail the morphology of EMGgg response to a negative pressure pulse using the ensemble averaged rectified but otherwise unprocessed EMG signal.

4.2 Methods

4.2.1 Subject Selection

21 young healthy non-smoking males, without a history of respiratory disease, sleep-disordered breathing, or medication use and with baseline FEV_1 >80% predicted gave informed written consent to participate in the study. The study was approved by the Daw Park Repatriation General Hospital and Adelaide University Human Research and Ethics Committees.

4.2.2 Measurements and Equipment

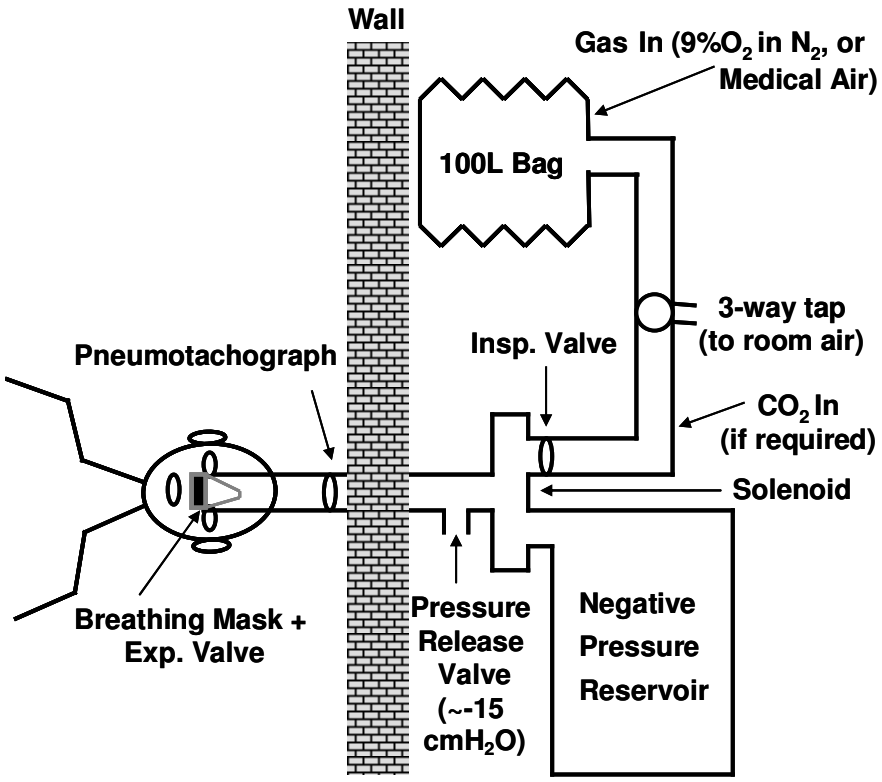
Subjects were instrumented with EEG (C3 and C4 referenced to linked ears), left and right electrooculograms and submental electromyogram for sleep staging and arousal scoring. In addition, EEG electrodes at Fz, Cz and Pz were applied to record RREPs. Electrodes were reapplied as necessary until impedance at each site was less than 5 kOhms. The nostrils were decongested with xylometazoline

hydrochloride nasal spray (Otrivin, Novartis Australasia, Rowville, Victoria, Australia) and anaesthetised (2% lignocaine). Two custom made air-perfused catheters (see reference (462) for further detail) were inserted via the most patent nostril and attached to pressure transducers (MP45, Validyne Engineering, Northridge, CA, USA). One catheter was advanced 1-2 cm below the base of the tongue under direct visualization (P_{epi}) the other to the level of the choanae (P_{cho}). Two fine wire Teflon coated intramuscular electrodes (316SS3T wire, Medwire, Mt Vernon, NY, USA) were inserted 4 mm either side of the frenulum to a depth of approximately 1-1½ cm after surface anaesthesia (4% lignocaine) to measure genioglossus EMG activity (EMG_{gg}). These procedures were similar to those previously described (394, 415). Surface electrodes were also placed overlying scalene, parasternal intercostal and diaphragm muscles as described previously (433). Subjects were fitted with a nasal mask (Gel mask, Respironics, Murrysville, PA, USA) with a two-way non-rebreathing valve attached (series 2600, Hans Rudolph, Kansas City, MO, USA). An additional pressure transducer was fitted to the mask (P_{mask}) and the expirate was continuously sampled to determine the end-tidal partial pressure of CO_2 ($PETCO_2$). SaO_2 (POET II model 602-3 Criticare Systems, Waukesha, WI, USA) and ECG was measured continuously. A pneumotachograph (PT36, Erich Jaeger, Germany) in series was used to record ventilatory parameters. UA negative pressure pulses (P_{mask} ~-10 cmH_2O, 250 ms) were delivered during early inspiration via a computer-controlled rapid actuating solenoid valve system (Iso star, SXE9575-A70-00, Norgren, Switzerland). Figure 14 is a schematic of the breathing circuit. Negative pressure pulse delivery was controlled via custom written software to ensure no pulses were presented during

QRS activation which would otherwise result in contamination of surface EMG recordings.

Data were acquired simultaneously on two separate recording systems. The first system (Compumedics E series, Abbotsford, Victoria, Australia) was used to determine sleep stage and to score arousals. All other data were acquired using the Windaq data acquisition system (DI 720 DATAQ Instruments Inc, OH, USA). In order to capture fast frequency reflex components and synchronise key stimulus magnitude parameters for event related analysis, inspiratory flow, ECG, EMG and pressure channels were sampled at 2 kHz and filtered at 30-1000 Hz. EEG for RREP measures (Fz, Cz, and Pz) were also sampled at 2 kHz and filtered at 30-100 Hz. The remaining channels that were not directly used for reflex and event related timing purposes were sampled at 200Hz. An event mark was simultaneously placed on both recording systems coincident with solenoid activation of each pulse allowing both data acquisition systems to be accurately linked in time.

Figure 14 Schematic of the Breathing Circuit for Study 3 (Chapter 4)



4.2.3 Protocol

4.2.3.1 Preliminary Visit

Initially, subjects attended a preliminary visit during the day for familiarization with the testing environment, recording equipment and staff and to obtain informed consent. Spirometry was performed to ensure normal lung function (JLab software version 4.53; Compactlab, Jaeger™, Wuerzburg, Germany).

4.2.3.2 Main Experimental Visits

On two separate occasions, approximately one week apart, subjects arrived at the laboratory 2.5 hours prior to their usual bedtime. Subjects abstained from alcohol and caffeine for at least 12-hours prior to each visit. Once all the equipment was fitted several negative pressure pulses were delivered for familiarization. Subjects were then asked to lie on their back, to keep their eyes open and stay awake for 10 minutes (for baseline wakefulness data collection) after which the lights were switched off and subjects were given the opportunity to sleep. During the night in the event that the subject became uncomfortable maintaining the supine posture they were given the opportunity to stretch before returning to sleep on their back.

After at least 5 mins of stable stage 2 sleep subjects were randomly allocated to breathe either normoxic (medical air) or isocapnic hypoxic (compressed dry ~11% O_2 in N_2) gas mixtures throughout the night. During hypoxia trials the inspired O_2

fraction was adjusted to maintain SaO_2 ~85% and a manual inspiratory bleed of CO_2 was employed as necessary to ensure isocapnia. Subjects remained blinded to the test gas condition. Following 15 mins of sleep under each gas condition UA negative pressure pulses were delivered every 2-10 breaths during stable sleep. In the event of an arousal, pulses were ceased until there was at least 1 minute of arousal free sleep. In the event that the subject woke during the night the subject was given a 5 minute opportunity to return to sleep while the experimental gas remained on. However, if the subject was unable to return to sleep within 5 minutes the subject was switched back to room air. Once stable sleep was achieved the subject was returned to the experimental gas condition and pulses recommenced following at least 10 minutes of stable sleep after returning to breathing the experimental gas. Upon awakening the following morning, the test gas remained on and approximately 50-60 pulses were delivered every 2-10 breaths during wakefulness to elicit EMG reflex and RREP responses.

4.2.4 Data analysis

A single trained sleep technician, blinded to the gas condition, defined the presence of arousals and sleep stage according to standard criteria (463, 464). Similarly, a single observer blinded to the gas condition identified the presence of K-complexes to brief pulses of negative pressure according to previously described criteria (306, 463). Custom designed software to detect the most rapid change in P_{mask} during pulse presentation was employed to align each individual pulse to an accurately identifiable and highly reproducible reference point for EMG and RREP event

related analyses. Briefly, on breaths pre-identified as having a negative pressure pulse presented, the software identified the point in P_{mask} at which the change in pressure with respect to time (i.e. slope) was most negative. Stimulus onset latency (time zero) was defined in the conventional manner as the last point preceding the sudden decrement in P_{mask} following solenoid activation. Negative pressure pulse stimulus magnitude was calculated as the minimum pressure after the initial "ringing" observed in the pressure channels (Figure 15).

For each subject, EMG and EEG trials free from movement artefact and arousal were grouped and ensemble averaged by sleep stage (wake, NREM and REM) for the normoxia and hypoxia visits. Raw EMG recordings were rectified without moving time averaging for each subject. All artefact free trials, including those with clearly defined single motor unit activity, were then ensemble averaged. Individual subject's averaged RREP waveform at each of the scalp electrode sites and the ensemble averaged rectified EMG reflex responses were visually inspected to identify, using custom designed semi automated software, the presence, timing and amplitude of each positive and negative component. Examples of typical EMGgg and EMGsc reflex responses and the criteria used to define the various reflex characteristics are displayed in Figure 15. EMG reflex amplitude data were expressed as a percentage of baseline calculated as the average EMG activity for the 100 milliseconds preceding pulse onset as described previously (436, 440). Excitation onset was defined as the point at which the rectified EMG signal crossed baseline prior to the clearly defined positive EMG waveform. Inhibition/suppression onset was defined as the first point at which the rectified EMG recording crossed

the baseline level following the peak of the excitation response if present. The first point at which the rectified EMG returned to baseline levels after the inhibition/suppression nadir was used to define the cessation of inhibition/suppression and the onset of the secondary excitation for EMGsc responses. Where available, EMGgg single motor unit discharges were examined during and immediately prior to negative pressure pulse application. The baseline activity of a motor unit was defined as the average firing frequency of the unit for the 100 ms immediately prior to negative pressure pulse onset. The firing frequency of the unit for the first and second impulse after pulse onset was also calculated. In order to perform group comparisons, baseline, first and second impulse firing frequency of 6 negative pressure pulses selected at random were averaged in each subject demonstrating clearly identifiable single motor unit EMGgg activity.

Ventilatory parameters were calculated on a per breath basis using custom designed software. Ventilatory parameters at the time of negative pressure pulse stimuli were derived for each subject by averaging values from the breath immediately preceding each pulse presentation.

4.2.5 Statistical Procedures

ANOVA for repeated measures were used to examine gas (hypoxia versus normoxia), state (wakefulness and sleep) and interaction effects for EMG reflex peak amplitudes and timing characteristics. ANOVA for repeated measures were also used to examine gas (hypoxia versus normoxia), electrode site (Fz, Cz and

Pz) and interaction effects for RREP waveform amplitudes and latencies. To explore the effect of NREM sleep on the early RREP components (Nf, P1, N1 and P2) ANOVA for repeated measures were used to compare gas (hypoxia versus normoxia) state (wakefulness and NREM sleep) and interaction effects at the electrode sites of maximal activation for each peak. Similarly, ventilatory parameters across study periods (wake versus sleep and between gas conditions) were explored using ANOVA for repeated measures. Where main ANOVA effects were observed, post-hoc comparisons were performed using Dunn-Sidak adjusted Student's paired t-tests (444). Statistical significance was inferred when $p<0.05$. All data are reported as means ± SEM.

4.3 Results

4.3.1 Anthropometric Characteristics and Sleep Architecture

A total of 7 subjects did not complete all of the study requirements. Four subjects had insufficient sleep on the first experimental visit (3 during hypoxia, 1 during normoxia) and were excluded from further participation. One subject slept poorly on their second visit (hypoxia) and was unable to return for a repeat visit. One subject demonstrated significant sleep-disordered breathing on the first visit (normoxia). One other subject successfully completed the first visit (normoxia) but was unable to return for their final visit. Thus, 14 subjects successfully completed all of the study requirements. The mean age and the body mass index of these 14 subjects were 24.1± 1.6 years and 24.2± 0.8 $kg.m^{-2}$ respectively. Subjects had normal lung

function (Mean FEV_1 102.2± 3.9 and forced vital capacity 106.8± 3.6 % predicted). All 14 subjects were able to successfully sleep in the supine posture for the entire data collection period. The background resistance of the breathing circuit was 2.5± 0.02 $cmH_2O \cdot l^{-1} \cdot s$. There were no differences in sleep architecture variables between gas conditions in the 14 subjects that completed both arms of the study (Table 4).

4.3.2 Ventilatory Characteristics

The ventilatory characteristics immediately prior to pulse presentation during wakefulness and NREM sleep are displayed in Table 5. By design SaO_2 was significantly lower during hypoxia experiments. There were no other significant gas or gas by state interaction effects in any other ventilatory parameter. During NREM sleep, $\dot{V}_I$ and V_{TI} were significantly reduced compared to wakefulness. P_{ETCO_2} levels increased and there was a marginal increase in F_B from the waking level. PIF remained unchanged from wakefulness levels (Table 5).

4.3.3 Reflex Responses to Brief Pulses of Negative Pressure

4.3.3.1 Genioglossus Negative Pressure Reflex

EMGgg reflex data during wakefulness under both gas conditions was not available in 3 subjects. In one subject there were insufficient replicate trials to generate rectified EMG reflex responses as post hoc sleep staging revealed the subject

spent the majority of the wakefulness period in stage 1 sleep. In two subjects one of the EMGgg intramuscular electrodes was dislodged prior to wakefulness measures (1 during a cough upon waking in the morning, the other upon removal of the mouth tape in the morning). Of the remaining 11 subjects, negative pressure pulse stimuli during wakefulness resulted in a short latency peak followed by prolonged suppression of the rectified EMGgg in the normoxia and hypoxia experiments. This pattern of reflex EMG response was maintained during NREM sleep in all 14 subjects under both gas conditions. Phasic EMGgg activity was observed in all of these subjects. Examples of the morphology of the EMGgg reflex response in two subjects during wakefulness and NREM sleep are displayed in Figure 16A. The number of stimulus presentations, peak reflex amplitudes, timing, stimulus properties during wake and NREM sleep are summarised in Table 6. The initial peak occurred earlier during NREM sleep compared to wakefulness under both gas conditions. After the initial peak phase there was a suppression of EMGgg amplitude below baseline that was significantly greater during NREM sleep compared to wakefulness. Stimulus magnitude was marginally larger during NREM sleep compared to wakefulness as measured by mask and choanal pressures (Table 6). Epiglottic pressure catheters were prone to blockage and did not provide reliable recordings in most subjects. Of the limited data available under both gas conditions, stimulus intensity at the level of the epiglottis was similar during normoxia and hypoxia during wakefulness (-6.7± 1.1 vs. -5.7± 0.9 cmH_2O p=0.969; n=4 subjects) and NREM sleep (-8.2± 0.6 vs. -8.3± 1.9 cmH_2O p=0.968; n=2 subjects). There were no differences in EMGgg reflex component amplitudes or latencies between gas conditions.

An example of the firing frequency of a prominent single EMGgg motor unit during and immediately prior to a single negative pressure pulse is displayed in Figure 18. Single motor unit activity was observed in 6 subjects. The average baseline firing frequency and the average of the first and second impulses after stimulus onset for 6 separate pulses per subject are displayed in Table 7. The firing frequency of the first impulse after negative pressure pulse onset did not change from baseline (p= 0.290) whereas the firing frequency of the second impulse was significantly reduced (Table 7).

Sufficient REM sleep to present negative pressure pulse stimuli was achieved in 5 subjects under both gas conditions. While replicate trials were limited (normoxia n= 6.5± 1.6 versus hypoxia n= 9.2± 2.2, p=0.913) the predominant reflex response was a prolonged period of suppression with (2/5) or without (3/5) any preceding excitation. Indeed, the suppression often approached complete silencing of the electromyogram. The rectified EMGgg reflex responses in two individual subjects during REM sleep are displayed in Figure 17. ANOVA for repeated measures exploring state (wake, NREM and REM) and gas (normoxia and hypoxia) effects revealed a significant decrease in genioglossus peak suppression amplitude from wake to NREM to REM sleep (63.3± 4.8 vs. 39.3± 8.9 vs. 16.0± 2.0 % of baseline respectively, p= 0.002). There were no other significant state, gas or interaction effects in reflex peak amplitudes or latencies for comparisons incorporating REM data.

4.3.3.2 Inspiratory Muscle Reflex Responses

Similar to other reports (436, 440), the signal to noise ratio for surface electrode recordings for EMGdi and EMGic was insufficient to discern reflex responses in this study. EMGsc reflex responses were reliably observed in 10 of the 14 subjects during wakefulness and NREM sleep under both gas conditions. In many instances the response consisted of reflex inhibition followed by excitation. The number of stimulus presentations, peak reflex amplitudes, timing, stimulus properties during wake and NREM sleep for the inhibition and subsequent excitation components are summarised in Table 8. The latency to the peak of the inhibitory response, the latency to the onset of the subsequent excitatory response and the latency to the peak of the excitatory response were significantly delayed during hypoxia compared to normoxia. Accordingly, the duration of EMGsc reflex inhibition was also significantly prolonged during hypoxia compared to normoxia. There was a marginally significant state effect for inhibition amplitude ($p=0.049$) whereby inhibition was most pronounced during wakefulness. There was a significant state by gas interaction effect for peak inhibition amplitude such that inhibition amplitude was most pronounced during hypoxia in wakefulness. Inhibition duration was greater during wakefulness compared to NREM sleep. The subsequent excitation onset also occurred later during wakefulness compared to NREM sleep ($p=0.044$, Table 8). There were no other state, gas or interaction effects for EMGsc reflex characteristics. There were insufficient replicate trials to generate EMGsc reflex responses during REM sleep.

In addition to the inhibition and subsequent excitation responses an initial short latency increase in EMGsc activity prior to the inhibition period was clearly observed in 2 of the 10 subjects during wakefulness and NREM sleep under both gas conditions. This initial increase in EMGsc activity was also present in other subjects, but its presence across experimental conditions was inconsistent. Nevertheless, a discernable increase in EMG activity above baseline levels was observed in 50% of subjects during normoxia and hypoxia trials during wakefulness. In NREM sleep it was observed in 80% of subjects during normoxia studies and 70% of subjects during hypoxia experiments. Where present, the amplitude and timing characteristics were quantified using the same criteria used for the initial EMGgg peak (Figure 15A). The onset latency of the initial increase in EMGsc activity for the overall average (normoxia, hypoxia wake and NREM sleep combined) of all cases where a peak was present was 26.4± 1.7 milliseconds. The overall peak amplitude was 165.5± 1.7 % baseline and occurred at 32.4± 1.6 milliseconds. This initial peak component was of short duration (13.3± 1.1 milliseconds). Examples of EMGsc reflex responses in two subjects during wakefulness and NREM sleep are displayed in Figure 16B.

4.3.4 RREPs

An average of 52.5± 3.2 pulses during normoxia and 51.3± 2.9 pulses during hypoxia (p=0.705) free from movement artefact were used to generate RREP waveforms at each scalp electrode site per subject during wakefulness. An average of 72.2± 7.5 pulses during normoxia and 67.8± 9.5 pulses during hypoxia (p=0.513)

free from movement artefact were used to generate RREP waveforms at each scalp electrode site per subject during NREM sleep. RREP waveform amplitudes and latencies to negative pressure pulse stimuli at electrode sites where activation was maximal during wakefulness and NREM sleep are displayed in Table 10 and Table 11 respectively. Group average RREPs during wakefulness and NREM sleep at Cz are displayed in Figure 19. N1 latency was significantly delayed during hypoxia compared to normoxia during wakefulness (Table 10). P2 amplitude was also significantly reduced during hypoxia compared to normoxia during wakefulness and NREM sleep (Table 10 and Table 11 respectively). There were no other gas related differences in RREP amplitudes or latencies. The early Nf peak, maximal at Fz, occurred slightly earlier during wakefulness compared to NREM sleep (46.5± 1.2 versus 49.6± 0.8 ms, p=0.005). The P2 peak, maximal at Cz, occurred later during wakefulness compared to NREM sleep (218.3± 12.3 versus 157.7± 3.7 ms, p=0.001). The amplitude of this peak was not different between wake and NREM sleep (30.4± 2.5 versus 39.4± 3.8 μv, p=0.090). The amplitude of the N1 deflection was significantly more pronounced during wakefulness compared to NREM sleep (-12.2± 2.6 versus 5.8± 2.2 μv, p=0.001). There were no other latency or amplitude differences between wakefulness and NREM sleep for the early RREP components at the electrode sites where activation was maximal. While replicate trials were limited during REM sleep an example of the RREP at Cz during REM sleep displayed in Figure 20 shows that RREP morphology appears similar to wakefulness.

Arousal probability to negative pressure pulse stimuli decreased progressively from stage 1 to SWS (Table 9). The proportion of negative pressure pulses that elicited arousal from NREM sleep was not different between normoxia and hypoxia ($p=0.834$, Table 9). Arousal probability during REM sleep was not different to any of the other sleep stages in the limited data available ($p \geq 0.102$) nor were there differences between gas conditions during REM ($p=0.298$). The likelihood of eliciting a K complex increased progressively from stage 1 to SWS (Table 9). K complex probability was significantly less during REM sleep compared to stage 2 and SWS but was not different compared to stage 1 ($p= 0.117$). The majority of negative pressure pulse presentations elicited a K complex during NREM sleep but the frequency of pulse-induced K-complexes was not different between normoxia and hypoxia in any stage of sleep (p=0834, Table 9).

Table 4 Sleep Architecture Data

	Normoxia Experiments	Hypoxia Experiments	p value
SOL (minutes)	19.4± 5.8	18.6± 2.4	0.906
TST (minutes)	241.2 ± 11.6	246.9± 7.7	0.633
Sleep efficiency (%)	70.9± 3.6	67.7± 2.8	0.402
Stage 1 (% TST)	11.9± 5.2	14.9± 3.4	0.319
Stage 2 (% TST)	52.7± 2.9	56.3± 2.6	0.319
SWS (% TST)	30.2± 3.8	25.1± 3.2	0.096
REM (% TST)	5.2± 1.3	3.7± 0.9	0.241
AI (arousals·hr^{-1})	21.6± 2.5	23.3± 3.8	0.634

Sleep onset latency (SOL), total sleep time (TST), slow wave sleep (SWS), rapid eye movement sleep (REM) and arousal index (AI). Data are means± SEM. N=14.

Table 5 Group Mean Ventilatory Characteristics Immediately Prior to Stimulus Presentation during Wakefulness and NREM Sleep

	Normoxia Experiments		Hypoxia Experiments	
	Wake	**NREM**	**Wake**	**NREM**
$\dot{V}_I$ **(l·min^{-1})**	9.3 ± 0.3	7.1 ± 0.3†	9.6 ± 0.5	7.8 ± 0.4†
V_{TI} **(l)**	0.76± 0.06	0.51 ± 0.03†	0.78 ± 0.04	0.53 ± 0.02†
F_B **(b·min^{-1})**	13.4 ± 0.5	14.2 ± 0.5†	13.1 ± 0.4	14.5 ± 0.6†
PIF (l·min^{-1})	30.6 ± 1.5	28.9 ± 3.1	32.4 ± 2.1	28.9 ± 2.1
P_{ETCO_2} **(Torr)**	41.7 ± 0.8	45.2 ± 0.7†	39.7 ± 0.9	44.1 ± 0.7†
SaO_2 **(%)**	97.9 ± 0.2	97.6 ± 0.1	86.2 ± 0.5*	85.9 ± 0.2*

Minute ventilation ($\dot{V}_I$), inspiratory tidal volume (V_{TI}), breathing frequency (F_B), peak inspiratory flow (PIF), end-tidal CO_2 (P_{ETCO_2}) and arterial blood oxygen saturation during wakefulness and NREM sleep. Data are means± SEM for the breath immediately prior to pulse onset. * denotes a significant difference compared to normoxia. † denotes significant difference compared to wakefulness. (N=14).

Table 6 Effect of Hypoxia on EMGgg Reflex Characteristics to Negative Pressure Pulse Stimuli during Wake and NREM Sleep

	Normoxia Experiments		Hypoxia Experiments	
	Wake	**NREM**	**Wake**	**NREM**
Excitation Phase				
Onset latency (ms)	24.9 ± 1.6	21.6 ± 1.4	27.1 ± 1.3	23.1 ± 2.0
Peak amplitude (% baseline)	236.4 ± 36.1	205.7 ± 14.2	226.0 ± 34.5	193.3 ± 9.3
Peak latency (ms)	36.7 ± 2.0	32.0 ± 2.0*	38.0 ± 1.4	34.2 ± 1.7*
Duration (ms)	24.2 ± 2.5	18.0 ± 1.6	22.3 ± 2.3	21.2 ± 2.1
Suppression Phase				
Onset latency (ms)	41.0 ± 2.3	33.8 ± 2.3	39.6 ± 2.7	38.2 ± 1.4
Nadir amplitude (% baseline)	67.4 ± 6.1	47.1 ± 4.5*	63.0 ± 5.6	41.7 ± 4.4*
Nadir latency (ms)	69.6 ± 5.0	64.2 ± 0.8	71.9 ± 9.0	66.9 ± 1.7
Duration (ms)	40.9 ± 7.4	40.9 ± 2.1	45.9 ± 9.5	40.2 ± 3.7
Stimulus Properties				
Pmask (cmH_2O)	-9.4 ± 0.3	-10.5 ± 0.4*	-9.1 ± 0.2	-10.3 ± 0.3*
Pcho (cmH_2O)	-8.5 ± 0.5	-9.3 ± 0.7*	-8.1 ± 0.4	-9.3 ± 0.6*
# Artefact free pulse presentations	56.6 ± 2.8	72.8 ± 7.5	53.1 ± 1.7	67.4 ± 7.6

* denotes significant difference compared to wakefulness. Values are means ± SEM. Data are presented only for the subjects in whom values for all the measured variables were available under all conditions (N=11).

Table 7 EMGgg Single Motor Unit Firing Frequency during Negative Pressure Pulse Stimuli

	Genioglossus Single Motor Unit Firing Frequency		
Subject #	**Pre-Stimulus Baseline (Hz)**	**First Post-Stimulus Impulse (Hz)**	**Second Post-Stimulus Impulse (Hz)**
1	17.5 ± 1.0	19.2 ± 1.4	12.8 ± 0.6
2	20.1 ± 1.5	20.5 ± 1.4	17.5 ± 1.7
3	18.4 ± 0.7	21.2 ± 1.9	12.1 ± 0.6
4	10.1 ± 0.3	9.5 ± 1.2	9.3 ± 0.8
5	22.7 ± 0.9	22.3 ± 1.2	17.1 ± 0.9
6	26.0 ± 2.2	25.5 ± 2.8	9.5 ± 0.5
Mean	19.1 ± 2.2	19.7 ± 2.2	13.0 ± 1.5*†

The average firing frequency of genioglossus single motor units during 6 pulse presentations per subject immediately prior and the first and second impulses after pulse onset respectively. * denotes a significant difference compared to baseline. † denotes significant difference compared to first post-stimulus impulse.

Table 8 Effect of Hypoxia on EMGSc Reflex Characteristics to Negative Pressure Pulse Stimuli during Wake and NREM Sleep

	Normoxia Experiments		Hypoxia Experiments	
	Wake	NREM	Wake	NREM
Inhibition Phase				
Onset latency (ms)	35.1 ± 3.2	36.1 ± 1.9	33.0 ± 2.7	39.3 ± 3.3
Nadir amplitude (% baseline)	62.4 ± 2.6	62.8 ± 4.0	50.0 ± 3.4†‡	61.5 ± 2.8*
Nadir latency (ms)	63.8 ± 8.5	60.1 ± 3.7	86.6 ± 5.5†	78.4 ± 3.6†
Duration (ms)	36.0 ± 7.5	35.0 ± 5.0	62.7 ± 8.6†	47.5 ± 5.0*†
Excitation Phase				
Onset latency (ms)	76.2 ± 9.4	71.7 ± 5.3	109.4 ± 8. 9†	90.7 ± 3.1*†
Peak amplitude (% baseline)	153.8 ± 8.0	145.3 ± 6.8	171.3 ± 9.7	154.3 ± 12.8
Peak latency (ms)	111.1 ± 11.0	109.3 ± 7.6	135.8 ± 10.8†	153.6 ± 21.0†
Stimulus Properties				
Pmask (cmH_2O)	-9.7 ± 0.3	-10.8 ± 0.4*	-9.1 ± 0.3	-10.5 ± 0.3*
Pcho (cmH_2O)	-8.0 ± 0.6	-10.3 ± 0.5*	-8.1 ± 0.5	-10.1 ± 0.4*
# Artefact free pulse presentations	54.9 ± 3.7	69.8 ± 8.9	52.3 ± 2.0	60.0 ± 8.4

* denotes significant difference compared to wakefulness. † denotes significant difference compared to normoxia. ‡ denotes a significant gas by state interaction effect. Values are means ± SEM. Data are presented only for the subjects in whom values for all the measured variables were available under all experimental conditions (N=10).

Table 9 Arousal and K Complex Probability to Negative Pressure Pulse Stimuli during Sleep

	Normoxia Experiments				Hypoxia Experiments			
	Stage 1	**Stage 2**	**SWS**	**REM**	**Stage 1**	**Stage 2**	**SWS**	**REM**
Arousal (%)	64.0± 10.2†‡	31.1± 7.2*‡	7.5± 2.9*†	32.1± 12.4	69.6± 9.3†‡	29.3± 5.6*‡	8.6± 2.9*†	8.3± 4.0
K Complex (%)	38.6± 10.6†‡	75.8± 6.4*‡§	87.9± 5.8*†§	4.7± 3.7†‡	35.8± 12.5†‡	76.9± 5.5*‡§	90.3± 5.4*†§	4.6± 3.0†‡

Percentage of negative pressure pulses that elicited arousal from sleep and K complexes during sleep. * denotes a significant sleep stage effect compared to stage 1; † stage 2; ‡ slow wave sleep (SWS) and § rapid eye movement sleep (REM). Data are means± SEM. N=14.

Table 10 Effect of Hypoxia on RREP Mean Amplitude and Latency Data at Maximal Sites of Activation during Wakefulness

Nf Maximal at Fz		P1 Maximal at Cz		N1 Maximal at Cz		P2 Maximal at Cz		P3 Maximal at Pz	
normoxia	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**
Amplitude (µv)									
-7.0 ± 0.9	-6.7 ± 1.0	12.6 ± 1.6	13.3 ± 2.1	-13.5 ± 3.1	-12.7 ± 2.0	32.9 ± 2.6	27.9 ± 2.9*	23.5 ± 3.4	22.3 ± 3.2
Latency (ms)									
46.4 ± 1.4	46.6 ± 1.2	81.2 ± 4.1	81.0 ± 3.7	122.9 ± 5.5	130.4 ± 5.3†	219.6 ± 13.0	217.1 ± 11.9	286.5 ± 11.3	283.5 ± 11.7

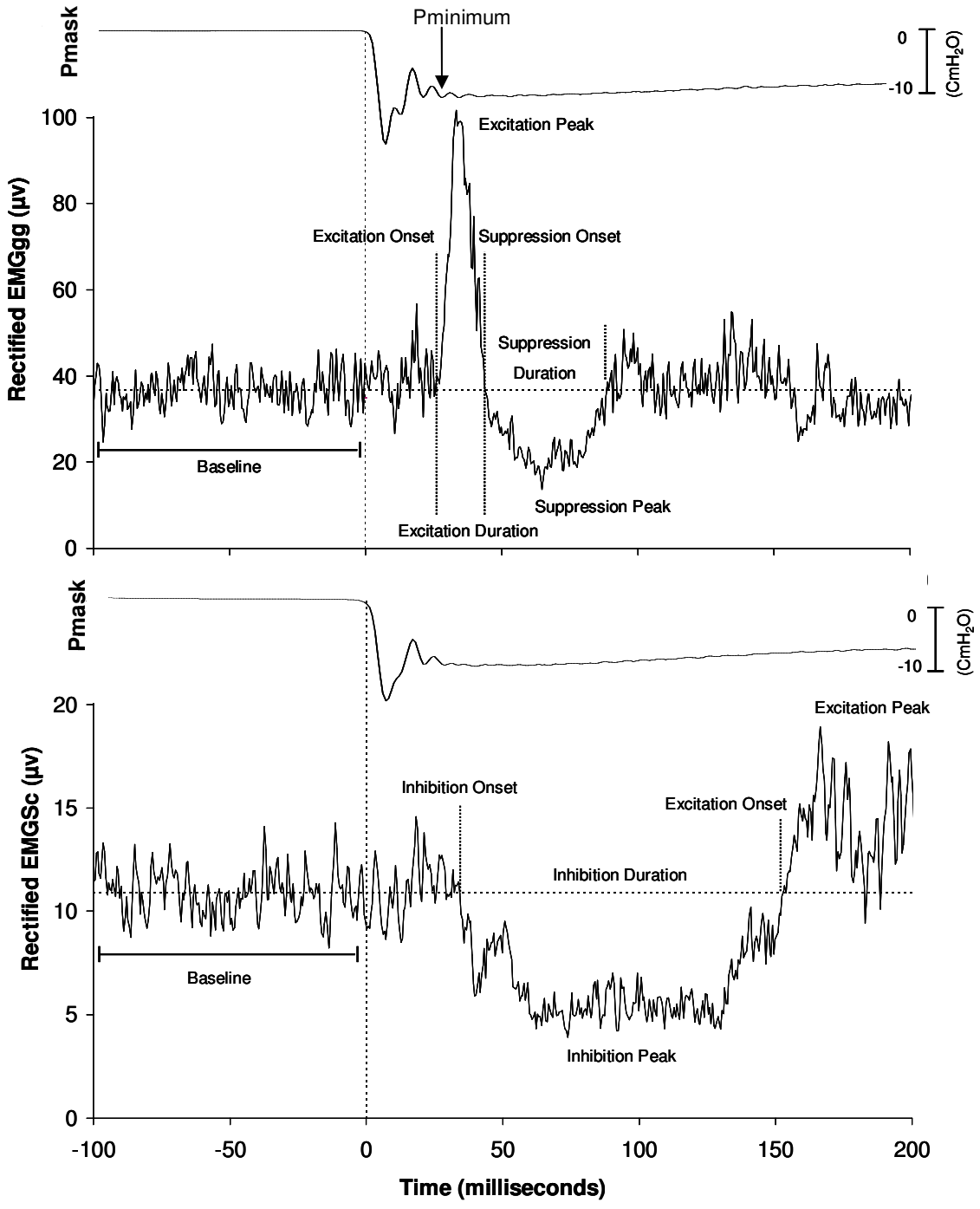
Respiratory related evoked potential mean amplitude and latency data at sites of maximal activation during isocapnic hypoxia and normoxia during wakefulness. Values are means ± SEM. N=14 subjects for all peaks except P3 which was present in 11 of the 14 subjects. Difference between normoxia and hypoxia; * $p<0.05$, † $p<0.01$.

Table 11 Effect of Hypoxia on RREP Mean Amplitude and Latency Data at Maximal Sites of Activation during NREM Sleep

Nf Maximal at Fz		P1 Maximal at Cz		N1 Maximal at Cz		P2 Maximal at Cz		N550 Maximal at Fz	
normoxia	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**	**normoxia**	**hypoxia**
Amplitude (µv)									
-5.6 ± 0.9	-6.7 ± 2.0	12.8 ± 1.8	9.6 ± 3.0	8.8 ± 2.2	2.7 ± 3.2	43.1 ± 3.6	35.6 ± 4.6*	-130.0 ± 14.4	-138.9 ± 13.8
Latency (ms)									
49.2 ± 0.9	50.0 ± 0.9	88.6 ± 4.3	88.8 ± 5.3	107.1 ± 2.6	107.0 ± 3.4	156.1 ± 4.9	159.4 ± 3.0	456.6 ± 15.1	470.3 ± 15.4

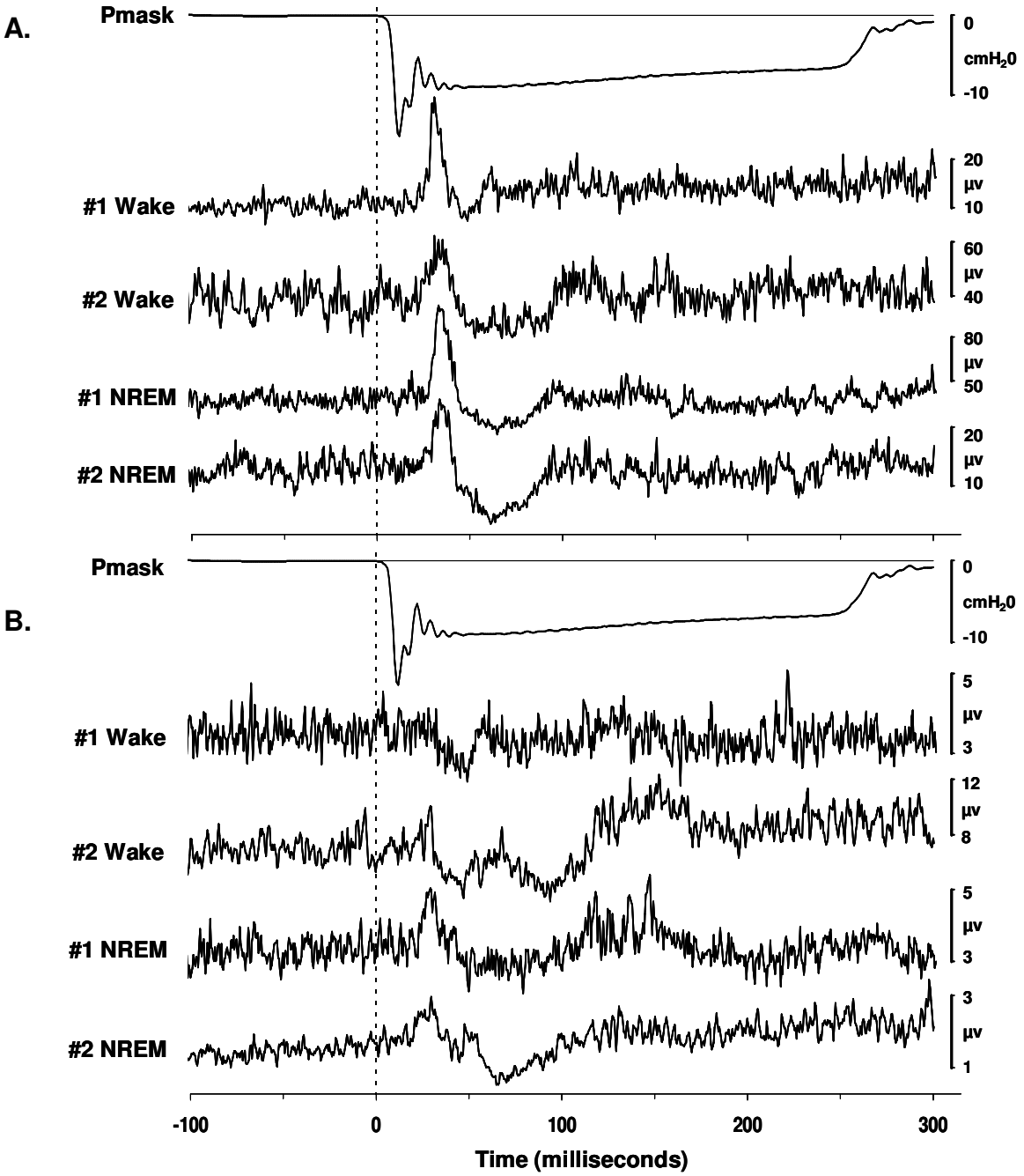
Respiratory related evoked potential mean amplitude and latency data at sites of maximal activation during isocapnic hypoxia and normoxia during NREM sleep. Values are means ± SEM. N=14 subjects for all peaks except P1 and N1 which were present in 10 of the 14 subjects. * denotes a significant difference between normoxia and hypoxia; $p<0.05$

Figure 15 Rectified EMGgg and EMGSc Reflex Peak and Latency Characterisation



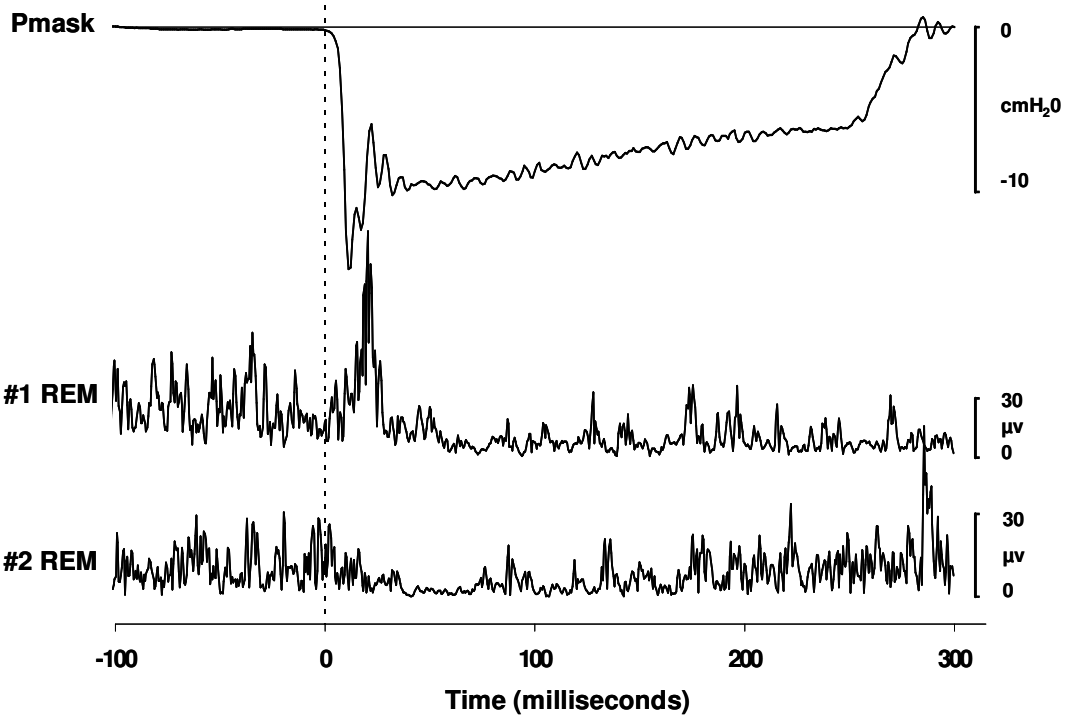
Characterisation criteria used to define EMG reflex components and stimulus properties (refer to the text for further detail).

Figure 16 Rectified EMGgg and EMGSc Reflex Responses during Wakefulness and NREM Sleep in Two Subjects



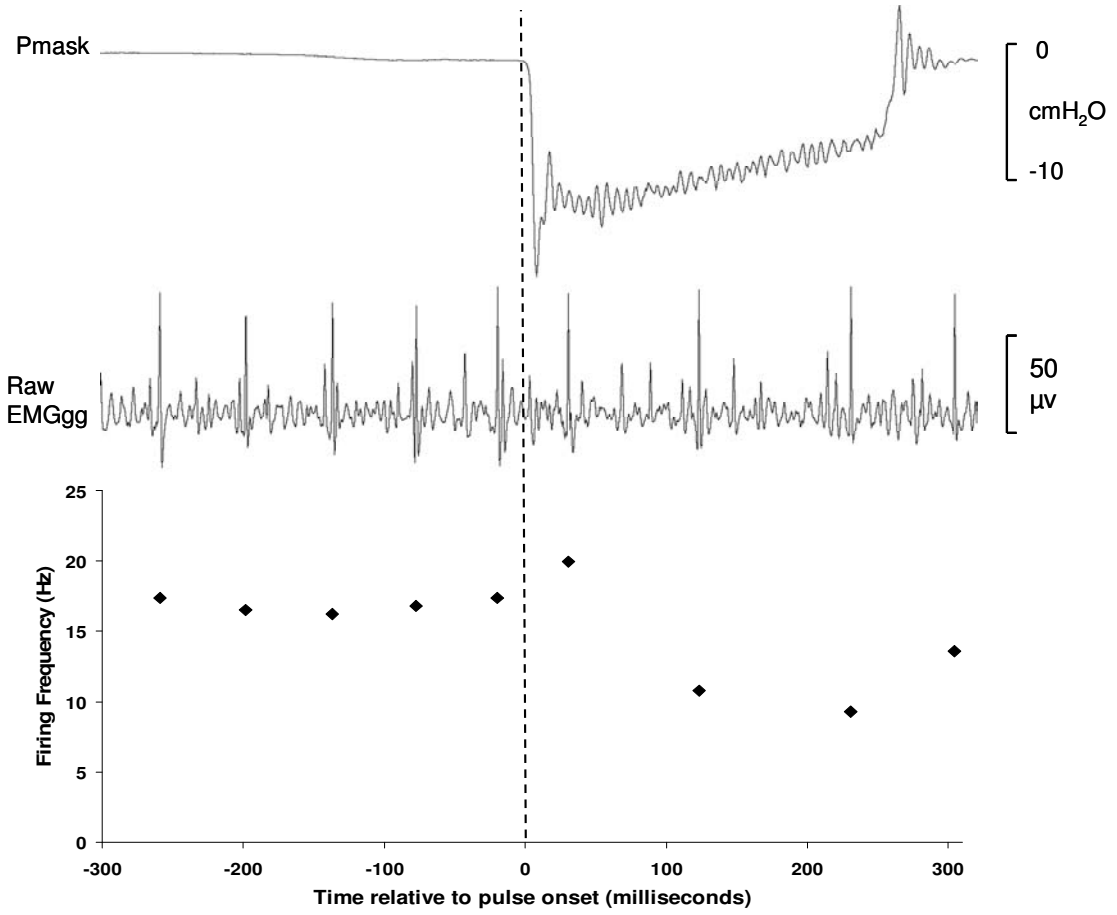
Ensemble averaged mask pressure and EMGgg (A) and EMGsc (B) reflex responses to brief negative pressure in two subjects during wakefulness and NREM sleep.

Figure 17 Rectified EMGgg Reflex Responses during REM Sleep in Two Individual Subjects



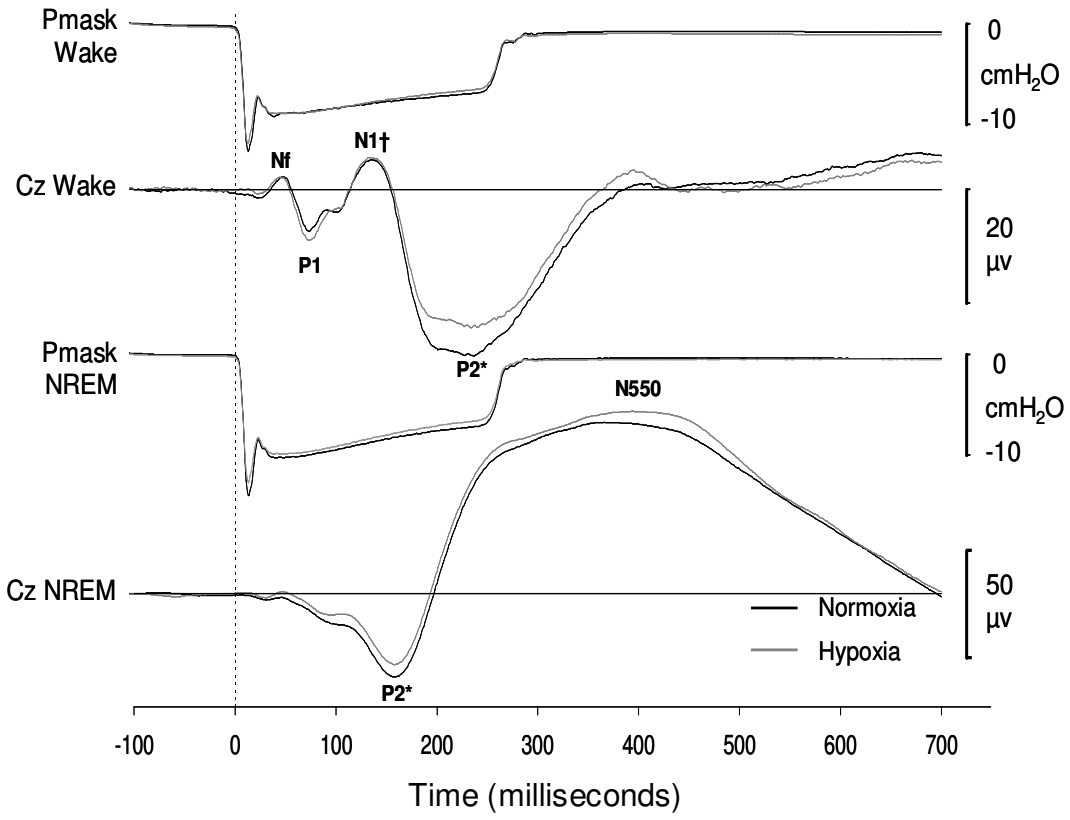
Ensemble averaged mask pressure and EMGgg reflex responses to brief negative pressure stimuli in two individual subjects during REM sleep.

Figure 18 Example of EMGgg Single Motor Unit Activity during a Negative Pressure Pulse Presentation



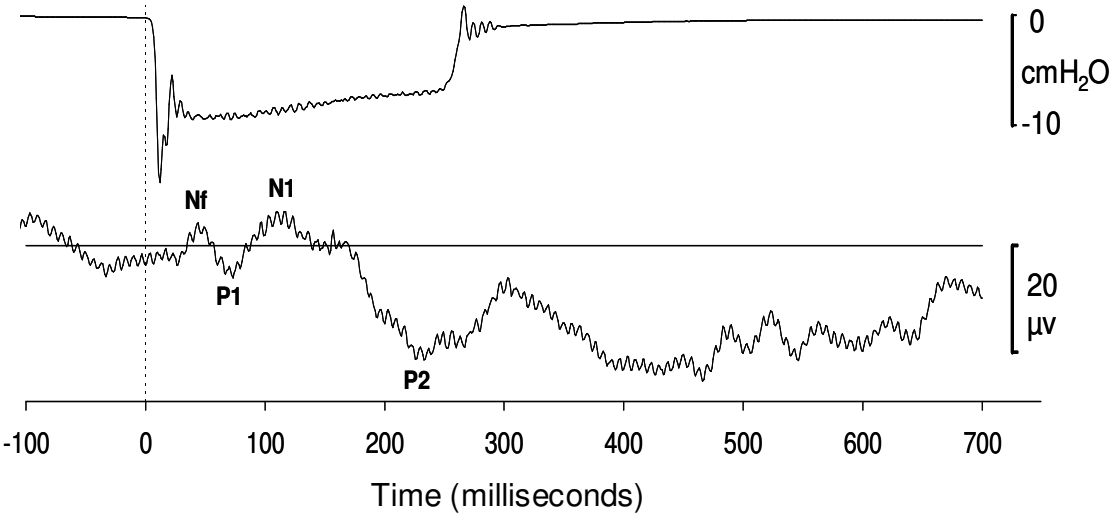
Example of mask pressure, Raw EMGgg recording with a prominent single motor unit and the firing frequency of the motor unit during a single pulse presentation. Notably there is a clear reduction in motor unit firing frequency after the first post-stimulus impulse.

Figure 19 RREP Group Average Waveforms during Wakefulness and NREM Sleep at Cz



Wakefulness and NREM sleep group mean average mask pressure and respiratory related evoked potential waveforms at Cz during isocapnic normoxia and isocapnic hypoxia. * Denotes amplitude difference between normoxia and hypoxia. † Denotes latency difference between normoxia and hypoxia. N=14.

Figure 20 Example of the RREP Waveform during REM Sleep in One Individual Subject at Cz



Ensemble averaged mask pressure and the respiratory related evoked potential at Cz in one individual subject during REM sleep. (Values derived from 16 replicate pulses during REM sleep).

4.4 Discussion

In this study, EMGgg and EMGsc reflex responses and the RREP to brief pulses of negative UA pressure during wakefulness and sleep were compared between conditions of mild isocapnic hypoxia (SaO_2 ~85%) and normoxia. Although the EMGgg negative pressure reflex was unaffected by mild overnight hypoxia, the latency of several components of the EMGsc reflex response to negative pressure was increased and EMGsc reflex inhibition duration was prolonged during hypoxia compared to normoxia. The amplitude of the P2 component of the RREP was also reduced during wakefulness and NREM sleep in the hypoxia condition. In addition, by presenting multiple replicate trials and expressing ensemble averaged rectified EMGgg reflex responses (without moving time averaging) a previously undescribed reflex morphology consisting of an initial increase in EMG activity followed by suppression has been observed with greater suppression during sleep compared to wake.

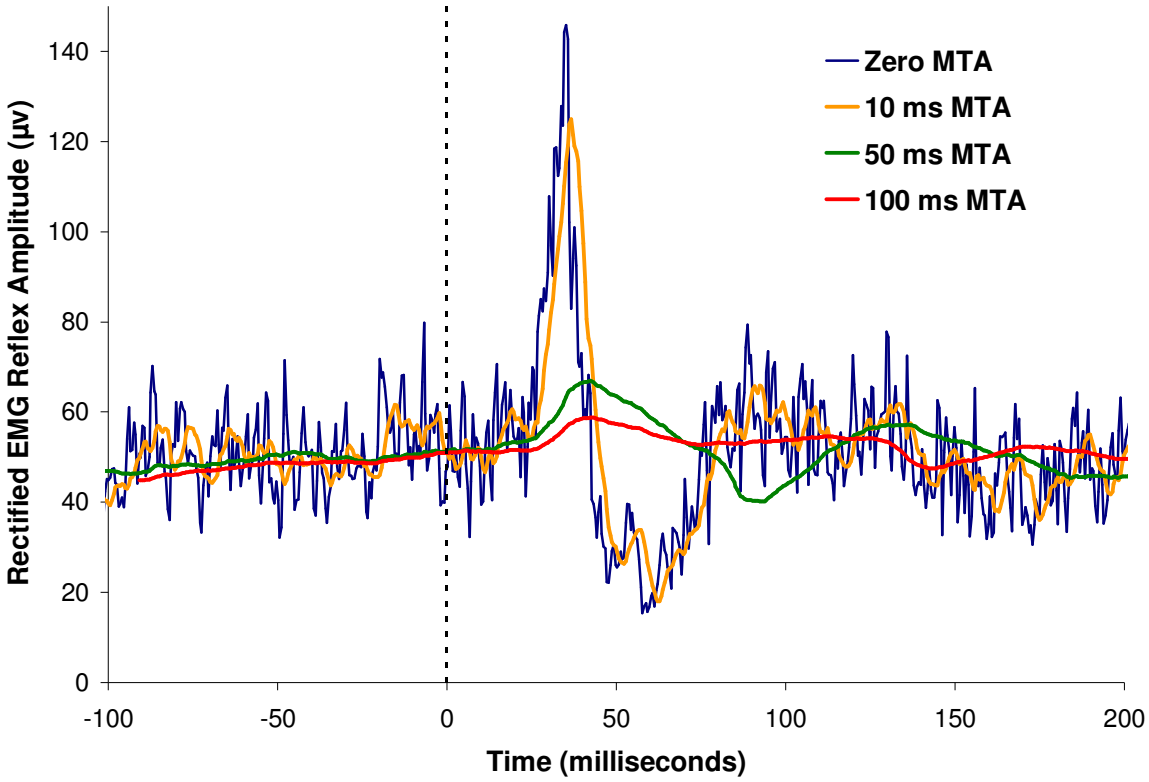
4.4.1 EMG Reflex Morphology to Negative Pressure Pulse Stimuli

4.4.1.1 EMGgg

The ensemble averaged rectified EMGgg response to brief pulses of negative airway pressure showed a previously unknown pattern, namely, an initial increase followed by a decrease from baseline EMGgg activity. This may not have been

observed in earlier studies for several reasons. Previous studies of the EMGgg negative pressure reflex in humans have been conducted using a combination of surface and intramuscular electrodes using multiple unit recordings (30, 393, 396-398, 400, 401, 407). A relatively poor signal to noise ratio produced by surface EMG electrodes and a small number of replicate trials (393, 398, 407) may have obscured these characteristics in previous studies. Subsequent studies that utilised intramuscular electrodes and increased replicate trial numbers likely improved the signal to noise ratio characteristics (30, 397, 400, 401) but nevertheless may have obscured a short latency inhibitory response via moving time averaging of the rectified EMG signal. Indeed, using a typical subjects' rectified EMGgg reflex response from the data derived in the current study and performing a 100 ms moving time average (as employed by many earlier studies) the suppression component of the reflex observed in the present study was effectively completely obscured (Figure 21).

Figure 21 Reflex Morphology; Effects of Moving Time Averaging of the Rectified Ensemble Averaged EMG Signal



Ensemble averaging of a rectified EMG reflex response (N=60 replicate trials) using zero, 10, 50 and 100 ms moving time averaging (MTA) windows respectively.

Where a reflex response consisting of an excitation phase followed by suppression is present on the rectified EMG it is possible that the suppression component is an epiphenomenon of motor unit synchrony rather than true reflex inhibition (465). Briefly, the excitation phase can cause otherwise out of phase motor units to become temporarily aligned as asynchronous units within the motor neuron population are nearly simultaneously brought to firing threshold as a result of the stimulus. This alignment can cause a subsequent suppression phase characterised by a relative lack of EMG activity whereby the previously asynchronous motor units are momentarily silent until they return to their respective pre-stimulus firing rates. In the current study, consistent with reflex inhibition rather than suppression, a consistent marked reduction in the firing frequency of single motor units was observed in the second post-stimulus impulse. While, difficult to definitively quantify using the sampling frequencies and techniques employed in the current study, the lack of change from baseline in the firing frequency of the first post stimulus impulse for the motor units examined suggests that the excitation phase of the EMGgg negative pressure reflex likely occurs primarily as a result of additional motor unit recruitment rather than an increase in the firing frequency of individual motor units. An alternate explanation for these findings is that the apparent excitation phase observed on the ensemble averaged rectified EMG is an aberrant peak caused by rectification of an inhibitory reflex as has been shown to occur in masseter reflex responses (466). However, this seems unlikely given the consistency with which the initial peak was observed and its relatively large amplitude. Recent human studies in which detailed quantification of EMGgg single motor unit activity was undertaken during normal respiration has highlighted the

heterogeneous nature of the genioglossus muscle and neural inputs (406). To definitively determine the neural mechanisms mediating the initial peak of the EMGgg negative pressure reflex and the precise role of negative pressure in modulating EMGgg activity a similar systematic exploration of the behaviour of single motor units within the muscle will be needed.

Functionally, these data suggest that the reflex response of the genioglossus muscle to transient negative pressure is not simply an excitatory response resulting in tongue protrusion as previously believed (389, 395, 467-469). While moment to moment excitatory modulation of UA EMGgg activity may be an important protective mechanism in maintaining UA patency during normal inspiration (389, 395, 467-469) the findings of this study raise the possibility that this response may be threshold dependent. For example, the response to relatively small negative pressures to the UA as would occur during normal tidal breathing, may be largely excitatory whereas the predominant response to large negative pressures may be inhibitory. The relatively long stimulus duration in the present study (250 ms) combined with the substantial stimulus amplitude is likely to be more akin to rapid airway occlusion than within-breath fluctuation in airway pressure to which EMGgg reflexes may normally be able to effectively respond. Consequently it is possible that shorter duration and less negative airway pressure stimuli would show a more pronounced excitatory response compared to the dominant inhibitory response observed in this study.

The precise functional role of EMGgg reflex inhibition is unclear and would appear to be counterproductive to respiratory homeostasis. However, it is possible that reflex inhibition in itself may be protective. The initial response appears to be excitatory which would tend to dilate the UA. However, if this initial response was not sufficient to overcome the impediment to respiration (whether it be UA collapse or inhalation of a foreign object) it would be counterproductive to continue making sustained inspiratory effort. Thus, the reflex inhibition observed may be a result of inhibition of the respiratory pattern generator inputs to this reflex arc in the same manner that respiratory drive muscles have been shown to be inhibited by brief respiratory load stimuli (431, 433, 434, 436, 440). In support of this hypothesis the latency of the onset of the reflex inhibition phase for EMGgg and EMGsc to negative pressure pulse stimuli were remarkably similar (~38 versus ~36 ms, respectively).

4.4.1.2 EMGsc

The response of several human inspiratory muscles to a sudden increase in respiratory load delivered during mid-inspiration during wakefulness has been reported to consist of an initial inhibitory phase (onset ~35-40 ms) followed by an apparent subsequent excitation (onset ~80-100 ms) (431-433). This is functionally different to the stretch or loading responses seen in limb muscles, which consist of reflex excitation without inhibition (437-439). In this study, the morphology and timing of the inhibitory and subsequent excitatory components of the EMGsc reflex response to a rapid onset negative pressure pulse delivered during early inspiration

was comparable to previous reports (433, 434, 436, 440). However, an initial increase in EMGsc activity was also variably observed. Given the short duration and inconsistency of this initial peak, the most likely explanation is that it is an aberrant peak caused by inhibition of motoneurons rather than an excitatory response (412, 466). An alternative possibility is that the reflex response of human inspiratory muscles to sudden respiratory load is influenced by posture such that the initial peak may represent a stretch reflex component. All previous studies have measured EMGsc reflex responses in the seated upright position rather than supine as employed in this study. Animal studies have demonstrated that the vestibular system can modulate respiratory pump muscle activity and reflex responses (470-472). While posture related changes may contribute to the genesis of this initial peak, this would appear unlikely to be a dominant factor given the variability in this components presence.

4.4.2 The Effect of Sleep on Reflex Responses to Negative Pressure Pulse Stimuli

4.4.2.1 EMGgg

Earlier studies investigating the effect of sleep on EMGgg reflex responsiveness to negative pressure either did not control for posture or studied subjects in the lateral position (397, 398). These studies found delayed latency and attenuation of the excitatory EMGgg negative pressure reflex response during NREM sleep. The present finding that the excitatory component of the EMGgg negative pressure

reflex in the supine posture was unaffected by sleep is different, but is in agreement with the recent findings of Malhotra and colleagues (401). This provides strong support for the concept that EMGgg reflex activity to negative pressure is posture dependent. In contrast to earlier studies, the latencies of the various EMGgg reflex characteristics were similar during NREM sleep compared to wakefulness, although the initial peak occurred slightly earlier during NREM sleep. While the reasons for the latency differences between studies are unclear they may also relate to postural and other methodological differences. However, the potential for postural related effects on EMGgg latency characteristics is difficult to establish given that latency data were not presented in the recent study by Malhotra and colleagues (401).

The finding of marked reflex inhibition during REM sleep, often in the absence of any preceding excitation, is in agreement with previous EMGgg negative pressure reflex findings by Shea and colleagues (400) and is consistent with earlier reports demonstrating reduced EMGgg activation to occlusive stimuli during REM sleep (473, 474). The lack of excitatory responsiveness of the largest UA dilating muscle to negative pressure during REM sleep is likely an important contributing factor to the increased severity of sleep disordered breathing observed in this sleep stage.

In contrast to the previous concept that sleep caused a suppression of the excitatory response to negative UA pressure (177, 178, 468), thereby increasing the likelihood of UA collapse, our data suggest it is the secondary inhibition phase of the EMGgg negative pressure reflex that may be more important. Greater reflex

inhibition of the EMGgg negative pressure reflex during sleep may be mediated by changes in sleep specific neuromodulators such as serotonin (405, 475).

4.4.2.2 EMGsc

This study is the first to examine the effects of NREM sleep on the EMGsc reflex response to a brief respiratory stimulus. The reflex morphology was similar between states. However, inhibition was more pronounced and of greater duration during wakefulness. The onset of the subsequent excitatory peak also occurred earlier during sleep compared to wakefulness. The reasons for state related changes in EMGsc reflex responses remain unclear. Many postural reflexes are reduced during sleep and the changes in EMGsc reflex activity observed in this study may similarly be the result of a global down-regulation of reflex activity. However, if one of the functions of this reflex response is to prevent the development of downstream negative pressures generated by inspiratory pump muscles that would otherwise tend to further collapse the UA as has been proposed (433, 436), a decrease in its efficiency during NREM sleep would likely be disadvantageous for UA stability.

4.4.3 The Effect of Hypoxia on Reflex Responses to Negative Pressure Pulse Stimuli

4.4.3.1 EMGgg

Sustained overnight hypoxia did not alter the EMGgg reflex responses to negative pressure. This finding is consistent with previous wakefulness reflex data (30) and a recent report demonstrating no change in baseline EMGgg activity during brief periods of isocapnic hypoxia alone (3 min, SaO_2 ~80-85%) or when combined with inspiratory resistive loading (~5-15 $cmH_2O.l^{-1}.sec$) during NREM sleep (429). Together these data suggest that the hypoglossal motor nucleus and the various components involved in the EMGgg negative pressure reflex arc are relatively insensitive to mild sustained isocapnic hypoxia. This is in contrast to recent observations of impaired sensory processing of respiratory load and suppression of the cough reflex during sustained hypoxia (33, 208, 450, 451, 461). The respiratory afferent pathways that are activated during respiratory loading, airway occlusion (to produce cough) and negative airway pressure pulses all relay through the NTS. Previous studies have suggested that the NTS may be a site of hypoxia-induced neural inhibition (53, 54). The different effects of hypoxia on the cough reflex, load sensation and the negative pressure UA reflex, suggest either that hypoxia does not exert its effects at this level of the NTS or that they relay through different nuclei that are differentially sensitive to hypoxia.

Intermittent hypoxia has been shown to cause marked attenuation of baseline EMGgg activity during wakefulness in humans and reduce excitatory hypoglossal nerve output in the rat (76, 419). Thus, it remains possible that the EMGgg negative pressure reflex may be impaired during intermittent hypoxia but not sustained hypoxia. This is worthy of future investigation given that intermittent hypoxia is a predominant feature of OSA.

4.4.3.2 EMGsc

Unlike the EMGgg reflex response that appeared to be relatively insensitive to the effects of hypoxia, several latency components of the EMGsc reflex response were delayed and the duration of inhibition was greater during hypoxia compared to normoxia. Even in the absence of hypoxia, patients with asthma and OSA also demonstrate similar changes in EMGsc reflex responses to sudden respiratory loading (434, 436). This altered reflex response has been proposed to be an adaptive response to repetitive exposure to increased respiratory load. In support of this hypothesis the duration and nadir of inhibition measured during wakefulness positively correlate with the RDI in OSA patients (436). The sensory receptors mediating this inhibitory reflex are more likely to be intramuscular (muscle spindles and tendon organs) than intrathoracic or upper and lower airway in origin (431, 433, 440). While pontomedullary inspiratory neurons are clearly involved, the precise CNS sites and synapses to this reflex arc are not known. Thus, hypoxia-induced changes in EMGsc reflex responses may be caused by a slowing of the sensory afferent traffic to the CNS and/or a net inhibitory effect at the level of the brainstem

to the neurons involved in this reflex response. This altered reflex response may be one of many that occur during hypoxia as part of a central chemosensitive inhibitory network (3).

4.4.4 The Effects of Sleep and Hypoxia on RREP Waveform Components to Negative Pressure Pulse Stimuli

Similar to a previous report by Webster and Colrain (242) the morphology of the early RREP waveform components was relatively unchanged between wakefulness and NREM sleep. This suggests that early sensory processing of respiratory stimuli remains intact during NREM sleep. A shift from the presence of the P3 sensory processing component visible during wakefulness overlying the secondary sensory association area of the cortex, to the predominance of a N550 component most visible frontally, is consistent with previous reports and is believed to reflect a marked state related change in sensory processing during NREM sleep (235, 242, 306). Sensory processing to respiratory stimuli during REM sleep using RREP techniques has not been previously described. The example observed in this study suggests that sensory processing during REM sleep is similar to that during wakefulness and is in agreement with findings during REM sleep in auditory ERP studies (476, 477).

Consistent with the findings of Webster and Colrain (242) and the hypothesis that the N1 peak is a marker of attention which decreases from wakefulness to sleep (243), the amplitude of the N1 deflection was also reduced during NREM sleep in

the current study. However, this is in contrast to earlier findings by Wheatley and White that demonstrated an increase in N1 amplitude during NREM sleep (245). These and other variations in the timing and amplitude of RREP components between studies likely reflect differences in stimulus properties and the experimental protocols used. In the limited number of studies available there have also been disparate findings regarding the effect of sleep on the P2 component of the RREP. One study reported a non significant trend towards a decrease in P2 amplitude with sleep (242) whereas others reported increased P2 amplitude from wakefulness to stage 2 sleep (235, 245). No significant change in P2 amplitude was observed in the current study between states although the P2 peak occurred earlier during NREM sleep compared to wakefulness. The most likely explanation for the difference in P2 latency in the current study is that there was some degree of overlap between the predominant later large negative component and P2 during NREM sleep.

Decreased amplitude of the P2 component of the RREP during hypoxia observed in wakefulness and NREM sleep is consistent with a recent RREP study conducted during wakefulness (451). Compared to the P1 and P3 peaks of the RREP, remarkably little is known regarding the functional significance and neural genesis of the P2 peak (243). P2 latency, preservation of the P2 peak in both stimulus attend and ignore conditions and the fact that this peak is maximal overlying the somatosensory area of the cortex suggest that it reflects a combination of exogenous and endogenous processes (236, 243). Based on auditory ERP studies it has been proposed that the P2 peak represents an early indexing phenomenon of

sensory stimuli that is necessary to elicit the later sensory processing components (478). However, despite reductions in P2 amplitude during wakefulness and NREM sleep during hypoxia, the amplitude of the later sensory processing components (P3 and N550 respectively) remained unchanged. This finding raises the possibility that the proposed model of P2 based on auditory induced ERPs may not be appropriate for respiratory stimuli. P2 has also been proposed to be importantly involved with the arousal process (243). However, if P2 is a marker of sensory processing involved in the arousal process a reduction in its amplitude would imply a decrease in arousal probability. This was not the case in the current study as arousal probability was the same during hypoxia and normoxia conditions. However, it is possible that a reduction in P2 amplitude does indeed reflect an arousal prevention mechanism but the nature of the stimulus employed (i.e. sudden relatively large negative pressure pulse) in the current study did not allow for changes in arousal probability to be observed. In support of this hypothesis a recent study by Hlavac and colleagues using a similar overnight mild hypoxia regime demonstrated an increased time to arousal during hypoxia to resistive load stimuli but not to the more abrupt stimuli of sudden airway occlusion (450). There has been some debate regarding the functional role of K-complexes as to whether or not they represent a sleep preservation phenomenon by providing a means of sensory processing to internal/external stimuli in the absence of arousal, or are inherently involved in arousal production (244). Consistent with a previous report using auditory ERPs (479) the likelihood of eliciting a K-complex to a negative pressure pulse increased from stage 2 to SWS in the current study. In the same manner that the nature of the stimulus used in this study may not have led to a

discernable change in arousal probability between gas conditions despite a reduction in P2 amplitude during hypoxia, an absence of between gas differences in K-complex probability may also be the result of this phenomenon.

The lack of a reduction in P1 amplitude with hypoxia in this study was somewhat unexpected and differs from the study reported in Chapter 2 (451). This is probably explained by differences in stimulus properties. The previous RREP study (451) utilised mid inspiratory resistive load stimuli whereas the current study used early inspiratory negative pressure pulses. While both stimuli may stimulate common receptor systems, mid-inspiratory resistive loading likely stimulates respiratory afferents at multiple levels including UA, lung and chest wall. On the other hand, sudden negative pressure pulse stimuli delivered to the nose might predominantly stimulate UA afferents. It also remains possible that the lesser degree of hypoxia employed in the current study (SaO_2 ~85% versus SaO_2 ~80%) may have contributed to these disparate findings. Nonetheless, the finding that P1 amplitude was not reduced in the current study suggests that UA afferents were not suppressed during mild isocapnic hypoxia. Consistent with this finding, the EMGgg negative pressure reflex was not affected by hypoxia. A lack of a reduction in P1 amplitude during hypoxia despite changes in EMGsc reflex responses suggests that this latter reflex response does not produce somatosensory cortical activation, leastwise under these particular experimental conditions.

4.4.5 Possible Relevance to Sleep Disordered Breathing

A diminished excitatory EMGgg negative pressure reflex during sleep has been postulated to contribute to the development of OSA in patients with an anatomically narrow UA. The finding that the amplitude of the initial excitatory component of the reflex measured in the supine posture was not different between wake and NREM sleep does not support this hypothesis. However, more pronounced EMGgg reflex inhibition following the initial excitation phase to negative pressure during NREM sleep would likely render the UA more prone to collapse. In the same way that inspiratory pump muscle reflex responses to respiratory occlusion are altered in OSA patients it would be interesting to investigate if the inhibition component of the EMGgg negative pressure reflex was similarly affected.

Increased reflex inhibition of the respiratory pump muscles to respiratory occlusion has been postulated to be protective by way of preventing greater downstream negative pressures during times of UA narrowing or obstruction. The finding of less reflex inhibition of EMGsc to negative UA pressure during NREM sleep combined with more pronounced reflex inhibition of EMGgg suggest that this combination is likely to be disadvantageous to UA stability during sleep. On the other hand, prolongation of the duration of reflex inhibition of the EMGsc reflex response to negative pressure, combined with the preservation of the initial increase in EMGgg reflex activity during mild isocapnic hypoxia in NREM sleep may be protective in modulating UA stability. However, the findings of the current study are likely most relevant to sleep disordered breathing characterised by sustained periods of

hypoxia. Intermittent hypoxia as occurs in OSA has been postulated to be deleterious to UA muscle function (421). To definitely address this issue, human studies of UA reflex responses during intermittent hypoxia are required.

4.4.6 Methodological Considerations

There are several important methodological considerations with regard to this study. Firstly, as this was a within subjects repeated measures study design we elected to study male subjects only due to the known influence of changes in respiratory stimulant hormones that occur throughout the menstrual cycle and their associated effects on genioglossus muscle activation (480). Further, the prevalence of sleep-disordered breathing is greater in men than women (173). While we would propose that the effects observed in this study in men would be similar in women if stage of menstrual cycle was controlled this has yet to be determined.

Although we hypothesize that more pronounced EMGgg reflex inhibition observed during sleep occurs due to changes in sleep specific neuromodulators it is possible that this difference may be explained by the marginally greater stimulus presented during NREM sleep. Similarly, the decreased latency of the EMGgg excitatory peak during NREM sleep compared to wakefulness may be the result of differences in stimulus properties. However, this would appear unlikely given that differences in stimulus magnitude were small (~1-2 cmH_2O) and other components of the reflex were not different between states. Another potential limitation is that negative pressure was applied nasally rather than generated distal to the UA as occurs in

OSA. While this approach stimulates additional receptor systems not implicated in UA collapse in OSA the available evidence suggests nasal and laryngeal receptors respond in a similar manner (401, 407). While, based on these findings it would appear unlikely, it remains possible that this type of stimulus evokes additional proprioceptive afferents that are known to cause suppression of EMGgg activity in animal models (481).

Subjects studied were heavily instrumented and it is possible that reflex responses may have been altered as a result. The inspiratory circuit added a small (~2 $cmH_2O \cdot l^{-1} \cdot sec$) resistance to inspiration and all subjects breathed nasally. However, given that all subjects experienced the same conditions, these factors are unlikely to account for the hypoxia-induced differences in EMGsc and RREPs observed.

The epiglottic pressure catheter was prone to drift, presumably because of build up of airway secretions on the catheter. Thus, we cannot be certain that negative pressure pulse stimuli at the pharyngeal airway were matched between gas conditions. However, most studies suggest that in the absence of changes in ventilatory drive respiratory muscle tone, respiratory mechanics and pharyngeal resistance are unchanged during hypoxia in humans (23, 24, 30). Further, the choanal and mask pressures during negative pressure pulse stimuli were not different between gas conditions, nor were ventilatory parameters on the breath prior to stimulus application. These data strongly support that negative pressure pulse stimuli were indeed similar between gas conditions.

4.4.7 Summary

In summary, this study has demonstrated that EMGsc reflex inhibition to brief pulses of negative pressure is prolonged during mild sustained hypoxia. This finding suggests that responses to hypoxia may serve to protect against further downstream negative pressures by inhibiting respiratory pump muscles during sudden respiratory loads to breathing. During wakefulness and NREM sleep, the P2 component of the RREP was reduced during hypoxia. Hypoxia did not change any of the measured EMGgg negative pressure reflex characteristics and the initial increase in EMGgg activity was preserved form wakefulness to NREM sleep. However, a previously undescribed EMGgg reflex morphology consisting of a short latency excitatory peak followed by suppression, likely inhibitory in origin was observed. The onset latency of the EMGgg inhibitory reflex component was similar to EMGsc reflex inhibition suggesting these reflex responses may share common neural pathways. EMGgg reflex inhibition to negative pressure pulse stimuli increased in magnitude progressively from wakefulness to NREM and appeared most pronounced during REM sleep. Greater EMGgg reflex inhibition to negative pressure stimuli during sleep raises the possibility that this may be a contributing mechanism to UA collapse in individuals with an anatomically narrow airway.

CHAPTER 5. SUMMARY AND CONCLUSIONS

Acute hypoxia is a common feature of many respiratory disorders including severe episodes of asthma, COPD, pneumonia and during sleep-disordered breathing in conditions such as sleep hypoventilation syndrome and sleep apnea. Many of these hypoxic diseases are associated with significant morbidity. In respiratory disease, hypoxia is invariably coupled with increased respiratory load. Compensatory protective mechanisms are activated to oppose these impediments to respiration. However, hypoxia has also long been associated with impaired neurocognitive function and recent studies have demonstrated that hypoxia suppresses respiratory load perception in healthy individuals and asthma patients. While the effects of hypoxia on ventilation have been well described the role of hypoxia in modulating respiratory sensation and protective respiratory reflexes is less well defined. The studies presented in this thesis were designed to assess several previously untested aspects of respiratory sensory processing and respiratory reflex responsiveness during acute sustained hypoxia.

Intact function of respiratory sensory processing and the ability to appropriately recognise and respond to increased respiratory load are vitally important for patients with respiratory disease. For example, an asthmatic or a patient with COPD experiencing an acute exacerbation must first recognise that their condition is worsening in order to take appropriate treatment measures. Indeed, blunted sensation of respiratory load has been implicated as an important factor

contributing to increased risk of morbidity and mortality in these disorders. Similarly, during times of increased respiratory load or airway obstruction in patients with sleep disordered breathing, appropriate sensory recognition is required in order to activate an appropriate compensatory response (i.e. arousal or increased UA dilator muscle activity). Thus, any factor that impairs the function of these protective processes, whether it be a pre-existing inherent trait or one that arises acutely, is likely to increase disease severity. Several recent studies have demonstrated that during acute sustained isocapnic hypoxia (SaO_2 ~80-85%) the ability to perceive increased respiratory load during wakefulness and NREM sleep is impaired. The respiratory related evoked potential to brief mid inspiratory resistive load stimuli was used in Study 1 (CHAPTER 2) to investigate the mechanisms underlying hypoxia-induced suppression of respiratory load sensation in healthy individuals. The observed reduction in the amplitude of the first and second positive peaks (P1 and P2) of the RREP during hypoxia suggests that sensory afferent transmission and early processing of respiratory information is impaired during acute sustained hypoxia. Under the same experimental conditions, hypoxia also impaired respiratory load perception to inspiratory resistive loads. These data provide further support that hypoxia suppresses respiratory load perception and suggest that this is mediated, at least in part, by suppression of respiratory afferent information prior to its arrival at the cortex.

The finding that hypoxia suppressed respiratory sensory traffic below the level of the cortex raised the possibility that other protective respiratory responses may be similarly affected. The cough reflex is one of several defensive respiratory reflexes

that are important for protecting the lungs from inhalation or aspiration of potentially injurious substances and for clearing excess secretions. A blunted cough reflex can be harmful or even fatal in the presence of severe respiratory disease. Hypoxia has been shown to impair cough reflex function to mechanical stimuli in animals but had not been previously tested in humans. In study 2 (CHAPTER 3), the effects of acute sustained hypoxia on the cough reflex threshold and cough tachyphylaxis to inhaled capsaicin was explored in healthy individuals. Acute sustained hypoxia suppressed cough reflex sensitivity to inhaled capsaicin but adaptation to a continuous cough stimulus was not affected by hypoxia. Tachyphylaxis to capsaicin is believed to be mediated at the sensory nerve ending. Together, these findings suggest that cough reflex sensitivity is likely impaired centrally (i.e. brainstem or cortex) rather than peripherally. Impaired function of the cough reflex during acute hypoxia in healthy individuals raises the possibility that this important protective response may be impaired during acute exacerbations of hypoxic-respiratory disease.

The genioglossus is the largest UA dilator muscle and has been shown to be reflexively augmented in response to negative UA pressure. A diminished response of this muscle during sleep has been postulated to be a contributing mechanism to OSA pathogenesis in individuals with an anatomically narrow UA. However, measurement techniques employed in earlier EMGgg reflex studies, including moving time averaging of the rectified EMG, raise the possibility that components of the EMGgg reflex response to negative UA pressure may have been obscured using these procedures. By presenting ensemble averaged EMGgg reflex

responses, without moving time averaging, a previously undescribed morphology of the EMGgg negative pressure reflex consisting of activation followed by inhibition was observed. Also in study 3 (CHAPTER 4), it was found that the initial genioglossus reflex activation to UA negative pressure was maintained from wakefulness to NREM sleep in healthy individuals. This suggests that the previously proposed sleep-induced loss of EMGgg reflex activity may be more complex than first believed. In fact in study 3, the secondary reflex inhibition of EMGgg activity was progressively more pronounced from wake to NREM sleep to REM sleep. Thus, while supporting the concept that UA dilator muscle function is impaired during sleep, these data suggest it may be mediated more by an increase in negative pressure reflex inhibition than by a reduction in reflex muscle excitation.

Unlike the cough reflex the function of the EMGgg negative pressure reflex was maintained during mild overnight hypoxia. This suggests that the hypoglossal motor nucleus and the respective components of the EMGgg negative pressure reflex arc are preserved during acute sustained hypoxia. Conversely, EMGsc reflex inhibition to negative UA pressure was prolonged during hypoxia. Reflex inhibition of respiratory pump muscles to sudden respiratory loading has been postulated to be protective by way of preventing greater collapsing negative pressure to the UA and preventing further inhalation of potentially injurious material. Should this be the case, this response may play a protective role during the advent of sudden respiratory loading. In the same study, in accordance with the findings of study 1, the amplitude of the P2 component of the RREP was reduced during hypoxia further supporting the role of hypoxia-induced impairment of early sensory

processing. However, the P1 component was not different between gas conditions suggesting that UA sensory transmission is not impaired during mild overnight isocapnic hypoxia.

Several questions have arisen from these studies. Firstly, as these studies were all conducted in healthy individuals it would appear important to determine whether protective reflex responses during hypoxia were similarly affected in the relevant patient populations. It would also appear important to determine the effects of intermittent hypoxia, the pattern typical of OSA, on UA and respiratory pump muscle reflex responses to respiratory stimuli. Given the findings of hypoxia-induced CNS impairment it would be clinically significant to undertake studies examining the effects of oxygen therapy on the arousal threshold and ventilatory adaptation during sleep in sleep hypoventilation patients. Examination into the neurochemical basis for hypoxia-induced suppression of respiratory sensation would likely provide important mechanistic insight. This could be achieved by employing either pharmacological neuroinhibitors (eg theophylline an adenosine antagonist; naloxone an endogenous opioid antagonist) or developing animal model(s) where anatomical site(s) and neurochemical mechanisms of neuroinhibition can be systematically investigated using microdialysis and injection techniques. Recent findings raise the possibility that the neuroinhibitory effects of hypoxia may be specific to respiratory afferent pathways and central processing (450, 482). Thus, to definitively address this issue it would be interesting to compare the effects of hypoxia on respiratory, tactile and auditory afferent pathways in the same experiment. Investigation into the underlying mechanisms

responsible for the EMGgg negative pressure reflex morphology observed in this study may provide important new insight into the pathogenesis of OSA. This would likely involve studying patients and performing detailed examination of the overall response of EMGgg muscle activity and respiratory pump muscles to negative UA pressure.

Together these studies have highlighted the complex interaction between hypoxia, respiratory sensory processing and the function of several protective respiratory reflexes. Sensory processing of respiratory load information and the cough reflex are impaired during acute sustained hypoxia which may be deleterious to patients with hypoxic respiratory disease. On the other hand, EMGgg reflex responses to negative UA pressure are maintained and prolongation of reflex inhibition of respiratory pump muscles may be protective to patients with sleep disordered breathing.

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