The role of intermittent hypercapnic hypoxia in the induction of high loop gain in obstructive sleep apnoea pathophysiology

by

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ABSTRACT

Intermittent hypoxia (IH) and unstable breathing are key features of obstructive sleep apnoea (OSA), the most common respiratory sleep disorder. Unstable ventilatory control is characterised by high loop gain (LG), and likely contributes to the propagation of apnoeas by promoting airway collapse during periods of low ventilatory drive. Currently, the contribution of inherent versus induced traits causing high LG in OSA remains unclear. OSA patients exhibit abnormal chemoreflex control which contributes to increased LG. These abnormalities normalise with continuous positive airway pressure (CPAP) treatment, suggesting induced rather than inherent trait abnormalities. Experimental IH, mimicking OSA, increases hypoxic chemosensitivity and induces long-term facilitation; a sustained increase in ventilatory neural output which outlasts the original stimulus. These neuroplastic changes induce the same abnormalities in chemoreflex control as seen in OSA patients, suggesting that high LG in OSA is largely induced by IH, and is reversible.

IH protocols are typically conducted on a background of poikilocapnia or isocapnia, in contrast to combined hypoxia and hypercapnia experienced in OSA. The level of concomitant CO₂ is thought to be critical for both the induction and expression of IH induced neuroplasticity. To more accurately mimic OSA, the effects of intermittent hypercapnic hypoxia (IHH) on ventilatory neuroplasticity and LG were investigated in the first two experiments contained within this thesis. The effect of CPAP treatment on LG in untreated OSA patients was investigated in the third and final study of this thesis.
In the first study, whether IHH during sleep induces LTF or increases chemosensitivity in healthy males was investigated. A randomised, separate day of intermittent medical air served as control. Unlike previous reports using isocapnic IH during sleep in healthy males, IHH did not induce LTF of ventilation or genioglossal muscle activity. Also, there was no change in the magnitude or slope of the ventilatory response to IHH from the first exposure to the last, to indicate any change in chemosensitivity. These findings suggest the effects of IHH differ to those of IH during sleep in healthy males.

During wakefulness LTF in humans is only expressed during mild hypercapnia. In the second study, the effect of IHH on LG was investigated in healthy males during wakefulness using a CO₂ pseudorandom binary stimulation technique to measure LG on a background of mild hypercapnia. There was no change in chemosensitivity during IHH or ventilatory LTF following IHH. There was no change in LG and although there was a trend towards a change in the ventilatory impulse response to a sudden change in CO₂ following IHH, this was not statistically significant. These findings further support that the effects of IHH during wakefulness differ to those of IH in healthy males.

In the third study, the effect of 6 weeks CPAP treatment on LG in previously untreated OSA males was investigated. Participants matched for age, sex, height, weight and BMI were also studied as controls. Helium dilution was used to assess supine functional residual capacity (FRC) and LG was compared prior to
commencing CPAP treatment and at 2 and 6 weeks after starting treatment, and at the same time points but without CPAP treatment in controls. LG was higher in the OSA patients versus matched controls, but there was no effect of CPAP treatment on LG. There was also no difference between patients and controls in FRC or controller or plant gain components of LG, although given that LG is the product of controller and plant gains, this could reflect a type II error. Patients exhibited reduced FEV1 and FVC and also higher supine abdominal height which positively correlated with AHI. Thus, this study confirmed that LG is higher in OSA patients versus matched controls, and supported previous work suggesting that central adiposity contributes to upper airway collapse. However, given no effect of CPAP on LG, larger cohorts and potentially alternative measures may be required to determine mechanisms driving elevated LG in OSA patients.

Although IH has previously been shown to induce neuroplastic changes to chemoreflex control that mirror abnormalities associated with high LG in OSA patients, the findings in this thesis suggest the effects of acute IHH differ to those of IH, both during sleep and wakefulness in healthy males. Potential causes for this disparity, and relevance of experimental findings to OSA pathophysiology are discussed. The effects of CPAP treatment on LG and implications for treatment options and CPAP adherence outcomes are also discussed.
PUBLICATIONS

Publications arising from this thesis


Conference abstracts


Other refereed journal articles


DECLARATION

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GLOSSARY OF ABREVIATIONS

AG  abdominal girth
AHI  apnea hypopnea index (events·hr⁻¹ sleep)
AIH  acute intermittent hypoxia
PaCO₂ arterial CO₂ partial pressure
F_B breathing frequency
CIH  chronic intermittent hypoxia
CPAP continuous positive airway pressure
CG  controller gain
EMGdia diaphragm electromyogram
diaLTF diaphragm long-term facilitation
ECG electrocardiogram
EEG electroencephalogram
EMG electromyogram
P_{ET}CO₂ end tidal partial pressure of CO₂
Pepi epiglottic pressure
T_E expiratory time
FRC functional residual capacity
FEV₁ forced expiratory volume in the first second
FVC forced vital capacity
EMGgg genioglossal electromyogram
ggLTF genioglossal long-term facilitation
HH hypercapnic hypoxia
HCVR hypercapnic ventilatory response
hLTF hypoglossal long-term facilitation
HVR hypoxic ventilatory response
TI
inspiratory time

$P_{iCO_2}$
inspiratory partial pressure of CO$_2$

IHH
intermittent hypercapnic hypoxia

IH
intermittent hypoxia

LTF
long-term facilitation

LG
loop gain

$P_{MASK}$
mask pressure

MAP
mean arterial pressure

$V_i$
minute ventilation

OSA
obstructive sleep apnoea

PIF
peak inspiratory flow

$pLTF$
phrenic long-term facilitation

PG
plant gain

PSG
polysomnography

PRBS
pseudorandom binary stimulation

PA
progressive augmentation

$SaO_2$
saturation of oxygen

$sLTF$
sensory long-term facilitation

$V_T$
tidal volume

$T_{TOT}$
total breath time

$uALTF$
upper airway long-term facilitation

$R_{UA}$
upper airway resistance

$vLTF$
ventilatory long-term facilitation

ROS
reactive oxygen species

SAH
supine abdominal height
CHAPTER 1. GENERAL INTRODUCTION

The role of high loop gain induced by intermittent hypoxia in the pathophysiology of obstructive sleep apnoea

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i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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| Signature | Date | 24 July 2015 |

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1.1 Introduction

Obstructive sleep apnoea (OSA) is characterised by repeated partial (hypopnoea) or complete (apnoea) collapse of the airway during sleep resulting in bouts of combined hypercapnia and hypoxia. OSA is the most common sleep disorder and is estimated to affect between 10% of men and 3% of women aged 30-49yrs, and 17% of men and 9% of women aged 50-70yrs (Peppard et al., 2013). OSA is associated with increased mortality and morbidities including cardiovascular disease (Bradley and Floras, 2009), diabetes (Kim et al., 2013), cognitive impairment (Yang et al., 2013), pathological daytime sleepiness (Sforza and Lugaresi, 1995) and increased frequency of driving and other accidents (George et al., 2002). Continuous positive airway pressure (CPAP) is the main treatment for OSA, however long-term adherence is poor, with ≤50% of patients accepting and tolerating CPAP long-term (Weaver and Grunstein, 2008). Consequently there is a strong ongoing need for the development of alternative treatments. OSA pathophysiology is now understood to involve multiple interacting factors including increased airway collapsibility (typically measured from airway critical closing pressure), a propensity to wake to airway obstruction (low arousal threshold), poor upper airway muscle recruitment responses and unstable ventilatory control (high loop gain, LG), with variable combinations producing differing OSA phenotypes between individuals (Wellman et al., 2013). Wellman and colleagues have recently proposed new diagnostic methods designed to quantify these causal factors in each patient, thus allowing treatments to be tailored to each individual (Wellman et al., 2013). High LG has been reported in 36% of CPAP treated patients (Eckert et al., 2013), and could play a significant pathogenic role in a greater proportion of
previously untreated OSA patients. Given the prevalence of LG disturbances, a key part of this individualised treatment approach could include pharmacological or non-pharmacological manipulation of LG (Edwards et al., 2012). However it remains unclear if high LG is an inherent causal trait in OSA and/or an induced effect exacerbating OSA and contributing to disease progression. A greater understanding of the inherent versus causal mechanisms underpinning high LG in OSA is therefore needed to effectively guide treatments designed to reduce LG.

LG includes “plant” (respiratory apparatus) and “controller” (chemoreflex) gain components, both of which can be abnormal in OSA. Increased plant gain may predominantly reflect obesity effects on lung volume that may normalise while using CPAP (Heinzer et al., 2006). However, OSA patients also exhibit abnormally elevated controller gain independent of BMI (Narkiewicz et al., 1999c, Salloum et al., 2010, Younes et al., 2007). These chemoreflex control abnormalities have been shown to normalise following ≥1 month of CPAP use (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006), supporting that high controller gain is predominantly induced rather than an intrinsic trait in OSA.

Several stimuli experienced during obstructed breathing events have been shown to induce lasting changes in ventilatory neural responses. Intermittent hypoxia (IH) induces neuroplastic changes in the carotid bodies (Peng and Prabhakar, 2004), brainstem (Morris et al., 2000), and cervical spinal cord (Dale-Nagle et al., 2010b), inducing increased hypoxic sensitivity (Peng and Prabhakar, 2004) and long-term facilitation (LTF) of various ventilatory nerves, manifesting as a sustained increase
in neural output to a given stimulus (Mitchell et al., 2001a). LTF of ventilatory neural output has been studied and demonstrated in a variety of species including humans (Harris et al., 2006), dogs (Cao et al., 1992), cats (Fregosi and Mitchell, 1994), goats (Turner and Mitchell, 1997), rats (Mahamed and Mitchell, 2008) and avian species such as ducks (Mitchell et al., 2001b). LTF is thus highly conserved across phylogenetically distant species suggesting an important adaptive mechanism in ventilatory neural control. However, as with many physiological processes in disease states, LTF could play both adaptive and maladaptive roles in OSA pathophysiology and both have been posited (Mahamed and Mitchell, 2007, Mateika and Narwani, 2008). On the one hand hypoglossal LTF may augment upper airway dilator muscle activity to help prevent airway collapse (Mahamed and Mitchell, 2007). However, experimental IH also induces the same abnormalities in chemoreflex control that are seen in OSA patients (Chowdhuri et al., 2010, Harris et al., 2006, Mateika et al., 2004), which increase controller and therefore overall LG, suggesting IH induced neuroplasticity may worsen OSA. These neuroplastic changes appear to gradually decay following return to room air breathing for several days (Peng and Prabhakar, 2004, Pialoux et al., 2009b, Peng et al., 2003, Brugniaux et al., 2011), much the same as chemoreflex abnormalities in OSA normalise with CPAP use (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006). These data support that normalisation of LG may simply require prevention of IH and allowing sufficient time for chemoreflex control, and therefore controller gain, to readjust. Although lowering LG may not cure OSA given other contributory factors, IH induced high LG likely exacerbates OSA in a feed-forward manner. Neuroplasticity effects on LG have several treatment implications;
including lowered pressures over time that could improve long-term CPAP adherence, the development of drug therapies targeting the cellular mechanisms contributing to raised LG, and future combination treatments where LG lowering may be achieved with short-term conventional treatments such as CPAP by simply preventing the inducing IH stimulus.

This review summarises the evidence to support a pathogenic role for abnormal chemoreflex control and high LG in OSA, and further evidence to support that these effects are predominantly induced by OSA and reverse with treatment. The main focus is on animal and human studies supporting that IH induced neuroplasticity is the main causal mechanism inducing high controller gain and therefore overall LG in OSA. Implications for treatment and future research are also discussed.

1.2 Sleep chemoreflex control

Resting end-tidal CO₂ (P_{ET}CO₂ an indirect estimate of PaCO₂) determines the position of eupnoea on the so called metabolic hyperbola, which governs the relationship between changes in CO₂ when ventilation changes at a given rate of metabolic CO₂ production. During sleep, ventilation below eupnoea decreases linearly with reducing P_{ET}CO₂ until an “apnoic threshold” 3-6 mmHg below eupnoea is reached, where ventilation is totally suppressed (Skatrud and Dempsey, 1983). Above eupnoea, ventilation increases linearly with P_{ET}CO₂ and the slope of this relationship indicates CO₂ chemoreflex sensitivity. Following hypocapnic central apnoea during sleep or anaesthesia (e.g. after withdrawal of mechanical hyperventilation to induce central apnoea), PaCO₂ must rise several mmHg above
not only the level of CO\textsubscript{2} which induced apnoea, but also to a level above the resting eupnoeic level called the ventilatory recruitment threshold, before rhythmic breathing is reinitiated (Dempsey, 2005, McGuire et al., 2007). This contrasts with wakefulness, where hypocapnia does not induce central apnoea, and ventilation is maintained at a stable baseline level below a CO\textsubscript{2} chemoreflex threshold (Fink, 1961), above which ventilation rises linearly with increasing P\textsubscript{ET}CO\textsubscript{2} (Duffin and Mahamed, 2003).

During hypoxaemia mammals exhibit a characteristic biphasic hypoxic ventilatory response (HVR). Initially, there is an acute HVR during which ventilation increases with decreasing arterial O\textsubscript{2} (PaO\textsubscript{2}). If hypoxia is sustained the acute response is followed by a decrease in ventilation called hypoxic ventilatory decline (Powell et al., 1998). The HVR modulates ventilation via the combined effects of O\textsubscript{2} and CO\textsubscript{2} on peripheral chemoreflex responses (Duffin, 2007, Duffin and Mahamed, 2003), with hypoxia increasing the sensitivity to CO\textsubscript{2} unless PaCO\textsubscript{2} is below the chemoreflex threshold, below which the HVR is suppressed (Mohan and Duffin, 1997). Increased minute ventilation (V\textsubscript{I}) during hypoxia results in a reduction of P\textsubscript{ET}CO\textsubscript{2} which tends to constrain the HVR. During sleep, hypoxia does not alter the apnoeic threshold, therefore the point of eupnoea moves closer to the apnoeic threshold, reducing the CO\textsubscript{2} reserve and increasing the slope of the ventilatory response to CO\textsubscript{2} below eupnoea (Xie et al., 2001).
1.3 Ventilatory control stability – Loop Gain

Loop gain is an engineering concept usefully applied in many negative feedback control systems, such as the chemoreflex control of breathing, to quantify the overall stability of the feedback control system. LG is the ratio of a control systems response relative to the magnitude of the disturbance eliciting the response, and incorporates three main components; controller gain, plant gain and the feedback delay between the controller and plant. In ventilatory control, controller gain reflects chemoreflex sensitivity \((\Delta V/\Delta PaCO_2 \text{ and/or } \Delta PaO_2)\), plant gain reflects the magnitude of change in blood gases per unit change in ventilation \((\Delta PaCO_2 \text{ and/or } \Delta PaO_2/\Delta V)\), and the delay gain is determined by circulatory delay and mixing of gases between the blood and tissue compartments (Dempsey et al., 2004). If the response to a ventilatory disturbance is smaller than the initial disturbance, LG is less than unity and ventilatory perturbations following a disturbance dampen over time. However, if LG is equal to unity or greater, disturbances become self-perpetuating and amplified respectively and respiratory control is inherently unstable (See (Khoo et al., 1982) for a review).

LG can be mathematically quantified by fitting measures of ventilation and blood gas disturbances to feedback models of ventilatory control to estimate gain parameters of the model. Measures obtained by traditional chemoreflex tests also provide some insight into controller, plant and overall LG. Chemoreflex effects on ventilatory control stability during sleep have been reviewed elsewhere (Dempsey, 2005, Dempsey et al., 2004) and therefore will only be discussed briefly.
LG is not a constant and changes dynamically in any non-steady state, such as over the course of obstructed breathing. This reflects a range of factors such as changing blood gases, lung volume, negative intra-thoracic pressures, upper airway dilator muscle activity and airway resistance, which can all influence the dynamics of chemoreflex responses (Younes, 2014). Traditional chemoreflex breathing tests measure only the gain component of overall LG under near steady-state conditions. These measures are nevertheless useful to consider in the context of respiratory control stability and overall LG.

When considering ventilatory control in the context of OSA the most important factors are CO₂ at eupnoea, chemoreflex sensitivity above eupnoea, the CO₂ apnoeic threshold and the modulatory effects of hypoxia on the CO₂ response curve. The position of eupnoea is most relevant as the slope above and below eupnoea during sleep or anaesthesia may not be the same (Dempsey, 2005), and even though central apnoea is not characteristic of OSA, the apnoeic threshold will determine the slope of the ventilatory response below eupnoea.

An elevated chemoreflex gain component of overall LG increases the magnitude of hyperventilation and hypocapnia after recovery from obstruction, and this could promote subsequent airway collapse through reduced upper airway dilator muscle activity that helps defend the airway from collapse (see (Dempsey, 2005, Dempsey et al., 2004) for review). Elevated LG may occur due to increased sensitivity (controller gain i.e. slope of the chemoreflex response curve) to either hypercapnia or hypoxia (above eupnoea) without any change in the position of eupnoea or the
apnoic threshold (Fig. 1.1 A). However, changes in the apnoic threshold and/or eupnoea can also narrow the CO$_2$ difference from these inflection points, and increase the sensitivity (controller gain) to CO$_2$ below eupnoea (Fig. 1.1 B), potentially exacerbating the reduction in upper airway muscle activity for any degree of hypocapnia. A leftward shift in the position of eupnoea without any change in controller gain above eupnoea would still increase the ventilatory response for any given level of CO$_2$ above eupnoea (Fig. 1.1 C1) to promote greater hyperventilation. However, if there was no change in controller gain below eupnoea (i.e. a concomitant leftward shift in the apnoic threshold), the CO$_2$ reserve between eupnoea and the apnoic threshold would increase due to the leftward steepening slope of the metabolic hyperbola (Fig. 1.1 C2). This effect could potentially help protect against respiratory instability. In addition, as eupnoea shifts leftward along the metabolic hyperbola plant gain is reduced (due to the changing slope of the metabolic hyperbola), such that a greater ventilatory increase would be required to achieve the same reduction in PaCO$_2$ (Fig. 1.1 D). Consequently, a leftward shift along the metabolic hyperbola without any change in controller gain either above or below eupnoea may promote respiratory stability via increased CO$_2$ reserve below eupnoea and the reduction in plant gain, despite the increased ventilatory response for any given level of CO$_2$ above eupnoea (Dempsey et al., 2004, Dempsey, 2005).

It is important to note that significant confusion may arise when considering ventilatory recruitment and CO$_2$ chemoreflex thresholds, since both terms have been used interchangeably to describe two different inflection points with different
neural mechanisms and effects on ventilatory control. The CO₂ chemoreflex threshold is only observed during waking hypocapnic breathing and demarcates the level of CO₂ at which the chemoreflex slope intersects the CO₂-independent wake level of ventilation (Duffin and Mahamed, 2003). A change in this threshold may not reflect any change in the neural mechanisms of chemoreflex control, but rather the neural mechanisms governing minimum wake ventilation associated with the so-called “wake stimulus” (Fig. 1.1 E). Given the slope above and below eupnoea while asleep may not be the same (Dempsey, 2005), changes in the waking CO₂ chemoreflex threshold may also not impact on chemoreflex control during sleep when the waking stimulus is absent. The neural mechanisms governing the ventilatory recruitment threshold, which demarcates the level of CO₂ required to re-instate rhythmic breathing during hypocapnia in sleep or anaesthesia, are uncertain. However, the ventilatory recruitment threshold can be dissociated from changes to ventilatory drive (Mahamed and Mitchell, 2008), suggesting a CO₂ “on switch” largely independent of chemoreflex drive (Fig. 1.1 F). Given central apnoea is atypical in OSA the ventilatory recruitment threshold may have little bearing on ventilatory control or LG in OSA pathophysiology.
Figure 1.1 Potential mechanisms via which changes to sleep chemoreflex control could increase loop gain.

A. Increased sensitivity/controller gain to either hypercapnia (above eupnoea) or combined hypercapnic hypoxia. B. A change in either eupnoea or apnoeic threshold to reduce CO₂ reserve and increase controller gain below eupnoea. C. A leftward shift along the metabolic hyperbola with no change in controller gain either above or below eupnoea; 1, increases the magnitude of ventilatory response to hypercapnia and 2, increases the CO₂ reserve below eupnoea. D. A leftward shift along the metabolic hyperbola also decreases plant gain; greater ventilatory overshoot is then required to achieve the same reduction in PaCO₂. E. Changes to the waking CO₂ chemoreflex threshold may be due to neural mechanisms
governing waking stimulation of ventilation, not chemoreflex control. F. The ventilatory recruitment threshold may be independent of chemoreflex drive, merely representing the CO$_2$ at which ventilation is reinstated after hypocapnic apnoea during sleep.

1.4 **Cyclical airway collapse via unstable ventilatory control**

During wakefulness the upper airways are protected by excitatory stimuli associated with arousal and brisk reflex responses which prevent airway collapse from intrapharyngeal negative pressure. The genioglossus is the largest upper airway dilating muscle and is thought to be critical for maintaining airway patency. OSA patients have more collapsible airways (Isono et al., 1997) and appear to maintain airway patency during wakefulness due to increased genioglossal muscle activity (Malhotra et al., 2000a) to compensate for increased loading and negative pressure driven by mechanoreceptor feedback (Fogel et al., 2000, Malhotra et al., 2000a). Neural regions associated with regulation of both arousal and ventilation, such as the reticular activating system, raphe nuclei or perhaps other supra-pontine regions, are also thought to provide tonic excitatory drive to breathing and is often called the “waking stimulus” (Orem et al., 2002, Horn and Waldrop, 1998). During wakefulness this stimuli maintains ventilatory rhythmicity below the arterial CO$_2$ (PaCO$_2$) required for chemostimulation (Fink, 1961). At sleep onset the waking stimulus is lost (Fink, 1961, Orem et al., 2002) and genioglossal responses to intrapharyngeal negative pressure changes dramatically decrease (Malhotra et al., 2000b, Wiegand et al., 1989). In conjunction with progressively decreasing chemoreflex sensitivity from wake to NREM to REM (Douglas et al., 1982a,
Douglas et al., 1982b, Schafer, 1998), sleep results in upper airway hypotonia, narrowing of the upper airway, an increase in upper airway resistance and CO₂ retention; causing PaCO₂ to rise 3-8 mmHg above the waking eupnoic baseline to establish a sleeping baseline (Henke et al., 1992). From this point ventilation is largely regulated by chemoreceptor drive.

In anaesthetised cats hypocapnia preferentially reduces hypoglossal more than phrenic neural drive (Haxhiu et al., 1986). During hypocapnia in anaesthetised rabbits, genioglossal muscle activity ceases before diaphragm activity, and during recovery from hypocapnia phasic activity returns to the genioglossus after the diaphragm. During hypercapnia however, increases in activity above baseline are higher in the genioglossus than in the diaphragm (Brouillette and Thach, 1980). This differential gain in upper airway versus inspiratory pump muscle drive could promote airway collapse in OSA, firstly by preferentially stimulating upper airway dilator muscles and facilitating hyperventilation post obstruction, and secondly by preferentially inhibiting drive to upper airway dilator muscles during the ensuing hypocapnia. High LG and unstable ventilatory control likely further exacerbates obstruction propensity by increasing the magnitude of change in ventilatory drive between the hyperventilation and subsequent hypoventilation phases.

Several lines of evidence support that hypocapnic airway hypotonia and high LG are both important pathogenic features in OSA. Patients treated for OSA with tracheotomy often continue to exhibit unstable breathing when the airway is totally bypassed (Guilleminault and Cummiskey, 1982, Onal and Lopata, 1982), as do
around 15% of OSA patients effectively treated with CPAP (Morgenthaler et al., 2006). Experimental stomal occlusion in tracheotomised OSA patients results in upper airway obstruction, arousal and sufficient hyperventilation to induce subsequent hypopnoeas and breathing pauses depending on the severity of hypocapnia (Iber et al., 1986). Furthermore, breathing pauses were prevented with added inspired hypercapnic hyperoxic gas (Iber et al., 1986), blunting peripheral chemoreflexes and preventing hypocapnic ventilatory depression. Electromyographic recordings in OSA patients also show that upper airway dilator muscles such as the genioglossus and alae nasi exhibit periodic reductions in drive, with nadir muscle activity coinciding with airway obstruction (Remmers et al., 1978, Suratt et al., 1985). Similarly, imaging of the airway during obstructive apnoeas shows the airway is enlarged during inspiration and passively collapses during end expiration (Morrell et al., 1998). Even in healthy participants not normally susceptible to sleep disordered breathing, mechanical hyperventilation to produce hypocapnia during sleep induces periodic breathing and both central and obstructive apnoeas (Badr et al., 1995, Sankri-Tarbichi et al., 2009), with pharyngeal narrowing occurring during the expiratory phase (Sankri-Tarbichi et al., 2009). Hypoxia also induces periodic breathing in healthy non-OSA participants due to hyperventilation induced hypocapnia, and peak upper airway resistance and obstruction occurs at the nadir of ventilatory drive (Onal et al., 1986).

1.5 High controller and loop gain in OSA – inherent or acquired?

The contribution of LG as an underlying cause of OSA in any individual patient likely depends on several factors and interactions with other mechanisms in OSA.
(Eckert et al., 2013, Wellman et al., 2013). An intrinsically high LG could be an important causal factor in some patients. However, an acquired elevation through obesity and plant gain effects, and/or through neuroplastic changes in controller gain would also be expected to exacerbate OSA in patients with pre-existing OSA.

Using pseudorandom binary stimulation with 4% CO2 to assess chemo-reflex control, Hudgel and colleagues found less stable breathing control in OSA patients compared to healthy weight non-OSA controls (Hudgel et al., 1998). This did not appear to be due to higher controller gain, implying higher plant gain, perhaps consistent with reduced lung volume in obese OSA patients versus non-obese controls (Hudgel et al., 1998). Plant gain and lung volume were not directly measured and it remains unclear if plant gain differs between OSA patients and non-OSA controls independent of weight. Nevertheless, obesity-related increases in plant gain, and intrinsically high controller gain both appear likely to contribute to OSA in at least some patients.

More recently with the use of proportional assist ventilation LG has been shown to correlate with the severity of OSA (Younes et al., 2001). When patients with a high LG were given supplemental oxygen to suppress peripheral chemoreceptor activity, both AHI and LG decreased, with no significant effect in patients with a low LG (Wellman et al., 2008). These findings strongly support that elevated LG through chemoreflex hypersensitivity plays an important causal role in many patients.
Chemoreflex responses in OSA are affected by a range of factors such as age (Kronenberg and Drage, 1973), gender (Jensen et al., 2005), weight (Narkiewicz et al., 1999a) and comorbidities such as diabetes (Weisbrod et al., 2005), hypertension (Trzebski et al., 1982) and cardiovascular disease (Narkiewicz et al., 1999b). However, when all of these factors were controlled Narkiewicz et al. found that sympathetic nerve activity was higher at baseline in awake OSA patients compared to well matched non-OSA controls. During hypoxia, OSA patients also exhibited a significantly greater increase in minute ventilation, heart rate and mean arterial pressure, indicating greater peripheral chemoreceptor sensitivity in OSA, but with no significant differences between groups in responses to hypercapnia (Narkiewicz et al., 1999c). Other well controlled (Foster et al., 2009) and large cohort (Sin et al., 2000) studies have also found no significant differences in waking hypercapnic ventilatory responses (HCVR) between OSA patients and non-OSA controls. However, obesity, a major risk factor in OSA, is an independent predictor of increased central (CO₂) waking chemoreflex sensitivity (Narkiewicz et al., 1999a). The mechanisms responsible for this are not certain, however the authors speculated that it may be due to leptin, as leptin is raised in obesity and mice deficient in leptin show severe blunting of the HCVR (O'Donnell et al., 2000). Although OSA does not appear to independently increase CO₂ chemoreflex sensitivity, obese OSA patients may nevertheless exhibit an increased sensitivity to both hypercapnia and hypoxia. Consistent with this, OSA patients have been shown to exhibit an increased dynamic ventilatory response to hypercapnic hypoxia during sleep (Younes et al., 2007). During sleep, OSA patients also exhibit a lower eupnoeic PETCO₂ but similar apnoeic threshold compared to healthy age, sex and
body mass index matched non-OSA controls (Salloum et al., 2010). These combined changes in OSA increase controller gain both above and below eupnoea during sleep, and reduce the CO₂ reserve between eupnoea and the apnoeic threshold.

Importantly, chemoreflex control abnormalities in OSA patients have been shown to normalise with the use of CPAP (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006). Spicuzza et al. found that 1 month of nasal CPAP significantly decreased the waking ventilatory response to normocapnic hypoxia in moderate to severe OSA from 1.08 ± 0.07 L/min/%SaO₂ prior to treatment to 0.53 ± 0.02 L/min/%SaO₂ after treatment. However, there was no effect on the normoxic hypercapnic ventilatory response (Spicuzza et al., 2006), a finding similar to that of Foster et al. who also found 1 month of CPAP had no effect on the HCVR (Foster et al., 2009). Given that OSA is not associated with an abnormal HCVR above eupnoea (Foster et al., 2009, Narkiewicz et al., 1999c, Sin et al., 2000), the lack of CPAP treatment effects on HCVR is not surprising. However, treatment effects below eupnoea and with hypercapnic hypoxia, a more relevant stimulus in OSA, have been demonstrated. Salloum et al. found 1 month of CPAP significantly increased the gap between eupnoea and the apnoeic threshold from 1.9 ± 0.8 mmHg to 3.7 ± 0.7 mmHg, significantly reducing controller gain below eupnoea during sleep (Salloum et al., 2010). Loewen et al. found that ≥5 months of CPAP significantly reduced the dynamic ventilatory response to combined hypercapnic hypoxia during sleep from 131% ± 95% to 52% ± 34% of baseline ventilation (Loewen et al., 2009). Additionally, OSA patients exhibiting treatment emergent
central sleep apnoea often show resolution within a few months of treatment, further supporting gradual treatment reversal of underlying unstable breathing control induced by OSA (Corcoran et al., 2009, Guilleminault and Cummiskey, 1982). In combination, these findings support that chemoreflex control abnormalities underlying increased controller gain and LG in OSA are predominantly not inherent, but rather induced by OSA itself and are reversible with normal treatment. Thus, elevated controller gain and overall LG appears likely to be both a cause of OSA in some patients, and a consequence of OSA that further exacerbates the disorder in a larger group of patients (Malhotra et al., 2014, Orr et al., 2014, Younes, 2014).

1.6 Neuroplasticity induced by OSA

Several physiological stimuli associated with OSA have been shown to induce neuroplasticity at different locations in the ventilatory neural network. The most thoroughly studied forms of ventilatory neuroplasticity are those induced by IH. While many different IH protocols have been used (Mateika and Sandhu, 2011), the pattern of IH most relevant to OSA is that of short periods (<5 mins) of moderate hypoxia (desaturation 70-90%) with sufficient time between repeated hypoxic episodes to achieve normal arterial re-oxygenation (1-5 min in humans). Studies fitting these criteria have used two main designs; acute intermittent hypoxia (AIH) with the number of episodes ranging from 3 to repeated application across an entire day, and chronic intermittent hypoxia (CIH), typically ranging from 3 days to several weeks with 8-10 hours of exposure per day to simulate recurring IH during
sleep. The type of exposure (acute vs chronic) influences the type and location of neuroplasticity induced by IH.

AIH can induce LTF in ventilatory motor neurons, characterised by a sustained increase in whole nerve recording burst amplitude upon returning to normoxia, which may persist for several hours following hypoxic exposure (MacFarlane and Mitchell, 2009). LTF is only induced following IH and not by the same cumulative duration of sustained hypoxia (Baker and Mitchell, 2000), or by intermittent hypercapnia (Bach and Mitchell, 1998). LTF following AIH in rats has been reported in the phrenic (Bach and Mitchell, 1996), hypoglossal (Fuller, 2005), glossopharyngeal (Cao et al., 2010) and laryngeal nerves (Bautista et al., 2012). Phrenic LTF (pLTF) is the most studied form of LTF and manifests as increased $V_I$, predominantly due to increased $V_T$, although increased breathing frequency is also sometimes reported (Ryan and Nolan, 2009a). The key stimulus inducing pLTF with IH appears to be peripheral chemoreceptor activation which stimulates serotonergic or adrenergic release from medullary raphe or locus coeruleus neurons (Kinkead et al., 2001), respectively, onto relevant respiratory motor neuron pools. In rats, there are two known G-protein coupled intracellular pathways which can be independently activated by AIH to induce pLTF, which are indistinguishable at the phenotypic level (either neural activity or ventilation). These pathways are called the Q and S pathways due to activation of either $G_q$ or $G_s$ protein coupled metabotropic receptors. The pathway activated depends on the severity of hypoxia experienced during IH, with the Q pathway activated by $PaO_2$ levels above 35mmHg while $PaO_2$ levels 30mmHg or below elicit the S pathway, with cross talk
inhibition between the pathways appearing to confer dominance of one pathway at a time (Nichols et al., 2012).

Episodic activation of the serotonin receptor 5-HT$_2$ or α1-adrenergic receptors elicits pLTF via activation of $G_q$ coupled receptors (Dale-Nagle et al., 2010a). Devinney et al. 2013 (Devinney et al., 2013) provide a detailed review of the intracellular mechanisms thought to underpin pLTF. In brief, the S pathway is activated by episodic activation of the serotonin receptor 5-HT$_7$ and adenosine 2A receptors coupled to $G_S$ receptors (Golder et al., 2008, Hoffman and Mitchell, 2013). The two $G$ receptors activate different intracellular pathways, however a key interaction appears to involve the regulation of NADPH oxidase activity and reactive oxygen species (ROS) formation (Devinney et al., 2013). The Q pathway is dependent on upregulation of NADPH oxidase, which in turn increases synthesis of ROS (MacFarlane and Mitchell, 2009). ROS regulate the balance of kinase and phosphatase activities and ROS formation during hypoxia/re-oxygenation in IH is thought to confer pattern sensitivity to pLTF by increasing kinase activation and phosphorylation of key proteins required for induction of pLTF (MacFarlane et al., 2008). During severe hypoxia the S pathway dominates due to constraint of the Q pathway via activation of protein kinase A, which is thought to inhibit NADPH oxidase activity and reduce ROS formation (Hoffman and Mitchell, 2013). The ultimate cellular changes resulting in pLTF via the Q and S pathway are still not certain, but glutamate receptor phosphorylation and/or an increase in glutamate receptor insertion into the motor neuron membrane may facilitate post-synaptic neurotransmitter uptake thereby conferring increased neural activity characteristic
of pLTF (Dale-Nagle et al., 2010a). Of particular note is that the Mitchell laboratory describe phrenic motor facilitation as a general term for enhanced phrenic motor output, whereas pLTF selectively refers to the form induced by AIH which activates the serotonergic Q pathway (Dale-Nagle et al., 2010a). For simplicity, we refer to all AIH induced phrenic motor facilitation as pLTF.

Hypoglossal LTF (hLTF) increases phasic genioglossal electromyographic activity, but has also been reported to facilitate both protrudor and retractor upper airway muscle activity in adult male rats (Fuller, 2005). Much less is known about hLTF than pLTF, although hLTF appears to share similar mechanisms to pLTF, with both dependent on episodic serotonin stimulation (Bach and Mitchell, 1996) and formation of ROS (MacFarlane and Mitchell, 2008). However in rats, pLTF is more consistently induced with IH, while hLTF is not induced in certain rat strains and is inhibited by low testosterone levels and vagal feedback (Baker-Herman and Strey, 2011).

Three or more days of IH (i.e. CIH) induces LTF of afferent carotid body activity, or so-called “sensory” LTF (sLTF) characterised by increased basal neural discharge (Peng et al., 2003), and also increases carotid body hypoxic sensitivity (Peng and Prabhakar, 2004). In rats and mice, CIH induced sLTF has been shown to be mediated by both serotonin and Angiotensin II. Episodic application of serotonin to ex-vivo carotid bodies activates 5-HT2 receptors which activate protein kinase C (Peng et al., 2006a), and episodic Angiotensin II application activates angiotensin type 1 receptors (Peng et al., 2011), both of which activate NADPH oxidase-2,
increase ROS production and induce sLTF (Peng et al., 2009, Peng et al., 2011). In adult male rats CIH has been shown to increase carotid body hypoxic sensitivity via an independent pathway to sLTF involving ROS mediated activation of endothelin converting enzyme, which then upregulates endothelin 1 leading to activation of endothelin A receptors (Peng et al., 2013). As with AIH induced motor facilitation, the ultimate mechanisms leading to sLTF and increased carotid body hypoxic sensitivity following CIH are also uncertain, but appear to be via independent mechanisms associated with ROS dependant recruitment of serotonin and endothelin-1, respectively (Prabhakar, 2011). Both sLTF and increased carotid body hypoxic sensitivity in rats are reversible within 10 days of returning to room air breathing (Peng et al., 2003, Peng and Prabhakar, 2004). Kumar et al. 2012 (Kumar and Prabhakar, 2012) provide a more comprehensive review of CIH induced carotid body neuroplasticity.

IH also induces changes at multiple sites within the brainstem involved in both the induction and maintenance of LTF and transduction of chemoafferent activity. Changes in firing rate and connectivity between raphe neurons, nucleus tractus solitarius and the ventral respiratory group neurons have been recorded in decerebrate and vagotomised cats following acute episodic carotid chemoreceptor stimulation and induction of pLTF (Morris et al., 2000). AIH in rats induces increased sympathetic nerve activity and pLTF, both of which were blocked by systemic pre-treatment with methysergide, a serotonin receptor antagonist (Dick et al., 2007). This suggests neuroplasticity within brainstem serotonergic pathways involved in sympatho-respiratory coupling may underlie the increased sympathetic
neural activity seen in OSA (Dick et al., 2007, Narkiewicz et al., 1999c). Carotid body sLTF following CIH would be expected to further augment sympathetic neural activity, which is thought to contribute to hypertension and cardiovascular disease in OSA (Prabhakar et al., 2007a). CIH in rats increases aminergic terminals and receptors in the hypoglossal and trigeminal (which innervates palatal muscles and contributes to airway patency) motor nuclei (Mody et al., 2011, Rukhadze et al., 2010). As aminergic excitatory drive to the hypoglossal motor neuron pool is state-dependant, being highest in wake and lowest in REM sleep (Fenik et al., 2005), CIH induced increases in aminergic terminals and receptors may contribute to the adaptive wake related increase in upper airway dilator muscle activity seen in OSA (Mezzanotte et al., 1992, Rukhadze et al., 2010).

In addition to IH, other stimuli experienced during repetitive obstructive apnoeas, such as episodic vagal feedback (Zhang et al., 2004), negative upper airway pressure (Ryan and Nolan, 2009c) and withdrawal of motor neuron activity (Baertsch and Baker-Herman, 2013), have been shown to induce LTF of various motor neurons and respiratory muscles in rats. Whether these forms of neuroplasticity also occur in humans has yet to be determined, so their relevance to OSA is uncertain.

1.7 Neuroplasticity in humans versus animal models

LTF appears to be particularly difficult to elicit in awake vagally intact preparations, and even under highly controlled experimental conditions in IH studies using anaesthetised animals, 10% of preparations fail to exhibit LTF (Fuller et al., 2000).
This may be partly due to the differential effects of vagal feedback on hLTF versus pLTF (Golder and Martinez, 2008). Arousal states may also be important given that LTF is difficult to induce during wakefulness, but progressively more readily induced in deepening NREM sleep in Lewis rats (Nakamura et al., 2010). This could reflect that raphe neurons fire at or near maximal activity during wakefulness and show reduced activity with decreasing arousal state, providing a greater dynamic range of firing potential during deep sleep (Mateika and Narwani, 2008). Sex, age and genetic variation are also known to influence whether LTF is induced in animals (Mitchell et al., 2001a), although similar sex differences in the manifestation of ventilatory LTF (vLTF) or sensitisation of the HVR have not been noted in humans (Syed et al., 2013, Wadhwa et al., 2008). Given many potentially important uncontrolled variables it is perhaps not surprising that many IH studies conducted in awake healthy humans have failed to elicit LTF (Diep et al., 2007, Jordan et al., 2002, Khodadadeh et al., 2006, Mateika et al., 2004, McEvoy et al., 1996). Studies in sleeping humans have also reported mixed results. Some report reduced upper airway resistance in OSA patients but no increase in ventilation (Aboubakr et al., 2001, Rowley et al., 2007), another reports increased genioglossal activity in non-OSA participants without increased ventilation (Chowdhuri et al., 2008), while another reports increased ventilation among snorers (Babcock and Badr, 1998). Variable results in humans may also be due to differences in hypoxia protocols, wake influences on ventilation and, perhaps most importantly, the level of concomitant CO₂ used during IH.
Olson and colleagues found that in unanaesthetised, unrestrained rats maintained in a poikilocapnic environment, increased ventilation following LTF induction lead to hypocapnia and subsequent central depression of LTF expression. When CO₂ was supplemented back to baseline levels, LTF was equivalent in magnitude to previous findings of phrenic LTF in anaesthetised, vagotomised and paralysed preparations (Olson et al., 2001). These data suggest that CO₂ levels are a critical determinant of LTF expression. Mateika and colleagues have proposed that since waking stimuli maintain ventilatory rhythmicity even below the arterial CO₂ required for chemostimulation (Fink, 1961), and as most earlier studies in humans had not determined the CO₂ chemoreceptor threshold prior to experimentation, low CO₂ during and after IH may have suppressed LTF expression (Mateika et al., 2004). The same group demonstrated that LTF of ventilation and peak genioglossus muscle activity could be elicited in awake healthy humans in the presence of sustained mild hypercapnia (5 mmHg), but that when normocapnia was reinstated LTF became masked with V₁ constrained to normocapnic baseline values (Harris et al., 2006). Given that hypoxia elicits cerebral vasodilation, Pierchala and colleagues theorised that prolonged hypoxia utilised in many human studies may result in cerebral hypocapnia and central ventilatory depression which may reduce the stimuli required to induce LTF. With the use of shorter isocapnic hypoxic exposures (<1 min) during sleep in healthy humans, the same authors demonstrated LTF and upper airway LTF (UALTF) via increased V₁ and V₉ and decreased upper airway resistance (Pierchala et al., 2008). Consequently, CO₂ levels and feedback appear to critically influence both the induction and expression of LTF.
Similar to animal studies, Mateika, Badr and colleagues have shown that LTF and increased HVR can be induced in humans, but with some notable differences. AIH in humans has been shown to induce both $\nu$LTF and $u$u$LTF$ (Harris et al., 2006, Lee et al., 2009, Pierchala et al., 2008) (thought due to pLTF and likely other pump muscle motor neurons, and hLTF, respectively) and to induce progressive augmentation (PA) of the HVR with each successive hypoxic episode (Harris et al., 2006, Lee et al., 2009). In humans, similar to animal studies, CO$_2$ feedback constrains $\nu$LTF in the presence of poikilocapnia (Olson et al., 2001, Harris et al., 2006) and AIH reduces eupnoeic $P_{ET}$CO$_2$ (Chowdhuri et al., 2010, Mateika et al., 2004). However, AIH also reduces the apnoeic threshold in rats (Mahamed and Mitchell, 2008), but not in humans (Chowdhuri et al., 2010). Consequently in humans, AIH reduces the CO$_2$ reserve between eupnoea and apnoea thereby increasing controller gain below eupnoea (Chowdhuri et al., 2010).

CIH in animals increases carotid body hypoxic sensitivity (Peng and Prabhakar, 2004) and the magnitude of pLTF induced by AIH (Peng and Prabhakar, 2003b), and greater $\nu$LTF and HVR with AIH in OSA patients compared to matched non-OSA is consistent with similar CIH effects in OSA (Lee et al., 2009). As with CIH in animals (Peng and Prabhakar, 2003b), these effects in humans appear to depend on ROS formation since the degree of oxidative stress during CIH correlates with HVR changes (Pialoux et al., 2009b), and administration of antioxidants prior to IH diminishes LTF and HVR responses in OSA patients (Lee et al., 2009). CIH induced $\nu$LTF and increased HVR in humans is also reversible within 4 days returning to room air breathing (Brugniaux et al., 2011, Pialoux et al., 2009b),
similar to the reversible nature of sLTF, pLTF and increased carotid body hypoxic sensitivity in rats upon return to room air breathing (Peng et al., 2003, Peng and Prabhakar, 2004, Reeves et al., 2003). Following 30 brief hypoxic voluntary apnoeas, awake humans exhibit increased muscle sympathetic nerve activity, blood pressure and acute HVR (Leuenberger et al., 2007). Similarly, AIH in rats induces serotonin dependent increased sympathetic nerve activity (Dick et al., 2007), while CIH in rats also induces hypertension (Lesske et al., 1997).

In rats and cats increased carotid body hypoxic sensitivity only occurs after a minimum of three days CIH (Rey et al., 2004, Peng and Prabhakar, 2004), while humans consistently show progressive augmentation of the HVR during AIH (Harris et al., 2006, Lee et al., 2009) and during CIH after the first day of exposure (Pialoux et al., 2009b). Whether these findings reflect species differences in carotid body neuroplasticity are uncertain. It is possible that the increased HVR seen in humans following AIH may be due to pLTF and sLTF raising basal ventilation, resulting in an increase in peak HVR. Alternatively, neuroplasticity within brainstem regions facilitating central integration of chemoafferent activity could increase the HVR (Dick et al., 2007, Reeves et al., 2003, Morris et al., 2000), without any change in carotid body chemosensitivity (Refer to (Duffin, 2007) for detailed discussion of variables affecting HVR measurements). The mechanisms of AIH induced increased HVR in humans and the contribution of central versus carotid body neuroplasticity warrants further investigation.
1.8 Does IH and LTF help stabilise the airway?

Given that hLTF increases upper airway muscle activity it has been suggested that hLTF could help to stabilise the airway and protect against OSA (Mahamed and Mitchell, 2007). However, since OSA persists without treatment and returns with treatment withdrawal, hLTF protective effects may be relatively modest. Mateika and Narwani have proposed that hLTF may be maladaptive and exacerbate OSA by further dilating the airways upon airway re-opening, increasing the magnitude of hyperventilation and hypocapnia, subsequently exaggerating the decrease in ventilatory and upper airway muscle activity and propensity for airway re-collapse (Mateika and Narwani, 2008). Rowley and colleagues partly tested this concept by examining upper airway resistance and the pressure at which the airway collapses during sleep in OSA patients before and after AIH exposure on CPAP. The authors found no evidence of vLTF or altered airway collapsibility, although upper airway resistance was significantly decreased suggestive of uALTF. As there was no change in airway collapsibility they concluded that uALTF does not help to stabilise the airway in OSA patients (Rowley et al., 2007).

A recent study of chronic effects of IH in OSA patients found that ten days of CIH induced vLTF and sensitised the HVR, and these changes were clearly not protective as OSA severity increased (Yokhana et al., 2012). HVR changes correlated with increases in AHI, mixed apnoea frequency and apnoea duration and were also accompanied by greater oesophageal pressure and VT after apnoea termination, indicating greater hyperventilation and more unstable ventilatory control following daytime CIH exposure (Yokhana et al., 2012). vLTF induced by
daytime CIH did not correlate with any sleep ventilatory parameter, or help to stabilize breathing in sleep. The same laboratory also reported that AIH presented during sleep in male and female OSA patients on therapeutic CPAP induced γLTF and increased AHI without increasing the HVR (Syed et al., 2013, Wadhwa et al., 2008). Increased AHI with increased HVR induced by experimental CIH (Yokhana et al., 2012) is consistent with CIH effects in untreated OSA where higher LG correlates with increased AHI (Younes et al., 2001), and hyperoxic suppression of carotid body activity reduces both LG and AHI (Wellman et al., 2008). In combination, these findings support that both γLTF and increased HVR induced by IH contribute to increasing LG, respiratory instability and AHI.

An important reason hLTF may not stabilise the airway in OSA could be that to do so would require hLTF expression independent of chemoreceptor drive for augmented muscle activity to be maintained during the post obstruction hypocapnic hypoventilation phase. Hypocapnic suppression of LTF has been demonstrated in both humans and rats (Harris et al., 2006, Olson et al., 2001), and most likely reflects post synaptic changes underpinning LTF at the motor neuron level. Given motor neuron LTF is thought to be due to facilitation of post-synaptic excitatory neurotransmission (Mitchell et al., 2001a), reduced excitatory stimuli during hypocapnia would render LTF incapable of amplifying motor neuron output. Nevertheless, although suppressed during hypocapnia, robust pLTF has been demonstrated once normocapnia or hypercapnia are re-instated (McGuire et al., 2007). Consequently, hLTF would be expected to be inhibited during hypocapnic hypoventilation and to be preferentially expressed during normocapnia and
hypercapnia. Although increased EMG activity via hLTF would not necessarily translate into airway opening, which also depends on complex interactions between external collapsing forces, intrapharyngeal pressure and upper airway dilating forces, the expression of hLTF only during normocapnia and hypercapnia would likely facilitate hyperventilation by further dilating the airways, potentially exacerbating post-obstruction hypocapnia. For this reason hLTF could promote cyclical obstruction, even without changes in chemoreflex control or vLTF.

1.9 IH increases LG

Despite a large body of work examining IH and LTF effects relevant to respiratory muscle control and OSA pathophysiology, currently there is only indirect evidence to support that LTF is expressed in OSA. A comparison of resting ventilatory parameters between normotensive and hypertensive OSA patients and non-OSA controls found that hypertensive OSA patients had a higher resting $V_T$, suggesting higher tonic resting chemoreceptor drive than in normotensive OSA patients and controls (Loredo et al., 2001). Modified rebreathing tests in OSA patients compared to age, race, sex and weight matched controls found OSA patients have a higher resting $V_I$ due to a higher $V_T$ (Mateika and Ellythy, 2003). OSA patients also exhibit a lower eupnoeic $P_{ETCO_2}$ (Salloum et al., 2010) consistent with vLTF producing a leftward shift in eupnoea along the metabolic hyperbola via $CO_2$ feedback.

Perhaps the strongest evidence that repetitive IH in OSA has long-lasting neuroplastic effects which increase LG comes from studies showing that abnormal chemoreflex responses induced by experimental IH are also present in OSA
patients and are reversed by CPAP treatment (Fig. 1.2). This suggests induced rather than inherent chemoreflex control disturbances. These disturbances include increased HVR (Narkiewicz et al., 1999c, Spicuzza et al., 2006), reduced eupnoeic $P_{ETCO_2}$ (Salloum et al., 2010) and an increased ventilatory response to combined hypercapnic hypoxia (Loewen et al., 2009, Younes et al., 2007, Mateika et al., 2004). These changes combine to reduce the CO$_2$ reserve between eupnoea and the apnoeic threshold, and increase controller gain both below and above eupnoea during hypercapnic hypoxia. Although the leftward shift along the metabolic hyperbola would decrease plant gain, this effect is likely to be small with the net effect remaining increased controller and overall LG. As a consequence, the magnitude of post-obstruction hyperventilation would be expected to increase, exacerbating post-obstruction hypocapnia and upper airway hypotonia, potentially promoting airway collapse and increasing OSA severity. This is further supported by findings of increased AHI following CIH in OSA (Yokhana et al., 2012), and reduced AHI and apnoea duration following 30 days of antioxidant treatment alone without CPAP, suggesting that ROS with IH contributes to cyclical airway obstruction in OSA (Sadasivam et al., 2011).
Figure 1.2. Schema of potential changes induced by IH in OSA and effects on ventilatory control during sleep.

Black lines represent ventilatory response to CO₂ during sleep under normoxic and hypoxic conditions in healthy normal controls. Grey lines represent chemoreflex control changes induced by experimental IH. Equivalent chemoreflex control changes are seen in OSA patients, and normalise following CPAP treatment. Under normal conditions, prior to IH, ventilation changes linearly with changing CO₂ above and below eupnoea down to the apnoeic threshold where ventilatory drive ceases. Hypoxia increases the sensitivity to CO₂ and causes eupnoea to shift leftward along the metabolic hyperbola, reducing the CO₂ reserve and increasing controller gain below eupnoea. IH induced ventilatory long-term facilitation (vLTF) increases minute ventilation but this effect is constrained by CO₂ feedback causing a leftward
shift along the metabolic hyperbola during poikilocapnic normoxia. Although IH
does not increase sensitivity (controller gain) to CO₂ above eupnoea, ventilatory
LTF reduces the CO₂ reserve and increases controller gain below eupnoea under
normoxia. CIH sensitises the ventilatory response to hypoxia, increasing controller
gain above eupnoea under hypercapnic hypoxic conditions. Due to CO₂ feedback,
under hypoxic conditions after IH the point of eupnoea would shift further left along
the metabolic hyperbola, further decreasing the CO₂ reserve below eupnoea and
further increasing controller gain below eupnoea. Dotted lines represent ventilatory
response to combined hypercapnic hypoxia (hypothetical chemoreflex drive at
airway re-opening) before versus after IH induced changes in chemoreflex control.
These changes would be expected to increase controller and overall loop gain to
cause a greater degree of post-obstruction hyperventilation, hypocapnia and upper
airway hypotonia, thereby promoting airway re-obstruction and increased AHI (Data
compiled from (Chowdhuri et al., 2010, Harris et al., 2006, Lee et al., 2009, Loewen
et al., 2009, Mateika et al., 2004, Narkiewicz et al., 1999c, Olson et al., 2001,
Pierchala et al., 2008, Salloum et al., 2010, Spicuzza et al., 2006, Younes et al.,
2007)).

It is possible that under certain conditions neuroplastic changes which increase
controller and LG (νLTF and increased HVR) may be depressed while hLTF
remains expressed. For example, despite airway deficits OSA patients frequently
exhibit prolonged periods of stable breathing in sleep with increased genioglossal
muscle activity, particularly during slow wave sleep (Jordan et al., 2009, McSharry
et al., 2013). This could at least partly reflect blunted chemoreflexes and lower
controller gain above eupnoea during slow wave sleep (Douglas et al., 1982a, Douglas et al., 1982b). As chemoreflex drive drops, $V_1$ decreases (Stradling et al., 1985) and $\text{PaCO}_2$ increases with a rightward shift along the metabolic hyperbola (Fig. 1.3). If the apnoeic threshold remains unchanged this would widen the $\text{CO}_2$ reserve and decrease controller gain below eupnoea. Although there would be a small concomitant increase in plant gain (i.e. a smaller ventilatory overshoot would cause a greater reduction in $\text{PaCO}_2$) this is unlikely to fully counteract reduced controller gain both above and below eupnoea. Consequently, overall LG would be expected to decrease during slow wave sleep leading to reduced hyperventilation and hypoventilation and more stable $\text{CO}_2$. More stable and elevated $\text{CO}_2$ would likely also facilitate ongoing expression of hLTF. When combined with an increased arousal threshold (more difficult to wake) in slow wave sleep (Berry and Gleeson, 1997), hLTF could more effectively augment upper airway muscle activity towards sustaining stable breathing without arousal. Thus, a combination of interacting factors may underpin markedly improved OSA severity in slow wave sleep.
Figure 1.3. Possible effects of slow wave sleep on LG and hLTF expression.  
A. During slow wave sleep chemoreflex sensitivity above eupnoea decreases, reducing $V_i$ and increasing $P_{ET}CO_2$, resulting in a rightward shift of eupnoea along the metabolic hyperbola. This would widen the CO$_2$ reserve and reduce controller gain below eupnoea. Reduced controller gain above and below eupnoea would reduce overall LG. In addition, an elevated arousal threshold ($T_A$ i.e. more difficult to awaken) in slow wave sleep allows for greater tolerance of augmented ventilation without arousal. B. Reduced LG would stabilise $V_i$ and $P_{ET}CO_2$. Higher and more stable CO$_2$ would facilitate continual expression of hLTF. In conjunction with reduced $T_A$, hLTF would also facilitate increased UA muscle activity without arousal, thus allowing more stable breathing in slow wave sleep.
1.10 Clinical significance

Treatments to reduce LG have significant future potential given frequent CPAP non-adherence (Weaver and Grunstein, 2008) and emerging methods to quantify variable and interacting deficits underpinning OSA (Eckert et al., 2013). Oxygen therapy and pharmacological agents such as acetazolamide show significant promise (Edwards et al., 2012, Wellman et al., 2008), although potential side effects may make long-term treatment unsuitable. Elevated plant gain from reduced lung volume in obesity may be difficult to correct without weight loss. However, controller gain abnormalities induced by IH could be more important in many patients. Given these appear to normalise with treatment (Salloum et al., 2010, Spicuzza et al., 2006), long-term pharmacological manipulation of LG may not be necessary, and other traits could potentially be treated with suitably targeted combination therapies following normalisation of LG. Reduced LG post-treatment might also influence CPAP requirements and warrant re-titration, potentially improving long-term CPAP tolerance and adherence.

The neuroplastic changes to chemoreflex control which increase controller gain and therefore LG are dependent on ROS production (MacFarlane and Mitchell, 2008, Peng et al., 2009, Peng et al., 2006b). Pre-treatment with antioxidants in both rats and humans can block IH induced neuroplasticity (Lee et al., 2009, Peng and Prabhakar, 2003a, Macfarlane et al., 2014). In addition, antioxidant administration without CPAP has been shown to reduce AHI and apnoea duration (Sadasivam et al., 2011). Furthermore, ROS are thought to play a causal role in many of the OSA
associated pathologies such as cardiovascular disease (Bradley and Floras, 2009), neurologic pathologies (Yang et al., 2013, Daulatzai, 2012) and diabetes (Polak et al., 2013). Antioxidant treatment alone may be sub-optimal, but in patients not able to tolerate CPAP or other treatments, could nevertheless help to stabilise breathing, reduce OSA severity and ameliorate other ROS mediated comorbidities.

1.11 Summary and future research directions

Unstable ventilatory control with high LG is now recognised to be one of the key non-anatomical factors underpinning cyclical airway obstruction in OSA (Wellman et al., 2013), predominantly via increasing the magnitude of post-obstruction hyperventilation promoting hypocapnic upper airway muscle hypotonia and ongoing obstruction. OSA patients exhibit an elevated HVR (Narkiewicz et al., 1999c) and a reduced eupnoic $P_{ET}CO_2$ (Salloum et al., 2010) which combine to increase controller gain and overall LG. These abnormalities are reversed with CPAP treatment (Salloum et al., 2010, Spicuzza et al., 2006), indicating they are an induced consequence of OSA rather than a primary underlying causal trait. Hence, LG is both an inherent and induced trait, and both a cause and a consequence of OSA (Orr et al., 2014, Younes, 2014, Malhotra et al., 2014). Although it has been suggested that IH induced $\vee$LTF and $h$LTF could potentially help protect against OSA (Mahamed and Mitchell, 2007, Mateika and Syed, 2013), several human studies do not support this, and have instead shown an increase in AHI and thus an exacerbation of OSA (Syed et al., 2013, Yokhana et al., 2012, Rowley et al., 2007). Due to ventilatory feedback in a poikilocapnic environment, $\vee$LTF reduces eupnoeic $P_{ET}CO_2$ (Olson et al., 2001) and CO$_2$ reserve (Chowdhuri et al., 2010, Harris et al.,
(2006), thereby increasing controller gain below eupnoea. In conjunction with IH induced sensitisation of the HVR (Harris et al., 2006), controller gain above eupnoea during hypercapnic hypoxia also increases (Mateika et al., 2004). This combination of IH induced VLTF and increased HVR is likely the mechanism causing elevated controller gain and LG in OSA. Motor neuron LTF is dependent on chemoreflex drive and is inhibited by hypocapnia (McGuire et al., 2007). Consequently, hLTF is most likely incapable of facilitating upper airway dilator muscle activity during the post-obstruction hypocapnic hypoventilation period, and possibly exacerbates hyperventilation during the hypercapnic hyperventilation phase by facilitating airway dilation.

CPAP treatment in OSA patients reduces the HVR and normalises eupnoeic $P_{ET}CO_2$, thereby reducing controller gain both above and below eupnoea (Salloum et al., 2010, Spicuzza et al., 2006). Similarly, IH induced sLTF and increased carotid body hypoxic sensitivity in rats (Peng and Prabhakar, 2003a, Peng and Prabhakar, 2004), and VLTF and increased HVR in humans is reversible within several days of returning to room air breathing (Brugniaux et al., 2011, Pialoux et al., 2009b). Collectively, these findings support that conventional CPAP treatment allows reversal of ventilatory neuroplasticity underpinning elevated LG. However, there remains an ongoing debate regarding the contribution of induced controller gain abnormalities versus inherent LG traits in OSA (Orr et al., 2014, Younes, 2014). Further work in this area appears to be warranted given elevated LG in many OSA patients (Eckert et al., 2013), CPAP reversal effects, non-CPAP treatment approaches to lower LG (Edwards et al., 2012), and ongoing uncertainty
regarding the contribution of inherent plant and controller gain versus induced
controller gain abnormalities in OSA.

Airway obstruction induces concomitant hypercapnic hypoxia rather than IH alone
and yet there are very few human or animal studies of the effects of intermittent
hypercapnic hypoxia on ventilatory neuroplasticity. The chosen level of isocapnia
during and following IH significantly impacts both the induction and expression of
LTF (Harris et al., 2006). Intermittent hypercapnia alone also induces a form of
noradrenergic dependent respiratory depression rather than serotonergic LTF
(Bach and Mitchell, 1998, Bach and Mitchell, 1996). Consequently the effects of
intermittent hypercapnic hypoxia may well differ from those of IH. Further studies of
intermittent hypercapnic hypoxia and the role of CO₂ in IH induced neuroplasticity
are needed to better understand the implications in OSA pathophysiology.

Due to the significant role that ROS play in neuroplasticity of ventilatory control
(MacFarlane et al., 2008, MacFarlane and Mitchell, 2009, Peng et al., 2011) and
comorbidities associated with OSA (Bradley and Floras, 2009, Polak et al., 2013),
and evidence that antioxidants can both block IH induced neuroplasticity (Lee et al.,
2009, Macfarlane et al., 2014, Peng and Prabhakar, 2003a) and reduce AHI in
untreated OSA patients (Sadasivam et al., 2011), future research investigating
potential therapeutic benefits of antioxidant treatment in OSA patients is warranted.
CHAPTER 2. Intermittent hypercapnic hypoxia during sleep does not induce long-term facilitation in healthy males

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Running Title: Intermittent hypercapnic hypoxia during sleep does not induce long-term facilitation in healthy males

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ii. permission is granted for the candidate to include the publication in the thesis; and

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2.1 Abstract

The role that intermittent hypoxia induced ventilatory neuroplasticity may play in the pathophysiology of obstructive sleep apnoea has been the focus of much research. However, although the level of concomitant CO$_2$ and arousal state are known to critically influence the effects of intermittent hypoxia, few studies have investigated the effects of intermittent hypercapnic hypoxia, a gas condition more relevant to sleep apnoea, and none have examined intermittent hypercapnic hypoxia effects during sleep. Thus, the primary purpose of this study was to investigate if intermittent hypercapnic hypoxia during sleep induces neuroplasticity (long-term facilitation and increased chemoresponsiveness) in humans. 12 healthy males were exposed to either intermittent hypercapnic hypoxia (24 x 30s episodes of 3% CO$_2$ and 3.0 ± 0.2% O$_2$) or control (same protocol but using intermittent medical air) during sleep in a randomised cross-over design with a two week intervening wash-out period. Minute ventilation, end-tidal CO$_2$, saturation of oxygen, breath times, upper airway resistance, and genioglossal and diaphragm electromyograms were compared between 10 min of stable stage 2 sleep preceding gas exposure, gas and intervening room air periods during the gas exposure protocol and during 1 hour of recovery breathing room air. Ventilatory changes were assessed from absolute value changes and as percent change from baseline during all stages of sleep as well as restricted to stage 2 sleep alone to control for potential confounding by sleep stage effects. There was no significant difference between conditions across time to indicate long-term facilitation of ventilation, genioglossal or diaphragm electromyogram activity, and no change in ventilatory responses from first to last gas exposure to suggest any change in chemoresponsiveness. These
findings do not support that short-term acute intermittent hypercapnic hypoxia during sleep induces neuroplastic changes in ventilatory control in healthy males.

2.2 Introduction

Obstructive sleep apnoea (OSA) is characterised by repeated episodes of upper airway obstruction causing concomitant hypercapnia and hypoxia. Experimental intermittent hypoxia (IH) can induce neuroplastic changes in ventilatory neural control (Harris et al., 2006, Peng and Prabhakar, 2004). Although much research has focused on the possible role of ventilatory neuroplasticity in OSA pathophysiology, it remains uncertain if neuroplasticity naturally occurs in OSA. In addition, both arousal state and the level of concomitant CO₂ utilised during IH are known to be important determinants of whether neuroplasticity is induced (Harris et al., 2006, Olson et al., 2001, Nakamura et al., 2010, Kinkead et al., 2001), yet very few studies of combined intermittent hypercapnic hypoxia (IHH) on ventilatory neuroplasticity have been published in either animals or humans (Diep et al., 2007, Waters and Tinworth, 2003), and none during sleep. Before the relevance of IHH to OSA pathophysiology can be determined, it first remains to be demonstrated that IHH, comparable to that occurring in OSA, can induce ventilatory neuroplasticity during sleep in healthy humans free from potential confounding effects of pre-existing OSA.

Long-term facilitation (LTF) of ventilatory motor neuronal output from hypoglossal and phrenic motor neuron pools can be induced following acute IH (AIH; ≥3 episodes to an entire day), and is characterised by a sustained increase in phasic
neural burst amplitude to the same level of chemical stimuli following IH (Bach and Mitchell, 1996, Mitchell et al., 2001a). Phrenic LTF (pLTF) manifests as an increase in minute ventilation ($V_i$) (McGuire et al., 2008a) and hypoglossal LTF (hLTF) as an increase in genioglossal electromyogram (EMGgg) phasic burst amplitude and reduced upper airway resistance ($R_{UA}$) (Chowdhuri et al., 2008). In intact animals these phenomena are referred to as ventilatory LTF ($\nu$LTF) and upper airway LTF ($\nu_{UA}$LTF). In rats ≥3 days of 8 hours of IH induce an increase in the carotid bodies hypoxic response (Peng et al., 2001). However during wakefulness humans experience an increase in the hypoxic ventilatory response following acute IH (Lee et al., 2009), although this has not been reported following AIH during sleep in humans.

Results of IH studies in humans have been highly variable (Diep et al., 2007, Jordan et al., 2002, Babcock and Badr, 1998, Harris et al., 2006). However, animal studies highlight that the presence or absence of neuroplasticity effects are highly sensitive to the induction protocol, with factors such as intensity of hypoxia (Nichols et al., 2012), duration and frequency/pattern of hypoxic episodes (Wilkerson and Mitchell, 2009, Mitchell et al., 2001a, Waters and Tinworth, 2003), level of concomitant CO$_2$ (Kinkead et al., 2001, Olson et al., 2001), age (Zabka et al., 2001a), sex (Zabka et al., 2001b, Zabka et al., 2005), genetics (Baker-Herman et al., 2010), arousal state (Nakamura et al., 2010), arousal from sleep (McGuire et al., 2008b) and negative pressure stimuli (Ryan and Nolan, 2009b) all known to be important determinants of LTF induction. Therefore, differing protocols could explain variable results in humans (Mateika and Sandhu, 2011). In humans few
studies have mimicked the blood gas disturbances experienced in OSA with all but one study assessing hypercapnic hypoxia as occurs during airway obstruction (Diep et al., 2007). However, this study only examined responses during wakefulness where wake ventilatory drive inputs could confound chemo respiratory drive, which dominates ventilatory control during sleep. Therefore, although IH appears to be capable of inducing LTF and increased hypoxic sensitivity in humans, it remains unclear if the more relevant stimulus of IHH during sleep can also induce LTF and alter chemosensitivity. Consequently, the aim of this study was to examine if a protocol designed to mimic the blood gas perturbations and frequent arousals experienced in OSA would induce LTF, increase hypoxic sensitivity, or other detectable changes in ventilatory control during sleep in healthy males, free from potential confounding by pre-existing neuroplastic changes in ventilatory control that could already be present in OSA patients.

2.3  Methods

2.3.1  Participants

Twelve healthy male volunteers between the ages of 18-45 gave written informed consent and participated in the study. All were non-smokers, demonstrated normal lung function (>80% predicted for both forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC); JLab software version 4.53; Compactlab, Jaeger, Wuerzburg, Germany), were not born or lived at high altitude, took no regular medications, were self-reported non-snorers and had no history of any other symptom suggestive of a sleep disorder. Participants were asked to abstain
from alcohol and caffeine for the whole day and to avoid strenuous exercise during the latter half of the day prior to each study. Participants were also asked to eat dinner at the same time of night prior to each study, which was a minimum of 4 hours prior to commencement of the study. The study was approved by the University of Adelaide and the Southern Health Human Research and Ethics Committees.

2.3.2 Study design

This protocol comprised of three night studies, each separated by a minimum of two weeks (Figure 2.1). The first night was split into two halves. During the first 3 hours participants underwent a standard PSG to rule out those with sleep disorders and those with difficulty sleeping in the laboratory environment. The second half of the first night was used to determine the subject specific experimental gas composition to use in subsequent experiments. The 2nd and 3rd nights comprised an experimental night where participants breathed IHH gas, and a control night of intermittent medical air, conducted in random order and separated by two weeks. If there was insufficient sleep to complete the protocol on any night, the subject returned a minimum of two weeks later for a fourth night to repeat the protocol. Participants slept in a sound-insulated room and were studied by 2 research staff from an adjacent room via continuous monitoring of respiration, sleep and a video display from infra-red camera overlying the bed.
Figure 2.1. Protocol flow diagram.
Sleep was measured by two channels of electroencephalogram (C₄/A₁, C₃/A₂), left and right electro-occulogram, submental electromyogram (EMG) and electrocardiogram. Respiration was monitored via measurement of nasal pressure (PTAF2, Pro-Tech Services, Woodinville, WA), oronasal airflow (nasal oral thermistor), arterial oxyhemoglobin saturation via ear pulse oxymetry (POET II model 602-3; Criticare Systems, Waukesha, WI), and chest and abdominal wall motion (piezo-electric bands). A tracheal microphone was used to monitor snoring. Body position and bilateral anterior tibialis electromyograms were also continuously recorded. Data were acquired on a Compumedics data acquisition system (E-series, Compumedics Inc., Melbourne, Australia).

For the second half of the preliminary night, all equipment from the PSG remained in place except the nasal cannula and thermistor were removed, the participant’s mouth was taped shut to prevent mouth breathing and a nasal mask (Gel mask, Respironics, Marrysville, PA) was fitted with a two way non-rebreathing valve attached (series 2600, Hans Rudolph, Kansas City, MO). CO₂ was continuously sampled at the mask (Capstar-100, CWE Inc., PA) and on the inspiratory side the breathing valve was connected to a pneumotachograph (PT16, Jaeger, Germany) to measure flow, which was attached to a Gatlin-shaped valve system (series 2440C, Hans Rudolph) for delivery of inspiratory gases. The Gatlin-shaped valve consisted of one port attached to the pneumotachograph and four inputs, three of which were open to room air while the fourth was connected to a foil bag (40 L, Scholle Industries, Adelaide, Australia) containing a hypercapnic hypoxic (HH) gas.
mixture. Each port could be rapidly occluded or opened with a solenoid and pneumatically driven balloon valve. Only one port input was open at a time and all changes between ports were conducted during expiration.

For the main experimental nights the same equipment, acquisition and analysis of sleep was used as described above. Participants were fitted with the same nasal mask as for the preliminary night along with mouth tape, with saturation of oxygen, flow and CO₂ recorded as above. Genioglossal EMG (EMGgg) was also recorded using two intramuscular electrodes (316SS5T wire, Medwire, Mt. Vernon, NY) inserted 4mm either side of the frenulum to a depth of 1-1.5 cm after surface anesthesia with 4% lignocaine (Eckert et al., 2007b). Surface diaphragmatic EMG (EMGdia) was recorded from a pair of surface EMG electrodes placed in the right sixth, seventh or eighth intercostal spaces adjacent to the costal margin that produced the highest inspiratory phasic activity. These positions were chosen to minimise intercostal and abdominal muscle artifact on the diaphragm signal. Epiglottic pressure (Pepi) was measured with a thin air-perfused nasal catheter [see (Hilditch et al., 2008) for further detail]. This catheter was advanced 1cm below the tongue base under direct visualisation, taped at the nose, and connected to a pressure transducer (MP45; Valindyne Engineering, Northridge, CA) after both nostrils were decongested with xylometazoline hydrochloride nasal spray (Otrivin, Novartis Australasia, Rowville, Victoria, Australia) and anesthetized (2% lignocaine spray). Mask pressure (P_{mask}) and end-tidal CO₂ (P_{ET}CO₂) were measured continually from the mask (P_{mask}; MP45, Valindyne Engineering, Northridge, CA, P_{ET}CO₂; Capstar-100, CWE). The mask was attached to the same breathing circuit
as for the preliminary night, but using a 300L foil bag in place of a 40L bag on the preliminary night.

Pneumotachograph airflow, EMGgg, EMGdia, Pepi, \( P_{\text{MASK}} \) and \( CO_2 \) signals beyond conventional channels recorded on the clinical sleep acquisition system (Compumedics) were recorded on a separate 32-channel Windaq (DI-720 DATAQ Instruments Inc., OH, USA) acquisition system at sampling rates of 1 kHz for EMG and timing signals and 200 Hz for all other channels. Time matching between recording systems was achieved through the use of a computer generated timing signal simultaneously recorded on both systems at 1 kHz on the Windaq system and 512 Hz on the Compumedics system.

2.3.4 Protocol

Preliminary night: Recordings began between 2200 and 0000 and finished a minimum of 3 hours later with a minimum of one complete sleep cycle (Figure 2.1). Participants were instructed to sleep in the supine position as much as possible. All studies were scored by one of two technicians using arousal and respiratory event scoring according to standard criteria (Chicago) (AASM Task Force, 1999), and using a total sleep apnoea-hypopnoea index (AHI) cut-off of \( \geq 15 \) events/hr for the diagnosis of OSA. Participants showing evidence of sleep or breathing disorders were excluded from further participation in the study and referred for separate clinical follow-up.

During the second half of the preliminary night participants slept in the lateral position to help achieve a more stable airway less likely to obstruct and cause
blood gas disturbances beyond those of the experimental gas delivery protocol. Participants chose either their left or right side and remained in that position for the duration of the protocol. A 40L foil bag was filled with a HH gas mixture starting with 6% O₂, 3% CO₂ and the balance N₂. During stable stage 2 sleep the breathing circuit was switched from room air to the bag for 30 seconds (Figure 2.1). The bag was then refilled with progressively lower O₂ content until consistent oxygen desaturations of 80-85% were achieved. This same gas mixture was then used for the subjects subsequent experimental study night.

Main protocol: Participants slept in the lateral position and on the same side as for the second half of the preliminary night. Following 10 minutes of stage 2 sleep (not necessarily continuous) participants were exposed to twenty four 30 s episodes of medical air or the subject specific HH gas determined from the preliminary night, initiated during sleep and separated by 2 min breathing room air (Figure 2.1). If the subject aroused at any stage subjects were immediately returned to room air and the HH delivery protocol was paused until a minimum of 2 min stable sleep resumed. The initial episode was always given in stage 2 sleep, however thereafter the protocol was continued irrespective of sleep stage. Recording continued while participants breathed room air for 60 min of recovery following the last HH episode.

2.3.5 Data Analysis

The inspiratory flow signal (pneumotachograph) was digitally integrated to give inspiratory tidal volume (\(V_T\)) and minute ventilation (\(V_I\)) breath-by-breath. Inspiratory (\(T_I\)), expiratory (\(T_E\)), total breath (\(T_{TOT}\)) times, breathing frequency (\(F_B\)), peak inspiratory flow (PIF), epiglottic pressure (Pepi) and upper airway resistance
(RUA) were determined on a breath-by-breath basis using custom software used previously (Eckert et al., 2006). Breath-by-breath measurements of expiratory (end-tidal) partial pressure of CO₂ (P_{ET}CO₂) were determined from the nadir and peak in mask CO₂ after adjusting for gas sampling delay. Both EMG_{gg} and EMG_{dia} were band-pass filtered (0.03 – 1kHz), rectified, and moving time averaged with a time constant of 100 ms. For each breath, the end-expiratory tonic (tonic) and peak inspiratory phasic (phasic) EMG activities were determined from the moving time averaged signal.

2.3.6 Selection of Breaths

Cross-correlation between breath-by-breath measures of V₁ and SaO₂, and V₁ P_{ET}CO₂ signals were used to calculate time-offsets to optimally adjust for SaO₂ circulatory delay, and circuit dead-space and sampling delays in SaO₂ and P_{ET}CO₂ signals respectively. This allowed temporal alignment of changes in V₁ to the corresponding changes in SaO₂ and P_{ET}CO₂ following the onset and offset of gas delivery. Data from periods with poor signal quality or clear EMG, Pepi, SaO₂ or P_{ET}CO₂ artifacts (e.g. wire dislodgement, blocked epiglottic pressure catheter, probe off) were excluded. Remaining data were then combined for analysis of all sleep stages combined as well as for stage 2 sleep alone to allow more direct comparisons with baseline stage 2 sleep data.

Following cross-correlation adjustments of SaO₂ and P_{ET}CO₂ data for signal delays, breath-by-breath ventilatory measurements were averaged over the 10 min baseline period, each 30 s of gas exposure, the last 30 s of each intervening room air period and each 5 mins over the hour recovery following the last gas exposure.
period. To examine potential changes in chemoreflex responses from the first gas exposure to the last, breath by breath measurements were compared between the 5 breaths preceding gas onset, the 6 breaths during gas exposure and then the breaths during the 70 s immediately after gas offset and return to room air breathing.

2.3.7 Statistical Analysis

For each variable; $V_I$, $V_T$, $T_I$, $T_E$, $T_{TOT}$, $F_B$, PIF, Pepi, $R_{UA}$, inspiratory and expiratory EMG for both genioglossus and diaphragm ($\text{Insp}$ and $\text{Exp}$ EMG$_{gg}$ and EMG$_{dia}$, respectively), expressed as absolute values and as percent change from baseline, linear mixed model analysis was used to examine day (IHH versus intermittent medical air control), time and day by time interaction effects. Three separate analyses were conducted for each variable to examine gas periods alone, all room air periods throughout the protocol (baseline, between gas episodes and recovery) and a third analyses to examine room air periods only during baseline and recovery to remove confounding effects of ventilatory measures which may not have reached baseline between gas exposures. Time and day (IHH vs control) were examined as repeated factors within subjects, using an auto-regressive covariance structure, and with subjects entered as a random effect, each with a separate intercept. Significant main and interaction effects were examined using post-hoc pairwise contrasts conducted within each linear mixed model incorporating Bonferroni correction for multiple comparisons. All values are reported as mean ± SEM. P-values <0.05 were considered statistically significant.
2.4 Results

2.4.1 Subjects

The physical characteristics of the 12 participants were; age, $21.8 \pm 0.8$ years; height, $179.6 \pm 1.9$ cm; weight, $74.7 \pm 2.4$ kg; body mass index, $23.2 \pm 0.5$ kg/m$^2$; FEV1, $96.8 \pm 2.9\%$ predicted, FVC, $98.2 \pm 3.3\%$ predicted and AHI $4.4 \pm 0.8$ /hr.

The final $O_2$ composition used during the main experimental night for all participants was $3.0 \pm 0.2\%$.

2.4.2 Sleep architecture

There was no significant difference between the control or experimental night in the amount of time spent awake or in each sleep stage (Table 2.1). However the percent of total sleep time spent in stage 2 was significantly lower on the experimental night ($E = 47.3 \pm 3.3\%$ versus $C = 57.8 \pm 3.1\%$, $p = 0.003$).

<table>
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<th>C</th>
<th>E</th>
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</table>

Table 2.1. Time (min) spent awake and in each sleep stage.

Time in mins spent awake and in each sleep stage for both the control (C) and experimental (E) night. Data are mean ± SEM.
2.4.3 Ventilatory measures – all sleep stages combined

2.4.3.1 Gas periods

An example of raw data is presented in Figure 2.2. In group data during HH exposures V̇i, P_{ET}CO_2, V_T, F_B and PIF were all higher while SaO_2 was lower, T_i, T_E, T_{TOT} were shorter and Pepi was more negative compared to during medical air control (Figure 2.3; mean ± SEM across all gas exposure periods for experimental [E] and control [C] V̇i, E = 10.9 ± 0.3 L/min versus C = 7.2 ± 0.3 L/min, p < 0.001; P_{ET}CO_2, E = 46.6 ± 0.5 mmHg versus C = 44.8 ± 0.5 mmHg, p < 0.001; V_T, E = 0.7 ± 0.0 L/min versus C = 0.5 ± 0.0 L/min, p < 0.001; F_B, E = 15.2 ± 0.4 b/min versus C = 13.7 ± 0.5 b/min, p < 0.001; PIF, E = 36.2 ± 1.0 L/min versus C = 26.0 ± 1.0 L/min, p < 0.001; SaO_2, E = 86.8 ± 0.4 % versus C = 96.9 ± 0.4 %, p < 0.001; T_i, E = 1.76 ± 0.04 s versus C = 1.83 ± 0.04 s, p = 0.001; T_E, E = 2.3 ± 0.1 s versus C = 2.7 ± 0.1 s, p < 0.001; T_{TOT}, E = 4.1 ± 0.1 s versus C = 4.5 ± 0.1 s, p < 0.001; Pepi, E = -5.8 ± 0.9 cmH_2O versus C = -3.8 ± 0.9 cmH_2O, p = 0.002). There were also significant main effects of time in T_E and T_{TOT} (p = 0.030 and 0.029, respectively) but no significant post hoc differences at any time point. There was a significant difference in P_{ET}CO_2 across the 24 gas episodes (main time effect p = 0.046) but the only significant post-hoc difference was between the 17th and 18th gas episodes (p = 0.007), and there was no significant interaction effect to support any different time effect between days. There was no significant difference in F_B between the control and experimental day for the first 5 gas episodes or from episode 10 to 12, however F_B was higher during all other gas episodes on the experimental day compared to control (main interaction effect p = 0.002) (Figure 2.3). However, there were no significant day by time interaction effects in any other variable to suggest
any change in the ventilatory response from the first to last HH episode. Similarly, when comparing only the first and last gas episodes, there was no significant difference in $V_i$, $P_{ET}CO_2$ or $SaO_2$ from first to last gas exposure to suggest any change in chemoresponsiveness following 24 HH exposures (Figure 2.4).
Figure 2.2. Example of raw data during IHH.

Example of raw data from one participant during two consecutive HH exposures showing inspiratory flow (Flow), electroencephelogram (EEG), diaphragm electromyogram (EMGdia), genioglossal electromyogram (EMGgg), mask CO₂ (P\textsubscript{ET}CO₂), oxyhemoglobin saturation of oxygen (SaO₂), epiglottic and mask pressure (Pepi and Pmask, respectively). 30s gas exposures are marked with vertical dashed lines. The horizontal dashed line shows mild hypercapnia (~2-3 mmHg) was achieved during gas exposure.
Figure 2.3. Ventilatory measures during the intermittent gas protocol and recovery.

Group data for ventilatory measures at baseline (B), throughout the intermittent gas protocol (G1 to G24) and 60 min of recovery (marked at 30 and 60 mins, R30 and R60, respectively). Panels show minute ventilation (V\textsubscript{i}), end tidal partial pressure of CO\textsubscript{2} (P\textsubscript{ET}CO\textsubscript{2}), oxyhemoglobin saturation (SaO\textsubscript{2}), tidal volume (V\textsubscript{T}) and breathing frequency (F\textsubscript{B}). Other variables not shown. Filled circles represent experimental data (HH) and open circles represent control data (intermittent medical air). Data expressed as mean ± SEM, n = 12. Symbols mark significant day by time post hoc differences for F\textsubscript{B}.
Figure 2.4. Breath by breath comparison of first versus last HH gas episode.

Breath by breath changes over the first HH gas episode (solid circles) to the last HH (open circles) for minute ventilation ($V_i$), end tidal partial pressure of CO$_2$ ($P_{ET}CO_2$) and oxyhemoglobin saturation ($SaO_2$). Left panel shows breaths preceding onset of gas exposure (marked by the bar at time 0s) and breaths during the 30s gas exposure. Right panel shows breaths during 70s immediately after gas exposure and return to room air breathing. Data are absolute values expressed as mean ± SEM, n = 12.
2.4.3.2 Room air periods

During the room air breathing periods (baseline, between gas episodes and recovery) $V_I$ was higher, $T_I$ and $T_{TOT}$ was shorter and both $F_B$ and PIF were higher on the experimental day versus control (Figure 2.3; mean ± SEM $V_I$, $E = 7.8 ± 0.2$ L/min versus $C = 7.3 ± 0.2$ L/min, $p < 0.001$; $T_I$, $E = 1.76 ± 0.04$ s versus $C = 1.83 ± 0.04$ s, $p < 0.001$; $T_{TOT}$, $E = 4.4 ± 0.1$ s versus $C = 4.6 ± 0.1$ s, $p = 0.004$; $F_B$, $E = 14.2 ± 0.4$ b/min versus $C = 13.7 ± 0.4$ b/min, $p = 0.004$; PIF, $E = 28.8 ± 0.7$ L/min versus $C = 26.2 ± 0.7$ L/min, $p < 0.001$). On both the control and experimental day $T_E$ and $T_{TOT}$ were shorter during all periods between gas exposures and recovery compared to baseline, and $\text{SaO}_2$ was consistently lower during baseline compared to most of the room air periods during the rest of the protocol (Figure 2.3 time effect for all three $p < 0.001$). However there was no significant day by time interaction effect for any variable to indicate that IHH had induced vLTF, ggLTF, diaLTF or uALTF.

2.4.3.3 Baseline and recovery

Similarly, following exclusion of room air periods between gas exposures to remove the possibility of contamination with ventilatory measures which may not have reached baseline between gas exposures, when only baseline and recovery periods were compared, $V_I$, $P_{ET}CO_2$ and PIF were higher and $T_I$ was shorter on the experimental IHH day compared to the control day (Figure 2.3; mean ± SEM for room air breathing measures during baseline and recovery on the experimental [E] and control [C] day, $V_I$, $E = 7.8 ± 0.3$ L/min versus $C = 7.3 ± 0.3$ L/min, $p = 0.003$;
\( P_{ETCO_2}, E = 44.7 \pm 0.5 \text{ mmHg} \) versus \( C = 44.1 \pm 0.6 \text{ mmHg}, p = 0.016; \) \( PIF, E = 28.5 \pm 0.9 \text{ L/min} \) versus \( C = 26.5 \pm 0.9 \text{ L/min}, p = 0.006; \) \( T_i, E = 1.8 \pm 0.0 \text{ s} \) versus \( C = 1.8 \pm 0.0 \text{ s}, p = 0.010 \). Additionally, \( SaO_2 \) was higher and both \( T_E \) and \( T_{TOT} \) were shorter during recovery compared to baseline (main time effect \( p < 0.001 \) for all three). However, there remained no significant day by time interaction effect for any variable to support the presence of LTF during the hour long recovery period following IHH.

### 2.4.3.4 Percent change from baseline

Overall findings were similar when each variable was expressed as a percent change from baseline to adjust for within-subject differences between baseline values in each condition (Figure 2.5). Compared to the control day, there was a greater change from baseline during HH gas exposures in \( SaO_2, P_{ETCO_2}, V_i, V_T, PIF \) (all \( p < 0.001 \)), \( T_E \) (\( p = 0.001 \)), \( T_{TOT} \) (\( p = 0.002 \)), \( F_B \) (\( p = 0.001 \)), \( Pepi \) (\( p = 0.041 \)), and both InspEMGdia and ExpEMGdia (\( p = 0.017 \) and \( p = 0.016 \), respectively). The only significant interaction effect was an increase in \( F_B \) during HH exposures from the 16th gas exposure which persisted for most of the remainder of the gas protocol (main time by day interaction effect \( p = 0.002 \)).

When all room air periods were analysed as percent change from baseline, many of the differences seen during gas exposures were evident during room air breathing periods. However by excluding the room air periods between gas exposures and analysing only the baseline and recovery room air periods as a percent change from baseline, fewer differences were evident. Both \( T_E \) and \( T_{TOT} \) were significantly different between days during recovery, exhibiting a greater
reduction from baseline on the control day versus the experimental day (main condition effect $p < 0.001$ and $p = 0.002$, respectively). Similarly, both InspEMGgg and ExpEMGgg showed an increase from baseline on the control day during recovery and a reduction from baseline on the experimental day, with the difference between days being significant ($p = 0.016$ and $p = 0.017$, respectively). These effects were evident throughout the whole of recovery, and showed no progressive change and thus no interaction effects. When comparing only baseline and recovery data as percent change from baseline there were also significant main time effects in Pepi ($p = 0.015$) and $\text{SaO}_2$ ($p = 0.002$). There was no significant post hoc effects for Pepi however $\text{SaO}_2$ showed a greater increase from baseline during only the first 3 room air recovery periods, which was no different between days.
Figure 2.5. Ventilatory measures during the intermittent gas protocol and recovery expressed as percent change from baseline.

Group data for ventilatory measures at baseline (B), throughout the intermittent gas protocol (G1 to G24) and 60 min of recovery (marked at 30 and 60 mins, R30 and R60, respectively) expressed as percent change from baseline. Panels show minute ventilation ($V_I$), end tidal partial pressure of CO$_2$ ($P_{ET}CO_2$), oxyhemoglobin saturation ($SaO_2$), tidal volume ($V_T$) and breathing frequency ($F_B$). Other variables not shown. Filled circles represent experimental data (HH) and open circles represent control data (intermittent medical air). Data showing mean ± SEM, $n = 12$. Symbols mark significant day by time post hoc differences.
2.4.4 Stage 2 sleep only

2.4.4.1 Gas periods

As with all sleep stages combined, analyses restricted to gas exposures during stage 2 sleep alone showed a significant condition by time interaction effect in \( F_B \) (\( p = 0.006 \)). Post-hoc contrasts showed significant differences between conditions in 13 of the 24 gas exposure periods when \( F_B \) was expressed as absolute values. As with all sleep stages combined, during only stage 2 sleep there was no similar interaction effect in \( V_I \) or any other measures during gas exposures to suggest a change in chemoreflex responsiveness during IHH.

2.4.4.2 Room air periods

Ventilatory data restricted to room air periods (baseline, between gas episodes and recovery) of stage 2 sleep only showed similar results to analyses from all sleep stages combined. When all room air periods were compared \( V_I, F_B \) and PIF were higher, \( T_I \) was shorter and \( SaO_2 \) and ExpEMGgg were lower on the experimental day compared to control (mean ± SEM for room air breathing measures during stage 2 sleep only on the experimental [E] and control [C] day, \( V_I, E = 7.7 ± 0.2 \) L/min versus \( C = 7.2 ± 0.2 \) L/min; \( F_B, E = 13.9 ± 0.4 \) L/min versus \( C = 13.5 ± 0.4 \) L/min; PIF, \( E = 28.7 ± 0.7 \) L/min versus \( C = 25.8 ± 0.7 \) L/min; \( T_I, E = 1.8 ± 0.1 \) s versus \( 1.8 ± 0.1 \) s, all \( p ≤ 0.001 \); \( SaO_2, E = 97.4 ± 0.2 \) % versus \( 97.8 ± 0.2 \) %, \( p = 0.013 \); ExpEMGgg, \( E = 88.0 ± 11.0 \) versus \( 135.2 ± 12.2 \) arbitrary units, \( p = 0.005 \)). There was a significant main time effect in Pepi (\( p < 0.001 \)), being significantly different between 10, 20 and 25 min of recovery. \( R_{UA} \) was higher during the last 10 min of recovery versus baseline (main time effect \( p = 0.034 \)). \( T_E \) and \( T_{TOT} \) were
longer during the baseline than all other time points except the last 5 min block of recovery (main time effect for both p = 0.001) with no difference between days (no day or interaction effect). During room air periods with only stage 2 sleep there was only a significant day by time interaction effect in SaO₂ (p = 0.031). SaO₂ was significantly higher on the control day during the room air periods between the 11th and 12th gas exposures and also during the first 5 and last 10 min of recovery.

2.4.4.3 Baseline and recovery

Similarly, when only the baseline and recovery periods with stage 2 sleep were examined differences remained essentially unchanged, except there was no longer any difference between conditions in SaO₂. There were significant main time effects for both Pepi and RUA (p = 0.004 and p = 0.022, respectively) with no significant post hoc effects. Comparing only baseline and recovery stage 2 sleep, Tₑ and TTOT were only significantly longer during baseline compared to the first 5 min of recovery (post-hoc contrasts for both p = 0.049), again with no differences between control and experimental days (no day or interaction effects). As with all sleep stages combined, in analyses restricted to only stage 2 sleep during the baseline and recovery room air periods there were no significant interaction effects for any measure to indicate LTF.

2.4.4.4 Percent change from baseline

When only stage 2 sleep data was expressed as percent change from baseline, the only significant interaction effect during gas exposures was seen in F_B (main interaction effect p = 0.015), with post hoc significance showing F_B was higher on the experimental day during gas episodes 13, 16, 19 and 20. However as with all
sleep stages combined, with data restricted to only stage 2 sleep there was no significant interaction effect during gas episodes for $V_1$ or any other measure to suggest a change in chemoreflex responsiveness during IHH.

With data expressed as percent change from baseline and with data restricted to stage 2 sleep comparing all room air periods, there were still significant differences between conditions. $\text{SaO}_2$, $\text{P}_{\text{ET}}\text{CO}_2$, $\text{InspEMG}_{gg}$ and $\text{ExpEMG}_{gg}$ were lower, $T_E$, $T_{\text{TOT}}$ were longer and $F_B$ was shorter on the experimental day (main condition effects $\text{SaO}_2$ $p = 0.003$, $\text{P}_{\text{ET}}\text{CO}_2$ $p = 0.003$, $\text{InspEMG}_{gg}$ $p = 0.009$, $\text{ExpEMG}_{gg}$ $p = 0.042$ $T_E$ and $T_{\text{TOT}}$ both $p < 0.001$ and $F_B$ $p = 0.004$). There was a significant main time effect in Pepi ($p < 0.001$) however no significant post hoc effect. There was also significant interaction effects in both the percent change from baseline in $\text{P}_{\text{ET}}\text{CO}_2$ and $\text{InspEMG}_{gg}$ ($p = 0.019$ and $p = 0.042$, respectively) during stage 2 sleep comparing all room air periods. There was a greater increase in the percent change from baseline in $\text{P}_{\text{ET}}\text{CO}_2$ on the control day during the 14th, 16th and 22nd room air periods between gas episodes, and also at 25 min of recovery, however at 30 mins there was a greater increase during the experimental day. $\text{InspEMG}_{gg}$ was higher during the control day throughout most of recovery.

When only stage 2 sleep data from the room air periods during baseline and recovery were analysed as percent change from baseline, there was still significant differences between days and across time, however no interaction effects. During recovery there was a significantly greater reduction from baseline in $T_E$ and $T_{\text{TOT}}$ while $T_I$ increased during recovery on the control day and decreased on the
experimental day (main day effects $T_E \ p = 0.001$, $T_{TOT} \ p = 0.013$, $T_I \ p = 0.045$). Both InspEMGgg and ExpEMGgg increased during recovery on the control day and decreased during recovery on the experimental day (main day effects InspEMGgg $p = 0.002$ and ExpEMGgg $p = 0.003$). Main time effects were seen in Pepi and $R_{UA}$ ($p < 0.001$ and $p = 0.026$, respectively) however there were no significant post hoc effects for either measure.

Thus, as with all sleep stages combined, baseline and recovery periods restricted to only stage 2 sleep showed no significant day by time interaction effects to suggest the presence of vLTF (Figure 2.6), either with data expressed as absolute values or as a percent change from baseline.
Figure 2.6. Minute ventilation during only stage 2 sleep at baseline and 15 min intervals of recovery.

Minute ventilation ($V_1$) during only stage 2 sleep in each 5 minute block during baseline and at 15, 30, 45 and 60 minutes of recovery during control (intermittent medical air) and the experimental day (IHH). Data are absolute values expressed as mean ± SEM, n = 12.
2.5 Discussion

Both vLTF and uALTF have previously been reported during sleep in humans without OSA following AIH on a background of sustained isocapnia (Pierchala et al., 2008, Shkoukani et al., 2002). However, the current study showed no evidence to support that acute IHH, which more closely reflects blood gas disturbances experienced in OSA, induces vLTF or uALTF. There was also no change in the ventilatory response during successive HH exposures to suggest sensitisation or depression of chemoresponsiveness. In combination, these findings support other literature to suggest that the concomitant level of CO₂ critically modulates neuroplastic effects of IH (Harris et al., 2006, Olson et al., 2001, Waters and Tinworth, 2003, Kinkead et al., 2001).

2.5.1 Statistical considerations

The absence of significant effects in this study could reflect type II error. However, significant increases in ventilation and reductions in RUA have been reported during sleep in healthy non-snorers following a very similar protocol with similar sample sizes (Pierchala et al., 2008), suggesting similar effects should have been detected in this study and that Type II error is unlikely. During sleep Pierchala and colleagues exposed 12 participants to 15 isocapnic hypoxic exposures of approximately 1 min in duration, reducing SaO₂ to 86.1 ± 0.4 % (Pierchala et al., 2008), comparable to the 86.8 ± 0.4 % achieved with the current protocol. Participants were also returned to room air breathing between gas exposures for a similar length of time (113.9 ± 11.5s) compared to the two minutes room air breathing between gas exposures in the current protocol, and recovery was also
monitored during room air breathing. Pierchala and colleagues reported that during recovery $V_I$ and $V_T$ were increased to $108 \pm 1.3\%$ and $105 \pm 1.7\%$ of control respectively, and $R_{UA}$ was decreased to $88 \pm 9.8\%$ of control following IH (Pierchala et al., 2008). These are small effects. Using the within-subject standard deviation in $V_I$ during baseline on both days of 1.05 L/min, the current study had sufficient power to detect a 1.34 L/min (15.4%) or greater increase in $V_I$, with 80% power and 2-tailed significance level of 0.05. Thus a smaller effect could have been missed due to Type II error. However, at 60 mins recovery following IHH, $V_I$ was only $102.4 \pm 0.8\%$ of baseline. Therefore although this study was not powered to detect the small increase in $V_I$ reported as vLTF by Pierchala et al. there was still no comparable change in $V_I$ following IHH in the current study.

2.5.2 Evidence for lack of vLTF and increased chemoresponsiveness

In the current study $V_I$ increased during experimental HH through increased $V_T$ and $F_B$, and $F_B$ was higher during the second half of gas exposures. With data analysed as absolute values and as percent change from baseline, and with all sleep stages combined and restricted to stage 2 sleep, increased ventilation was evident during room air breathing periods on the experimental day. However, when data were restricted to only baseline and recovery room air periods, most of those differences were no longer evident, suggesting that breathing was not fully recovered during the room air periods between HH exposures. As there was no similar change in $V_I$ across time during gas exposures, the progressive increase in $F_B$ during HH exposures most likely reflects incomplete $F_B$ recovery between acute gas
exposures rather than a change in chemoresponsiveness with successive HH exposures.

As breathing did not recover fully between gas exposures, room air breathing periods between gas exposures were excluded and only baseline and recovery room air breathing periods were analysed to specifically look for LTF. With all sleep stages combined and with data restricted to only stage 2 sleep, breathing was elevated across the whole protocol on the experimental day, suggesting a systematic difference at baseline between days. Participants were blinded to conditions, and the protocol employed a randomised crossed-over design, so this difference remains difficult to explain. When analysed as percent change from baseline to adjust for baseline differences, both $T_E$ and $T_{TOT}$ showed a greater reduction from baseline on the control versus the experimental day during recovery. This was not an effect of change in sleep stage as this was seen with all sleep stages combined and with data restricted to stage 2 sleep. During recovery, $T_I$ measurements restricted to stage 2 sleep and expressed as a percent change from baseline increased on the control day and reduced on the experimental day. This difference between days in $T_I$ was not evident with all sleep stages combined. As there was no accompanying change in $F_B$, $V_T$ or $V_I$ during recovery, either with all sleep stages combined or with data restricted to stage 2 sleep, this may indicate a persistent change in the pattern of breathing but not ventilatory drive itself, or could perhaps reflect a Type I error.
2.5.3 Neuroplastic mechanisms of CO₂

The main difference in the protocol used by Pierchala and colleagues and that of the current study was the level of concomitant CO₂ during hypoxic exposures, where Pierchala et al. supplemented CO₂ only during hypoxia to maintain isocapnia (Pierchala et al., 2008), compared to supplemental CO₂ throughout hypoxia to induce concomitant hypercapnia in the current study. Thus, one possibility is that the combination of hypercapnia with hypoxia may inhibit neuroplasticity which would otherwise be induced with isocapnic IH alone; a possibility already speculated in some detail by Kinkead and colleagues in their review (Kinkead et al., 2001). When it was first discovered that IH induced serotonin dependant pLTF and hLTF (Bach and Mitchell, 1996), the same authors tested the effects of other respiratory stimuli and found intermittent hypercapnia had an opposing effect, inducing long-term depression characterised by a sustained reduction in peak neural burst amplitude and frequency despite maintenance of P\textsubscript{ET}CO₂ (Bach and Mitchell, 1998). Pre-treatment with α\textsubscript{2}-adrenergic antagonists blocked long-term depression (Bach and Mitchell, 1998), suggesting that intermittent hypercapnia induces ventilatory long-term depression via activation of locus coeruleus neurons, as opposed to the induction of LTF following IH via activation of serotonergic raphe neurons (Kinkead et al., 2001). Interestingly, following pre-treatment with α\textsubscript{2}-adrenergic antagonists (both yohimbine and RX-821002) some rats expressed pLTF (Bach and Mitchell, 1998). Chemoafferent stimulation by either hypercapnia or hypoxia activates caudal raphe neurons, which provide serotonergic innervation of respiratory motoneurons such as the phrenic and hypoglossal nerves (Kinkead et al., 2001). This led the authors to propose that blockade of α\textsubscript{2}-adrenergic
receptors may have allowed facilitation via hypercapnic carotid chemoafferent activated serotonergic pathways (Bach and Mitchell, 1998). Thus, it appears that hypercapnia and hypoxia may act in a push-pull fashion, via apposing adrenergic and serotonergic pathways (Kinkead et al., 2001).

Although intermittent hypercapnia induced long-term depression has only been demonstrated in rats following severe hypercapnic exposures (10% inspired \( \text{CO}_2 \), raising \( P_{\text{ET-CO}_2} \) to 80-95 mmHg) and not following moderate hypercapnia (3-5% \( \text{CO}_2 \), raising \( P_{\text{ET-CO}_2} \) to 60 mmHg) similar to that used in the current study (Bach and Mitchell, 1998), it is possible that the activation of both serotonergic and adrenergic pathways during the combination of IHH may have had counteracting effects (Kinkead et al., 2001), resulting in the lack of either facilitation or depression in this study. The only other study we are aware of to have also examined the effects of IHH on ventilatory neuroplasticity in humans found fifteen 30s episodes of breathing 6% \( \text{O}_2 \) and 5% \( \text{CO}_2 \) separated by 90s of breathing air during wakefulness also failed to induce vLTF (Diep et al., 2007). However, hypercapnic adrenergic pathways clearly do not always negate facilitatory serotonergic pathways, given that simulated apnoeas in rats inducing IHH has been shown to induce serotonin dependent LTF of both phrenic and hypoglossal nerve activity to a similar magnitude as IH alone (Mahamed and Mitchell, 2008). In addition, a study of IHH induced ventilatory neuroplasticity in piglets found that cycle duration of gas exposures was a critical determinant of whether depression, facilitation or no net change in ventilatory output was induced. Waters et al. (Waters and Tinworth, 2003) found that continuous exposure for 24 min, and 24 min of 2 min cycles did
not induce changes to ventilation post gas exposure. However, 8 min cycles induced vLTF and 4 min cycles induced ventilatory long-term depression (Waters and Tinworth, 2003). Therefore, although the IHH protocol utilised in the current study during sleep and that of Diep et al. (Diep et al., 2007) in awake humans both failed to elicit vLTF, these findings do not preclude that more variable blood gas disturbances experienced in OSA may nevertheless induce ventilatory neuroplasticity. It is also possible that facilitation could occur in some patients while depression could occur in others, and that both facilitation and depression may occur within patients at different times.

### 2.5.4 Opposing hypoxia induced neuroplastic pathways

Although AIH induced pLTF is commonly referred to as being serotonin dependent, recent studies have identified multiple neurotransmitters and intracellular pathways capable of inducing phenotypically identical LTF in both phrenic and hypoglossal activity (Dale-Nagle et al., 2010a, Nichols et al., 2012). Similar to opposing adrenergic and serotonergic pathways elicited by hypercapnia and hypoxia, pathways influenced by hypoxia alone appear to function similarly via cross-talk inhibition (Nichols et al., 2012). Both 5-HT$_2$ and α$_1$-adrenergic receptors are coupled to G$_q$-coupled metabotropic receptors (Dale-Nagle et al., 2010a). Episodic activation of either 5-HT$_2$ (both sub-types A and B (MacFarlane et al., 2011)) and α$_1$-adrenergic receptors (Huxtable et al., 2014) with specific agonists induces pLTF. This has been designated the Q pathway, due to the necessity of G$_q$-coupled receptors (Dale-Nagle et al., 2010a). Episodic activation of either 5-HT$_7$ or adenosine A$_{2A}$-receptors can also induce pLTF, however these pathways are only activated with more severe hypoxic exposures (< 4% O$_2$) (Nichols et al., 2012,
Hoffman and Mitchell, 2013). These receptors are coupled to G_s-coupled metabotropic receptors and activate a different intracellular pathway, called the S pathway (Hoffman and Mitchell, 2013). Therefore in response to IH, serotonin, epinephrine and adenosine all appear to be capable of inducing pLTF indistinguishable at the phenotypic level (Devinney et al., 2013). However, it appears that a delicate interplay between these neurotransmitters and intracellular pathways exists. Activation of 5-HT_7 receptors without AIH induces pLTF (Hoffman and Mitchell, 2011), and their activation is necessary for the increased pLTF induced by AIH following CIH conditioning (McGuire et al., 2004). However 5-HT_7 receptor inhibition enhances pLTF (Hoffman and Mitchell, 2013). Additionally, while short-term sustained hypoxia does not normally induce pLTF (Baker and Mitchell, 2000), blockade of the serine/threonine kinase Akt (which functions in the S pathway) with intrathecal application of okadaic acid does induce serotonin dependent pLTF (Wilkerson et al., 2008). Therefore, it appears that during AIH the S pathway constrains the full expression of pLTF via the Q pathway, and at some point during CIH the S pathway becomes dominant (Navarrete-Opazo and Mitchell, 2014, Hoffman and Mitchell, 2013). In contrast, during short-term sustained hypoxia the S pathway completely inhibits the Q pathway, resulting in no net change in neural output (Navarrete-Opazo and Mitchell, 2014). It is unknown whether the same pathways and interactions occur in humans during the induction of vLTF following AIH. However, it seems likely that at least a similar regulatory mechanism would have evolved in humans, as this intricate system allows for fine tuning of ventilatory output in the face of considerable variability in blood gas exposure conditions. Therefore, the use of 3% O_2 utilised in this protocol may have activated
a similar inhibitory pathway contributing to a lack of any detectable change in ventilation post IHH.

2.5.5 Other possible mechanisms for lack of LTF

In rats sleep fragmentation inhibits both the induction of vLTF and sensitisation of the hypoxic ventilatory response via adenosine A₁ receptors (McGuire et al., 2008b). Sleep stage is also thought to alter neuroplastic responses to IH, as in both animals and humans LTF has been difficult to induce during wake as opposed to sleep or anaesthesia (Nakamura et al., 2010, Jordan et al., 2002). LTF is more easily induced with progressively deeper sleep stages in Lewis rats (Nakamura et al., 2010). However a recent study in humans found the magnitude of vLTF is enhanced during wake versus sleep when IH is conducted during mild hypercapnia (Syed et al., 2013). Therefore, the inhibitory effect of wake on the induction of LTF may be secondary to lower $P_{ET}CO_2$. Despite the uncertainty of arousal state on neuroplasticity, it is possible that arousals and varying sleep stages during the protocol could also have impeded neuroplasticity in this study.

Pierchala et al. successfully induced vLTF during sleep in healthy males, using gas exposures conducted only while subjects were in stable stage 2 or stage 3 (slow wave % = 20–50%) sleep, with arousals and wakefulness only occurring for $3.2 \pm 2.6$ min (out of 15 min) during IH, and only for $2.1 \pm 3.9$ min (out of 20 min) of the recovery period (Pierchala et al., 2008). This equates to arousals and wakefulness occurring during approximately 15% of the total protocol time and is comparable to the sleep quality achieved in the current study. In this study gas exposures were also only conducted during stable sleep, and on the experimental night participants
spent approximately 25 – 50% of the night in stage 2 or slow wave sleep and were awake for 26.9 ± 10.0 mins out of approximately 160 mins (Table 2.1; approximately 14%). Importantly, the amount of time spent in slow wave sleep and wakefulness was not significantly different between nights. Given comparable sleep quality to that reported by Pierchala et al., poor sleep appears unlikely to explain the lack of vLTF in the current study. Furthermore, arousals, sleep fragmentation and reduced deep sleep are major features of OSA. Consequently, if sleep fragmentation and reduced deep sleep did impair the development of LTF in humans, LTF would be of limited relevance in OSA patients. On the other hand OSA patients do consistently exhibit treatment reversible abnormalities in chemoreflex control (Narkiewicz et al., 1999c, Salloum et al., 2010, Younes et al., 2007, Loewen et al., 2009, Spicuzza et al., 2006), increased sympathetic neural activity, hypertension (Narkiewicz and Somers, 1999) and state dependent changes in genioglossal activity (Jordan et al., 2009, McSharry et al., 2013, Mezzanotte et al., 1992, Rukhadze et al., 2010) suggestive of IH induced neuroplasticity effects (Deacon and Catcheside, 2014). Thus, there do appear to be neuroplasticity effects in OSA despite frequent arousals and sleep fragmentation.

We elected to use 3% CO₂ based on the work by Younes and colleagues (Younes et al., 2007) who found that the combination of 3% CO₂ with hypoxia for approximately 30s was the highest level tolerated during sleep without inducing arousal in most patients (Younes et al., 2007). The timing, number and intensity of hypoxic episodes was designed to mimic gas disturbances typically experienced in OSA, corresponding to an AHI of 24 /h with desaturations to around 80-85%.
However, more severe and longer exposures to IH can degrade neuroplasticity due to increased ROS production and activation of inflammatory pathways (Huxtable et al., 2013, Huxtable et al., 2011). Due to the considerable diversity in IH protocols in the literature it is difficult to determine at what point IH might begin to elicit pathological effects, although it has been suggested that protocols using >15 episodes and severe hypoxia of 3-8% O$_2$ more consistently induce pathological symptoms including neuronal apoptosis and loss of neuroplasticity (Dale et al., 2014, Navarrete-Opazo and Mitchell, 2014). Thus, the increased number of episodes utilised in this protocol (24 versus 15 (Pierchala et al., 2008)) and the use of more severe hypoxia (3% O$_2$) may have counteracted LTF effects via increased ROS production. However, Mateika and colleagues have reported that during the induction of vLTF, ventilation during the periods between hypoxic exposures gradually increases, which then continues to increase during recovery following the final exposure (Mateika and Narwani, 2009). Given the absence of any increase in ventilation over time and corresponding reduction in $P_{ETCO_2}$, increased ROS production appears unlikely to explain the absence of LTF in the current study.

In addition to both hypoxia and hypercapnia, other respiratory stimuli experienced during apnoeas, such as negative upper airway pressure pulses (Ryan and Nolan, 2009c), intermittent vagal feedback (Zhang et al., 2004) and periodic withdrawal of neuromuscular activity (Baertsch and Baker-Herman, 2013) have also been found to induce LTF in various motor neurons and ventilatory muscles. Negative pressure pulses initiate ggLTF in rats of similar magnitude as IH, both alone or in conjunction with IH (Ryan and Nolan, 2009c). In humans, early studies found that vLTF was
more easily induced in snorers vs non-snorers (Babcock and Badr, 1998). During IH in the supine position increased ventilatory drive would tend to increase flow limitation and negative upper airway pressure which would be greater in those with more compliant airways prone to snoring. Therefore it may be that in humans negative upper airway pressure during IH is an important stimulus for inducing vLTF. Participants in this study slept laterally specifically to help stabilise the airway to reduce potential confounding of obstruction effects on desaturations and ventilatory responses during IHH episodes. It is possible that reduced negative upper airway pressure stimuli associated with lateral versus supine positioning resulted in the lack of LTF in the current study.

Body position is known to significantly influence OSA severity, and is generally considered to predominantly act through anatomical effects. The possibility of vestibular influences on ventilatory and upper airway control are rarely considered (Younes, 2003) and body position effects on ventilatory neuroplasticity have yet to be investigated. The medial and inferior vestibular nuclei innervate the rostral ventrolateral medulla, which is critically involved in cardiovascular, ventilatory rhythm generation, chemoreception and upper airway muscle control (Stocker et al., 1997). Vestibular nuclei also innervate raphe neurons (Yates et al., 1992), which are critically involved in serotonergic LTF induced by IH. Vestibular neurons regulate cardiorespiratory responses to hypercapnia, although they do not appear to be involved in hypoxic responses (Hernandez et al., 2004). In addition, lateral positioning decreases genioglossal and tensor palatini responsiveness to negative pressure pulses in humans (Malhotra et al., 2004). Thus, the vestibular system
modulates respiratory and upper airway muscle control, both during movement and maintaining variation in ventilatory regulation in different body positions (Monahan et al., 2002, Siniaia and Miller, 1996, Feroah et al., 2002), and has also been shown to regulate cardiac and ventilatory control in response to various physiological challenges. Vestibular influences on cardiac and ventilatory reflexes also appear to be modulated by sleep stage, which is thought to underlie positional and sleep dependence of sudden infant death syndrome (Harper et al., 2000). Therefore, it is also possible that lateral positioning impeded neuroplasticity via input from the vestibular system and this hypothesis may warrant further investigation.

2.5.6 Conclusion

Finally, it is also possible that the findings of this study and that of Diep et al. (Diep et al., 2007) reflect a true absence of IHH mediated ventilatory neuroplasticity in humans, unlike IHH induced LTF already established in rats and piglets (Mahamed and Mitchell, 2008, Waters and Tinworth, 2003). Given many potential confounding variables, it remains uncertain from this study if IHH during sleep could induce neuroplasticity in humans under different circumstances. Failure to show LTF with the current protocol could reflect that the intensity, duration, number or frequency of hypoxic exposures, concomitant hypercapnia, arousals, sleep fragmentation, insufficient upper airway negative pressure or perhaps vestibular effects associated with lateral positioning prevented the induction of LTF. Treatment reversible abnormalities in ventilatory control do support the presence of neuroplasticity in untreated OSA patients, and that these may have deleterious effects on respiratory and upper airway muscle control thereby contributing to OSA pathophysiology.
(Deacon and Catcheside, 2014). Further work is needed to investigate the effects of IHH and possible interactions with airway obstruction, as occurs in OSA, on ventilatory, chemoreflex and upper airway control. A better understanding of neural mechanisms underpinning unstable chemoreflex ventilatory control in OSA is needed to better guide the development of future potential treatments for correcting unstable ventilatory control.
CHAPTER 3. **No effect of intermittent hypercapnic hypoxia on loop gain in awake healthy males**

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**Running Title:** No effect of intermittent hypercapnic hypoxia on loop gain in awake healthy males

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<td>Publication Details</td>
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| Name of Principal Author (Candidate) | Naomi Deacon |
| contribution to the Paper | Key investigator: development of hypothesis and protocol, and execution of experiments, analysis and authorship. |
| Overall percentage (%) | 90% |

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Date: 24 July 2015

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By signing the Statement of Authorship, each author certifies that:

1. the candidate’s stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate to include the publication in the thesis; and
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3.1 Abstract

In both humans and animals, intermittent hypoxia has been shown to induce chemoreflex control changes with the potential to increase loop gain and exacerbate cyclical apnoeas and hypopneas in obstructive sleep apnoea. In awake humans sustained mild hypercapnia appears to be a necessary pre-requisite for intermittent hypoxia to induce these neuroplastic effects. However, in obstructive sleep apnoea intermittent hypercapnia and hypoxia (IHH) occur concurrently and it remains unclear if this more relevant stimulus in humans can induce sustained chemoreflex changes beyond the initial stimulus. This study tested the hypothesis that IHH induces ventilatory long-term facilitation and increases loop gain in awake humans. 15 healthy males underwent a pseudorandom binary stimulation (PRBS) breathing test using 4% CO₂ in medical air to evaluate ventilatory control parameters including loop gain before and after exposure to intermittent hypercapnic hypoxia (24 x 30 sec episodes of 3% CO₂ and 3% O₂ in N₂ separated by 2 min of room air breathing) or control (medical air in place of 3% CO₂/O₂) during wakefulness in random order 2 weeks apart. Minute ventilation in the 10 min baseline period prior to each PRBS test was increased and PĒTCO₂ decreased following both IHH and control exposures, with no difference between conditions. There was also no facilitation of ventilation during or between each IHH exposure to indicate long-term facilitation, or any change in controller gain or loop gain following IHH. Consequently, this study does not support that acute IHH is sufficient to induce neuroplastic changes in ventilatory control in healthy awake males.
3.2 **Introduction**

Obstructive sleep apnoea (OSA) is the most common respiratory sleep disorder and affects approximately 17% of men and 9% of women aged 50-70 yrs (Peppard et al., 2013). OSA is associated with a range of serious adverse outcomes including pathological sleepiness with increased accident risk (George et al., 2002), impaired cognitive function (Morrell and Twigg, 2006), cardiovascular disease (Somers et al., 2008) and diabetes (Kim et al., 2013). OSA is characterised by repeated partial or complete collapse of the upper airway during sleep resulting in bouts of combined hypercapnia and hypoxia. A body of evidence now supports that instability of the chemoreflex control of ventilation contributes to OSA pathogenesis by promoting cyclical patterns of ventilatory drive, with the airway being susceptible to collapse during periods of low neural drive to upper airway dilator muscles (Remmers et al., 1978, Suratt et al., 1985, Gleeson et al., 1989).

Loop gain (LG) is an engineering measure of a feedback control systems behaviour usefully applied to quantify the stability of an individual’s ventilatory control system, where a higher LG indicates less stable control (Khoo, 2000a). LG is influenced by two main components; “plant” gain (PG) which reflects the effectiveness of the lungs to change blood gases, and controller gain (CG) which reflects chemoreceptor sensitivity to blood gas disturbances, with their product determining the overall LG of the respiratory system. Abnormalities in chemoreflex control, which increase CG and therefore overall LG, have been shown to be induced by OSA given that these abnormalities are reversed following continuous positive airway pressure (CPAP) treatment (Loewen et al., 2009, Narkiewicz et al., 1999c, Salloum et al., 2010, Spicuzza et al., 2006, Younes et al., 2007). However, the
mechanisms responsible for inducing chemoreflex control abnormalities and their contribution to overall ventilatory control instability in OSA currently remain uncertain (Orr et al., 2014, Younes, 2014).

Intermittent hypoxia (IH) induces two well characterised forms of ventilatory neuroplasticity; motor neuron long-term facilitation (LTF) which is characterised by a sustained increase in peak neural burst amplitude, and sensitisation of the carotid bodies hypoxic response (Peng and Prabhakar, 2004, McGuire et al., 2005). LTF of phrenic and/or other pump muscle motor neuron pools increases minute ventilation (i.e. ventilatory LTF), and hypoglossal LTF increases upper airway muscle activity such as in the genioglossus (Baker-Herman and Strey, 2011). The potential role these forms of neuroplasticity may play in OSA pathophysiology remains contentious, with both beneficial and pathogenic roles being posited (Mateika and Narwani, 2009, Mahamed and Mitchell, 2007). However, experimental IH in both humans and rats has been shown to induce chemoreflex control abnormalities, which increase CG and LG, that are equivalent to those exhibited in OSA patients (Chowdhuri et al., 2010, Mateika et al., 2004, Narkiewicz et al., 1999c, Saloum et al., 2010). This suggests that IH induced neuroplasticity contributes to elevated LG in OSA patients.

As ventilatory responsiveness to hypoxia is dependent on CO₂ levels (Corne et al., 2003), the induction and expression of ventilatory neuroplasticity is highly dependent on concomitant CO₂ levels, both during and following IH (Harris et al., 2006, Olson et al., 2001). During wakefulness, ventilatory LTF has only been induced in humans in the presence of sustained mild hypercapnia (Harris et al.,
However, OSA patients experience intermittent hypercapnia and hypoxia (IHH) together interspersed with room air breathing, and there is some evidence that the effects of intermittent hypercapnia may oppose those of IH (Kinkead et al., 2001). Consequently, to help clarify the role that IHH-induced neuroplasticity might play in modulating ventilatory control stability to blood gas disturbances of more relevance to human OSA than IH alone, the purpose of this study was to test if acute exposure to IHH induces ventilatory LTF and/or changes chemoreflex control and LG.

### 3.3 Methods

#### 3.3.1 Participants

Healthy males without OSA were studied during wakefulness to specifically test for IHH effects on ventilatory control without potential confounding by pre-existing OSA, state/arousal, or hormonal or other influences between genders. Fourteen healthy non-obese male volunteers ≥18 years of age gave written informed consent and participated in the study. All were non-smokers and demonstrated normal lung function with forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) ≥80% predicted (JLab software version 4.53; Compactlab, Jaeger, Wuerzburg, Germany). None of the participants had participated in previous experiments with hypoxia, were born or had lived at high altitude, had known medical conditions or took any regular medications. All self-reported normal sleep and none reported regular snoring or any other history or symptoms suggestive of a sleep disorder. To confirm the absence of sleep breathing disorders all participants performed an overnight airflow recording and oximetry study in the home setting (ApneaLink, Resmed Inc, Martinsried, Germany) to exclude an oxygen desaturation...
index of ≥5 events per hour. The study was approved by the Southern Health Human Research and Ethics Committee.

3.3.2 Protocol

To control for potential circadian and metabolic effects participants returned to the laboratory at 11 am, before eating lunch, on two separate experimental day visits; an IHH experiment and an intermittent medical air (control) experiment, conducted in random order and separated by at least two weeks. Participants were blinded to the experimental conditions and were asked to abstain from alcohol the night before, and caffeine and strenuous exercise in the morning prior to each study. Participants were also instructed to get 8 hours sleep the night before each study, eat a similar breakfast at the same time on both mornings at least 4 hours prior to each study, and to stay awake throughout each experiment. Participants relaxed in the laboratory for 1 hour prior to commencement of the study at 12pm. A pseudorandom binary stimulation (PRBS) of carbon dioxide test described by Khoo et al. (Khoo, 2000b) was used to determine loop gain. This was conducted before and after exposure to IHH, with approximately 5-10 minutes break between IHH and LG measurements (Figure 3.1).
Figure 3.1. Pseudorandom binary stimulation and intermittent hypercapnic hypoxia protocol.
Panel A shows the protocol sequence. Panel B shows a raw sample of data during the pseudorandom binary stimulation of 4% CO₂. In the bottom tracing the bars represent switching between 4% CO₂ and returning to room air.

3.3.3 Measurements
Electroencophalograms (C4-A1 and C3-A2) and left and right electrooculograms were monitored throughout the protocol to confirm wakefulness (Compumedics, E-Series, Melbourne Australia) and the electrocardiogram and arterial oxyhemoglobin saturation (SaO₂) were monitored continuously via ear pulse oximetry (POET II model 602-3; Criticare Systems, Waukesha, WI). Carbon dioxide was continuously sampled at the mask via a capnograph (Capnostream20, Oridion, Israel) calibrated before each experiment against a known standard (8% CO₂, alpha-standard gas, BOC gases Australia) and N₂. Each participant was fitted with a nasal mask (Gel mask, Resprionics, Murraysville, PA) fitted with a two way non-rebreathing valve.
(series 2600, Hans Rudolph, Kansas City, MO) and their mouth carefully taped
(with easily removable tabs) to ensure nasal breathing. A pneumotachograph
(PT16, Jaeger, Germany) connected to the inspiratory side of the breathing valve
and calibrated via 1-L syringe manoeuvres was used for quantitative inspiratory
flow and volume measurements. The pneumotachograph was connected via 1 m of
tubing (Hans Rudolph, 35mm clean bore) to the common outlet port of a Gatlin-
shaped valve system with 4 inlet ports (series 2440C, Hans Rudolph) for delivery of
inspiratory gases. Each port could be rapidly open or closed via a computer
controlled solenoid valve system controlling pneumatic balloon occlusion. Only one
port was open at a time and all switches between ports were conducted during
expiration. Three of the inlet ports were open to room air while the fourth was
connected to two separate foil bags (300L, Scholle Industries, Adelaide, Australia)
connected via 3-way stop-cocks to enable closure of each bag and manual
switching between bags at the inlet port. One bag was filled with a mixture of
medical grade CO₂ blended with medical air at flow rates adjusted to achieve 4%
CO₂ as measured by the pre-calibrated CO₂ analyser. The bag was then partially
filled and flushed to washout dead-space, and then filled to near capacity
immediately prior to the experiment. The second bag was filled with either medical
air (control experiments) or a blend of 3% CO₂, 3% O₂ (Oxygen Analyser OM-11,
Beckman Instruments Inc, Fullarton, California, USA; pre-calibrated using 100% N₂
and a known standard; 9% O₂, alpha-standard gas, BOC gases Australia) balance
N₂ for intermittent hypercapnic hypoxia experiments.
3.3.4  *Pseudorandom binary stimulation protocol*

Participants lay supine and were instructed to keep their eyes open, to stay still and to breathe normally. Following a 10 minute baseline period to record stable breathing on room air, the PRBS protocol commenced. This consisted of a sequence of 63 breaths alternating between room air and 4% CO$_2$ in a pre-determined repeatable pseudorandom sequence spanning the full range of switching frequencies of between 1 and 6 consecutive breaths of CO$_2$. The same sequence was repeated a total of 8 times without interruption between adjacent sequences.

3.3.5  *Intermittent hypercapnic hypoxia protocol*

Following the PRBS protocol and a further 10 minutes of stable breathing each participant received a sequence of 24 separate 30 s episodes of either IHH (3% CO$_2$, 3% O$_2$ balance N$_2$) or medical air (control experiments) separated by 2 min of room air breathing. Supplemental oxygen was added into the inspirate if required to prevent desaturation below 80%. Immediately after the last gas exposure the participant was allowed a 10 min break, followed by a repeat of the PRBS test, including the initial 10 min baseline period.

3.3.6  *Data analysis and statistical procedures*

Inspiratory, expiratory and total breath times were determined from the flow signal, and digital integration used to measure inspiratory tidal volume ($V_T$) and minute ventilation ($V_I$) breath-by-breath. Breath-by-breath measurements of inspiratory and expiratory (end-tidal) partial pressure of CO$_2$ ($P_{IC}CO_2$ and $P_{ET}CO_2$ respectively)
were determined from the nadir and peak in mask CO$_2$ after adjusting for gas sampling delay.

The first pseudorandom binary stimulation sequence and any sequence containing breaths contaminated by movement artefact, swallows, sighs or sleep were excluded from analysis.

CG, PG, overall LG, feedback delay and other parameters of a simplified model of CO$_2$ chemoreflex control of breathing were estimated by fitting breath by breath measures of V$_1$, P$_1$CO$_2$ and P$_{ET}$CO$_2$ during the PRBS test to a model of the CO$_2$ chemoreflex control of breathing described by Khoo (Khoo, 2000b), with a minor modification to allow estimation of LG and related parameters. LG is frequency dependent (Khoo et al., 1982) and since the natural frequency of the system varies between individuals it is useful to calculate and examine LG at relevant fixed frequencies such as 1-2 cycles/min and at the natural frequency of the system. Thus the main outcomes of this analysis were gain parameters estimated at 1 cycle/minute and at the natural frequency, and the estimated impulse response curve of the system to a sudden change in CO$_2$. Goodness-of-fit between the original PRBS analysis described by Khoo versus the modified analysis was assessed via the coefficient of determination ($r^2$) and residual sum of squares (RSS) between the change in measured ventilation versus the model estimated change in ventilation breath-by-breath. LG parameters were compared between conditions and time (control or IHH, and pre and post PRBS tests) using linear mixed model analysis, and also as percent change from baseline using Student’s paired t-tests.
During each IHH exposure, ventilation and arterial oxyhaemoglobin saturation only began to change on the last breath and peaked in the 30 s following the exposure (Fig. 1A). Ventilatory data for room air periods were therefore compared using mean values calculated over the 30 s preceding each gas exposure and gas period data calculated as mean values over the 30 s following each gas exposure. The only exceptions were $P_{ETCO_2}$, which was calculated as mean values during each gas exposure given minimal delays in $P_{ETCO_2}$ responses, and minimum $SaO_2$ which was determined from the 30 s following gas exposure given an approximate 30-sec circulation delay between gas exposure and $SaO_2$ responses. Differences between IHH versus control conditions, changes over time, and interaction effects in $V_i$, $P_{ETCO_2}$, $SaO_2$, $V_T$ and breathing frequency ($F_B$) from room air periods between gas exposures and the 24 gas exposure periods were examined using linear mixed model analysis for gas exposure and room air periods separately. Time and experimental condition (IHH vs control) were examined as repeated factors within subjects using an auto-regressive covariance structure, and with subject entered as a random effect, each with a separate intercept. To test for the presence of LTF, $V_i$ and $P_{ETCO_2}$ from the 10 minute baseline period prior to each PRBS protocol were compared between gas conditions and pre- vs post-IHH or control gas exposures using both absolute values entered into a linear mixed model analysis, and as a percent change from baseline between gas conditions using Student’s paired t-tests. All statistical analysis was performed using SPSS (IBM SPSS Statistics Version 22). All data are presented as means ± SEM. P-values <0.05 were considered statistically significant.
3.4 Results

3.4.1 Subjects
One participant was unable to maintain wakefulness during the protocol and their data were excluded. The physical characteristics of the 14 remaining participants were; age, 25.5 ± 2.0 years; height, 181.1 ± 2.1 cm; weight, 79.1 ± 2.3 kg; body mass index, 24.1 ± 0.5 kg/m²; FEV1, 98.1 ± 2.7% predicted and FVC, 101.6 ± 2.5% predicted.

3.4.2 Intermittent hypercapnic hypoxia
Figure 3.2A shows a recording of the IHH protocol from a representative participant. Group data for ventilatory measures during the intermittent gas exposure protocol are presented in Figure 3.2B and Table 3.1. During IHH gas exposures there were statistically significant increases in $V_I$, $P_{ETCO_2}$ and $V_T$ and significant decreases in $SaO_2$ and $Fb$ (all $p < 0.001$). During gas periods $V_I$ significantly decreased over time ($p = 0.040$) by 1.25 ± 0.33L/min from the first to the last exposure ($p = 0.037$). However there were no significant gas condition by time interaction effects in $V_I$ ($p = 0.846$) or any other ventilatory variable during gas exposures. During room air periods between gas exposures $P_{ETCO_2}$ was slightly, but statistically significantly higher during the IHH compared to the control condition ($p = 0.005$), indicating the absence of any post IHH hypocapnia. In addition, $V_T$ was significantly higher ($p = < 0.001$) and $Fb$ was significantly lower ($p = < 0.001$) during room air periods between gas exposures on the IHH versus the control condition, indicating resting ventilation had not been reached during the 2 minutes between hypercapnic hypoxic exposures. There was no significant change in any ventilatory variable from the first room air period preceding the first gas exposure to the last
room air period. There were also no significant interaction effects between day and time in any ventilatory parameter during room air periods between gas exposures.
Figure 3.2. Ventilatory measures during intermittent gas protocol.

A. Compressed polygraph from a representative participant during the complete IHH gas protocol, with each 30 second gas exposure marked in the top panel by solid bars, room air periods by spaces, showing inspiratory minute ventilation ($V_1$), mask partial pressure of CO$_2$ (mask CO$_2$), mask partial pressure of O$_2$ (mask O$_2$) and saturation of oxygen (SaO$_2$). B. Group data for minute ventilation ($V_1$), end tidal CO$_2$ ($P_{ET}CO_2$), minimum oxygen saturation (SaO$_2$), tidal volume ($V_T$) and breathing frequency (Fb), during the intermittent gas protocol for both control and IHH. The 24 episodes of either hypercapnic hypoxic gas or medical air are shown on bottom panel. Data shown as mean ± SEM, n = 14.
Table 3.1. Ventilatory measures during intermittent medical air or IHH.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IHH</th>
<th>Room air</th>
<th>IHH</th>
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</thead>
<tbody>
<tr>
<td>$V_I$ (L/min)</td>
<td>8.2 ± 0.3*</td>
<td>11.5 ± 0.3*</td>
<td>8.3 ± 0.3</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>$P_{ETCO_2}$ (mmHg)</td>
<td>40.4 ± 0.8*</td>
<td>43.2 ± 0.8*</td>
<td>40.4 ± 0.8+</td>
<td>41.3 ± 0.8+</td>
</tr>
<tr>
<td>Min $SaO_2$ (%)</td>
<td>97.0 ± 0.6*</td>
<td>86.4 ± 0.6*</td>
<td>97.1 ± 0.2</td>
<td>97.0 ± 0.2</td>
</tr>
<tr>
<td>$V_T$ (L)</td>
<td>0.6 ± 0.0*</td>
<td>1.0 ± 0.0*</td>
<td>0.6 ± 0.0+</td>
<td>0.7 ± 0.0+</td>
</tr>
<tr>
<td>Fb (br/min)</td>
<td>14.9 ± 0.7*</td>
<td>13.0 ± 0.7*</td>
<td>14.9 ± 0.7+</td>
<td>13.8 ± 0.7+</td>
</tr>
</tbody>
</table>

Ventilatory measures during room air periods between gas exposures and during gas exposures during both the control (intermittent medical air) and intermittent hypercapnic hypoxia (IHH). * P < 0.05 during gas exposures control versus IHH. + P < 0.05 during room air periods between gas exposures control versus IHH. Data are mean ± SEM.
3.4.3  *Room air breathing and LTF*

There was a significant increase in $V_I$ during the 10 minute baseline breathing period prior to PRBS tests from pre to post gas exposure ($p = 0.022$), and a concomitant reduction in $P_{ETCO_2}$ ($p = 0.004$, Fig. 3.3). However, there were no significant $V_I$ or $P_{ETCO_2}$ differences between IHH versus control conditions or condition by time interaction effects to support the presence of post-IHH LTF. Expressed as percent change from baseline, the $V_I$ increase was $6.0 \pm 2.7\%$ in the control condition and $6.6 \pm 3.3\%$ following IHH, and the $P_{ETCO_2}$ decrease was $2.3 \pm 0.8\%$ in the control condition and $2.4 \pm 1.0\%$ following IHH, with no significant differences between conditions.
Figure 3.3. Room air breathing prior to PRBS, before and after IHH.
Minute ventilation significantly increased ($V_1$) and end tidal CO$_2$ ($P_{ETCO_2}$) significantly decreased during 10 minutes room air breathing prior to pseudorandom binary stimulation (PRBS) before (white bars) and after (black bars) intermittent gas exposure. Bars represent main time effect. There was no significant interaction effect of time by day to indicate ventilatory long-term facilitation. Data represent means ± SEM, n=14.

3.4.4 PRBS
There was no difference in $r^2$ or RSS of measured versus model fit estimates of the change in ventilation using the Khoo versus modified PRBS analysis model ($r^2$ 0.247 ± 0.020 vs 0.253 ± 0.020, p=0.492) so the latter model was used for the remaining analyses to allow for separate assessment of CG, PG and overall LG.

There were no significant differences in any PRBS derived parameter between gas conditions and no condition by time interactions; expressed either in absolute values (Table 3.2) or as a percent change from baseline (Figure 3.4). There was a significant breath number but no pre versus post gas condition, time or breath
number interaction effects on the feedback control model estimated impulse response to a sudden change in inspired CO₂ (Figure 3.5).

Table 3.2. Gain parameters pre and post medical air or IHH.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IHH</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>LGₙ</td>
<td>0.82 ± 0.08</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>LG₁</td>
<td>0.64 ± 0.08</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>CG₁</td>
<td>0.27 ± 0.03</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>PG₁</td>
<td>2.40 ± 0.20</td>
<td>1.96 ± 0.16</td>
</tr>
</tbody>
</table>

Loop gain (LG), controller gain (CG) and plant gain (PG) measured at the natural frequency (n) and frequency of 1 cycle of periodic breathing per minute. Values shown as mean ± SEM (n = 14) for control (medical air) and intermittent hypercapnic hypoxia (IHH). There was no significant change in LG, CG or PG following IHH (P > 0.05).
Figure 3.4. Percent change from baseline for LG parameters.
Percent change from baseline for loop gain (LG), controller gain (CG) and plant gain (PG) measured at the natural frequency (n) and frequency of 1 and 2 cycles of periodic breathing per minute. Control of med air (grey) versus intermittent hypercapnic hypoxia (IHH, black). There was no significant change in LG, CG or PG at any frequency following IHH (P > 0.05). Data represent means ± SEM, n=14.
Figure 3.5. Impulse response curves.
The estimated ventilatory response per mmHg change in CO₂ from the pseudorandom binary stimulation to 4% CO₂ represented as a 63 breathe impulse response. There was no significant interaction effect of time by day by breath number and therefore no significant effect of intermittent hypercapnic hypoxia (IHH) on chemoreflex response to normoxic CO₂. Grey represents before gas exposure, black represents after gas. Data represents mean ± SEM, n = 14.
3.5 Discussion

The main finding of this study was no change in ventilatory response to repetitive hypercapnic hypoxic gas exposures over time to indicate any sensitisation or depression of chemo-responsiveness induced by an acute exposure to IHH. No changes in PRBS-estimated CO₂ chemo-reflex response parameters following IHH is consistent with this main finding. Although V₁ increased and PₑTCO₂ decreased during room air breathing following gas exposure, perhaps suggestive of ventilatory LTF, this change occurred following both IHH and the intermittent medical air control condition, indicating the importance of a room air control and that this effect cannot be attributed to ventilatory LTF induced by IHH. The lack of any significant changes across time in V₁, PₑTCO₂, V₉ and F₈ during room air periods between IHH further supports the absence of ventilatory LTF in the present study. There were also no changes in LG or CG following exposure to IHH to support any post IHH changes in dynamic chemoreflex responses.

While it is possible that the absence of LTF and changes in dynamic ventilatory control measures in this study reflects type II error, we believe this is unlikely. Within-subject variability in ventilation across previous LTF inducing protocols are difficult to discern from published reports and there were no previously published studies of dynamic chemoreflex control or LG following IH, IHH or the induction of neuroplasticity to help power this study. However, based on 80% power, a two-tailed significance of 0.05 and standard deviations of within-subject differences in baseline V₁ and PₑTCO₂ before versus after the control condition of 0.8 l/min and 1.2 mmHg, 14 participants should have been sufficient to detect a 0.7 l/min (~8%) and 1 mmHg (~2.4%) greater change in ventilation and PₑTCO₂ from baseline with
IHH versus control. The estimated minimum detectable changes in LG, CG and peak impulse response based on variance of the measures were in the order of 26%, 19% and 22% respectively. Using 2 groups of 13 participant, Lee et al. reported that with $P_{ETCO_2}$ maintained 4 mmHg above eupnoea, ventilatory LTF induced by IH during wakefulness increased $V_i$ from $14.8 \pm 0.5 \text{ l/min}$ to $20.4 \pm 1.3 \text{ l/min}$, representing a 38% increase in the ventilatory response to the same degree of mild hypercapnia (Lee et al., 2009). Similarly, Harris et al. reported that in 11 healthy participants with $P_{ETCO_2}$ maintained 5 mmHg above eupnoea, ventilatory LTF increased $V_i$ from $19.6 \pm 1.0 \text{ l/min}$ to $28.4 \pm 1.7 \text{ l/min}$, representing a 45% increase in the ventilatory response (Harris et al., 2006). Given similar sample sizes and previous reports of moderate to large LTF effects, albeit with concomitant hypercapnia, inadequate power appears unlikely to account for the absence of LTF or dynamic CO$_2$ control response changes in this study.

LG is the ratio of the magnitude of a ventilatory response to the magnitude of the disturbance which elicited the response, which in a simplified model equals the product of CG and PG. An elevated LG is a key feature of unstable respiratory control in both central and obstructive sleep apnoea (Younes et al., 2001, Eckert et al., 2007a). Given that obesity, a key risk factor for OSA, compresses lung volume, increased PG via lung volume effects likely contributes to elevated LG in OSA. However, independent of weight, OSA patients exhibit an increased hypoxic ventilatory response (Narkiewicz et al., 1999c) and a reduced eupnoic $P_{ETCO_2}$ and CO$_2$ reserve (Salloum et al., 2010), which combine to increase CG both above and below eupnoea (Salloum et al., 2010, Younes et al., 2007). Following 1-5 months of CPAP treatment these abnormalities have been shown to normalise (Loewen et al.,
2009, Salloum et al., 2010, Spicuzza et al., 2006), indicating CG abnormalities are not inherent traits in OSA patients, but are induced by OSA itself.

Increased hypoxic sensitivity is by definition an increase in CG and without a concomitant reduction in PG, IH induced increased hypoxic sensitivity must therefore increase LG. Ventilatory LTF increases the ventilatory response for the same magnitude of chemostimulation, and must therefore also increase LG (as LG = \( \Delta \) of ventilatory response / \( \Delta \) of disturbance which elicited it). Despite increased LG indicating more unstable control, LTF is often posited to have stabilising effects on ventilation in OSA (Mateika and Syed, 2013). It has been suggested that hypoglossal nerve LTF may help brace the airway open, and that ventilatory LTF could prevent ventilatory depression promoting ongoing central and/or obstructive apneas/hypopneas (Mateika and Syed, 2013). However, IH has also been shown to reduce eupnoeic \( P_{ET}\text{CO}_2 \), which in humans reduces the CO\(_2\) reserve and increases LG via an increase in CG below eupnoea (Chowdhuri et al., 2010). These effects clearly destabilise ventilatory control and have been described as a separate mechanism to LTF (Mateika and Syed, 2013), although reduced eupnoeic \( P_{ET}\text{CO}_2 \) reflects ventilatory LTF expression combined with ventilatory feedback which partially constrains its expression (Olson et al., 2001, Harris et al., 2006). This effect of LTF also reflects chemoreflex control abnormalities in OSA patients which increases CG below eupnoea and ameliorates following OSA treatment (Salloum et al., 2010). Therefore it is likely that IH induced ventilatory LTF and increased hypoxic sensitivity are the main mechanisms through which elevated CG and LG are induced in OSA patients. In support of this view, two recent studies found that IH induced ventilatory LTF was associated with an increase rather than a
decrease in AHI in OSA patients (Syed et al., 2013, Yokhana et al., 2012). Opposing theories regarding the potential role of ventilatory LTF in OSA pathophysiology and the treatment implications (should LTF be harnessed or inhibited) formed the basis of this study.

Mateika and colleagues suggest that ventilatory LTF in awake humans can only be induced following acute IH if mild hypercapnia (3-5 mmHg above baseline) is maintained throughout the protocol (Harris et al., 2006, Lee et al., 2009, Wadhwa et al., 2008, Syed et al., 2013). These authors note that IH protocols in animals are most commonly conducted during anaesthesia, during which $P_{ET\text{CO}_2}$ is typically maintained several mmHg above the apnoiec threshold of the nerve preparation (phrenic or hypoglossal) under study (Harris et al., 2006). This ensures that hypocapnia secondary to increased $V_t$ during poikilocapnic IH does not feedback to inhibit ventilatory drive and neurotransmitter release at motor neurons required for the induction of LTF. During wakefulness the hypoxic ventilatory response is inhibited when $P_{ET\text{CO}_2}$ is below the chemoreceptor threshold, which is at or just below eupnoic $P_{ET\text{CO}_2}$ (Corne et al., 2003, Duffin and McAvoy, 1988). Only when CO$_2$ is raised above the chemoreflex threshold does chemoreflex drive dominate ventilatory output. Consequently Mateika and colleagues have proposed that if isocapnia is maintained at eupnoea, ventilatory drive may be dominated by wakefulness rather than chemoreflex drive, which may prevent the induction of LTF (Harris et al., 2006). With this reasoning Mateika and colleagues maintained $P_{ET\text{CO}_2}$ 5 mmHg above eupnoea throughout IH and were able to demonstrate ventilatory LTF for the first time in awake humans (Harris et al., 2006). Following return to room air breathing, ventilatory LTF reduced $P_{ET\text{CO}_2}$, which via ventilatory...
feedback, constrained the expression of LTF and hence $V_1$ back to baseline values (Harris et al., 2006). Although this was the first reported finding of ventilatory LTF in awake humans, these data were consistent with previous reports from the same laboratory following poikilocapnic IH (Mateika et al., 2004). Despite reporting no increase in $V_1$ and therefore no ventilatory LTF, $P_{ET}CO_2$ significantly decreased during the room air breathing recovery period following poikilocapnic IH (Mateika et al., 2004). When $P_{ET}CO_2$ was raised above the CO₂ chemoreflex threshold, the ventilatory response to combined hypercapnic hypoxia was significantly increased (Mateika et al., 2004). These findings suggest that LTF was present in awake humans although constrained under poikilocapnic conditions, and that hypercapnia is not essential to induce LTF in awake humans. Rather it appears that it is the maintenance of CO₂ during recovery which is essential for the full expression of LTF. This is further supported by findings in awake unanaesthetised rats which also required supplementation of CO₂ back to baseline levels to reveal the full expression of ventilatory LTF (Olson et al., 2001). However, once CO₂ feedback was accounted for, the magnitude of LTF was equivalent following IH during either isocapnia or poikilocapnia (Olson et al., 2001).

Given these findings, we reasoned that combined hypercapnic hypoxia would provide a stronger stimulus than IH alone for the induction of ventilatory motor neuron LTF. During room air periods between IHH exposures $P_{ET}CO_2$ remained significantly elevated indicating that hypocapnic constraint of LTF is highly unlikely. Rather than supplementing CO₂ following IHH exposure to prevent the possibility of hypocapnia constraining the expression of LTF, we opted to compare baseline and recovery breathing periods following both IHH and normoxic normocapnia control
during more physiologically relevant conditions of poikilocapnia. This also avoided
the possibility of artefactually inferring LTF by preferentially driving ventilation with
raised CO₂ only following IHH. These data cannot discount that more sustained
hypercapnia may remain a pre-requisite for acute LTF induction and expression,
but then the relevance to gas exchange disturbances in OSA would still remain
unclear.

The magnitude of change in both V₁ and PₑT₈CO₂ following both IHH and
intermittent medical air was similar during both days and could perhaps reflect a
circadian effect. Spenger et al. found no circadian changes in ventilation under
constant laboratory conditions (Spengler and Shea, 2000), but a significant but very
small reduction in PₑT₈CO₂ during the afternoon, that was independent of Vₑ and
driven by changes in metabolic rate (Spengler et al., 2000). Circadian effects
therefore appear unlikely to explain these findings and it is more likely that
augmented ventilation and reduced PₑT₈CO₂ under both IHH and control conditions
reflects an artefact of the study protocol itself, such as cumulative and carry-over
(e.g. minor discomfort) or perhaps anticipatory effects associated with the study
procedures. This finding highlights the importance of the control condition.

Similar to effects of chronic IH that increase the chemosensory response of carotid
bodies to hypoxia in rats (Peng et al., 2001), humans exhibit sensitisation of the
hypoxic ventilatory response with acute exposure to IH and so called progressive
augmentation of ventilation with successive hypoxic exposures during acute IH
(Lee et al., 2009). Whether progressive augmentation of ventilation in humans is
driven by neuroplasticity at the carotid bodies, or brainstem regions involved in the
central integration of chemosensory feedback is not known. LTF is highly sensitive to the protocol and several early studies in humans have also failed to induce LTF (Jordan et al., 2002, Mateika et al., 2004, McEvoy et al., 1996). It is only in more recent studies that LTF has been successfully induced in humans both during wake and sleep (Brugniaux et al., 2011, Dale et al., 2014, Harris et al., 2006, Pierchala et al., 2008). However increases in the hypoxic ventilatory response are more consistently reported following acute IH and with a broader range of hypoxic protocols (Katayama et al., 2009, Koehle et al., 2007, Lee et al., 2009), suggesting that increased hypoxic sensitivity is more readily induced and less sensitive to the pattern of IH. Although we did not test the normocapnic hypoxic ventilatory response, had hypoxic sensitivity increased, the ventilatory response to successive hypercapnic hypoxic exposures should also have increased. The absence of such an effect suggests the IHH protocol used in this study is not sufficient to induce sensitisation of the hypoxic ventilatory response.

It is possible that the lack of ventilatory LTF and increased hypoxic ventilatory response in this study was due to the combination of hypercapnic hypoxic exposures. Intermittent hypercapnia induces a form of ventilatory neuroplasticity called long-term depression, characterised by a sustained reduction in peak neural burst amplitude despite maintenance of $P_{ETCO_2}$ (Bach and Mitchell, 1998). However, this has only been demonstrated in rats following severe hypercapnic exposures (10% inspired CO$_2$, raising $P_{ETCO_2}$ to 80-95 mmHg) and not following moderate hypercapnia (3-5% CO$_2$, raising $P_{ETCO_2}$ to 60 mmHg) similar to that used in the current study. Pre-treatment with alpha2-adrenergic antagonists blocked this response (Bach and Mitchell, 1998), suggesting that intermittent
hypercapnia induces ventilatory long-term depression via activation of locus coeruleus neurons, as opposed to the induction of LTF following IH via activation of serotonergic raphe neurons (Kinkead et al., 2001). Thus it has been proposed that the combination of both intermittent hypercapnia and hypoxia, as inevitably occurs with obstructed breathing in OSA, may have opposing effects in a push-pull fashion that could ultimately produce no net effect (Kinkead et al., 2001). Consistent with the findings of this study Diep et al. reported that in awake humans 30 s episodes breathing 6% O₂ and 5% CO₂ separated by 90 s of breathing air did not induce ventilatory LTF (Diep et al., 2007). In contrast, simulated apneas in rats, lasting 25 s with 5 min between intervals to induce hypercapnic hypoxia, has been shown to induce phrenic and hypoglossal LTF (Mahamed and Mitchell, 2008). Furthermore, despite milder desaturations than IH, the magnitude of LTF was similar to that induced by poikilocapnic IH alone. This led the authors to conclude that the combination of a hypercapnic hypoxic stimulus may be a more potent stimulus for induction of LTF than IH alone (Mahamed and Mitchell, 2008). Waters and Tinworth investigated the effects of varying cycle durations of IHH on ventilatory LTF (Waters and Tinworth, 2003) in piglets, in the only other study in either humans or animals that we are aware of to have investigated the effects of combined IHH on ventilatory neuroplasticity. Continuous exposure for 24 min, and 24 min of 2 min cycles did not induce changes to ventilation post gas exposure. However, 8 min cycles induced ventilatory LTF and 4 min cycles induced ventilatory long-term depression (Waters and Tinworth, 2003). These findings are consistent with noradrenergic and serotonergic pathways acting in a push-pull fashion, with the duration and timing of gas applications determining whether IHH induces facilitation, depression or no change in post-stimulus ventilation. Thus, it may be
the pattern and timing of exposures, rather than concomitant hypercapnia *per se*, which prevented the induction of ventilatory LTF in this study.

We opted to use 30 s exposures with 2 min intervening room air periods to mimic gas disturbances commonly experienced in OSA, corresponding to an AHI of 24 /h with desaturations to around 80-85%. However if this pattern of IHH is not sufficient to induce neuroplasticity (either LTF or increased hypoxic sensitivity) it may be that only certain patterns associated with milder or more severe OSA are needed to explain pathological neuroplastic changes in patients with treatment reversible elevated CG. This contention is supported by previous work showing LG correlates with AHI, being higher in severe OSA versus mild/moderate OSA (Younes et al., 2001). In OSA, obstructed breathing events show marked variability in the duration, severity, inter-event interval and therefore frequency of blood gases disturbances. Considering the potential interacting effects of concomitant CO$_2$ levels (whether that be poikilocapnic, isocapnic, hypercapnic or intermittent), timing and severity of IH exposures, it is important that future work be directed towards determining the mechanisms and interactions of both hypercapnia and hypoxia underpinning ventilatory neuroplasticity. Given two opposing theories regarding the role of LTF in OSA pathophysiology, with one postulated to be protective and the other to augment LG and therefore exacerbate breathing disturbances, further work is needed to clarify the role of ventilatory LTF in altering LG.

One technical limitation of this study is that the PRBS test utilized a normoxic hypercapnic stimulus to evaluate CO$_2$ chemoreflex control above eupnoea. Chowdhuri *et al.* showed that IH increased hypocapnic CG and LG below eupnoea,
due to a reduction in eupnoeic P_{ET}CO_2 and the CO_2 reserve (Chowdhuri et al., 2010). However, above eupnoea IH increases hypoxic sensitivity but not hypercapnic sensitivity (Mateika et al., 2004), suggesting IH induces a leftward shift in eupnoea along the isometabolic hyperbola even though the gain of the response above eupnoea may remain unchanged. Theoretically, this should increase the ventilatory response for any level of CO_2 above eupnoea. Consequently, we theorized that LTF expression potentially masked without sustained hypercapnia (Harris et al., 2006) would also become apparent in dynamic CO_2 control responses to 4% CO_2 during normoxia. However, PRBS of normoxic hypercapnia may not accurately assess IH induced neuroplastic effects on LG given it fails to examine hypoxic chemosensitivity or chemoreflex control effects below eupnoea. Methods to more comprehensively assess dynamic LG including hypoxic CG influences remain to be developed and evaluated. Existing methods using mechanical ventilation or CPAP manipulations are better suited to sleep than wake measures and have other limitations, such as more problematic estimates in non-OSA participants, and the provision of only overall LG without separate evaluation of CG and PG (Sands et al., 2014, Younes et al., 2001, Terrill et al., 2014). Thus, despite some limitations, the PRBS technique appears to remain the best suited established method for assessing plasticity in the main CO_2 related elements of LG and CG in OSA patients versus controls during wake.

In conclusion, supplementation of CO_2 prevents hypocapnic constraint of LTF expression during ventilatory augmentation in both awake rats and humans (Harris et al., 2006, Olson et al., 2001). For this reason we hypothesised that a combined hypercapnic hypoxia stimulus, which is clearly more relevant to OSA than either IH
alone or IH plus sustained mild hypercapnia, would help to facilitate LTF expression by preventing post-hypoxia hypocapnia. We further anticipated that elevated CO$_2$ during the PRBS test would help to unmask any LTF expression and associated dynamic chemoreflex control disturbances potentially constrained without concomitant hypercapnia. However, as ventilatory LTF and sensitisation of chemoresponsiveness was not induced by IHH, whether these neuroplastic changes increase dynamic CG and LG could not be determined. The absence of LTF and ventilatory control changes in this study supports previous findings showing that the effects of IHH differ to IH, and that the timing of gas exposures may critically modulate the neuroplastic effects of IHH. As hypercapnia and hypoxia are experienced together in OSA, and due to the conflicting hypotheses regarding the possible roles of neuroplasticity in OSA pathophysiology and possible treatments, further work investigating neuroplastic effects of IHH in both humans and animals remains necessary to help guide development of future treatments.
CHAPTER 4. **High loop gain suggestive of weight distribution effects on dynamic CO$_2$ control in obstructive sleep apnoea**

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**Running Title:** Elevated loop gain in OSA versus matched controls

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By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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4.1 Abstract

Elevated loop gain is a key non-anatomical pathological trait contributing to obstructive sleep apnoea. This study sought to determine controller versus plant gain components of elevated loop gain, including lung volume, in obstructive sleep apnoea patients, and to examine the effect of treatment on loop gain parameters over time. Measures of loop gain, lung volume and lung function were examined using a 4% CO₂ pseudorandom binary stimulation method, helium dilution and spirometry respectively, and compared between newly diagnosed male obstructive sleep apnoea patients and case-controls matched for age, sex, height, weight and BMI. Patients then started continuous positive airway pressure therapy, with repeat measurements after 2 and 6 weeks of treatment. Loop gain was higher in patients versus controls (0.25 ± 0.02 versus 0.17 ± 0.02, p = 0.045) and there was a greater peak response and faster CO₂ decline in patients. However, there was no effect of treatment on loop gain, controller gain or the dynamics of the CO₂ response. Patients exhibited a larger supine abdominal height (29.5 ± 1.3 cm versus 24.2 ± 0.3 cm, p = 0.004) which positively correlated with AHI (r² = 0.639, p = 0.003). FEV1 and FVC were also reduced in OSA patients compared to controls (92.2 ± 1.7 versus 102.9 ± 3.5 percent predicted, p = 0.021; 93.4 ± 3.1 versus 106.6 ± 3.6 percent predicted, p = 0.015, respectively). These results suggest that differences in weight distribution and perhaps lung function in obstructive sleep apnoea patients may contribute to increased LG and OSA severity.
4.2 Introduction

Obstructive sleep apnoea (OSA) is a prevalent sleep disorder characterized by repeated partial or complete collapse of the upper airway during sleep causing bouts of hypercapnia and hypoxia, typically associated with arousal, hyperventilation and subsequent ongoing cyclical obstruction and arousal (Dempsey et al., 2010). The resultant sleep fragmentation and blood gas disturbances are associated with major neurocognitive, safety and cardiovascular sequelae (Morrell and Twigg, 2006, Somers et al., 2008). Recent data show that OSA affects approximately 17% of men and 9% of women aged 50-70 yr (Peppard et al., 2013), with prevalence appearing to have increased over time in line with population trends towards obesity (Young et al., 2009); a major risk factor for OSA.

The pathophysiology of OSA is now understood to involve several interacting factors which vary between individuals (Wellman et al., 2013). Recent work has begun to focus on treatments better targeted to each contributing factor (Edwards et al., 2012, Heinzer et al., 2008), including unstable ventilatory control and elevated loop gain (LG), a key non-anatomical pathophysiological trait thought to contribute to OSA (Wellman et al., 2013).

Unstable ventilatory control results in fluctuations in blood gases and neural drive to both ventilatory pump and upper airway dilator muscles (Remmers et al., 1978, Sankri-Tarbichi et al., 2009). Consequently elevated LG may render the airway susceptible to collapse during periods of low drive. LG has two major components; controller gain and plant gain (CG and PG, respectively), and in the context of ventilatory control these predominantly reflect chemoreflex sensitivity and the effectiveness of the lungs to change blood gases respectively (Khoo, 2000a). LG is
the ratio of the magnitude of a ventilatory response to the magnitude of the disturbance which elicited it. Hence, the higher the LG, the more prone the system is to self-perpetuating oscillations (Khoo, 2000a). Several recent studies investigating treatments to reduce LG, such as acetazolamide (Edwards et al., 2012) and oxygen therapy (Wellman et al., 2008), have confirmed that reducing LG can significantly reduce the apnoea-hypopnea index (AHI) and thus the severity of OSA. However these therapies essentially act to counteract high LG, rather than to treat the cause of high LG itself, such that a greater understanding of underlying mechanisms is needed towards refining future LG based treatments.

Two main factors that can increase LG are low lung volume, which increases PG, and elevated chemoreflex sensitivity (i.e. increased CG) (Dempsey et al., 2004). Hudgel et al. found that dynamic ventilatory control was less stable in OSA patients compared to normal weight controls and speculated that obesity effects on lung volume were likely important (Hudgel et al., 1998). However, lung volume was not measured to allow this to be examined in any detail. The only other published study to have compared LG between OSA patients and non-OSA controls found LG was elevated in both obese OSA patients and obese non-OSA controls compared to lean non-OSA controls, but with no difference between obese OSA patients and obese controls (Sands et al., 2014). Although lung volume and CG versus PG contributions to overall LG were not determined in this study, these findings indicate that elevated LG is not independently associated with OSA. These data support a role of obesity dependent reduced lung volume and increased PG as a key mechanism increasing LG in OSA patients. However, there is no published literature comparing lung volumes between OSA and morphologically matched
non-OSA controls. Therefore it is not certain whether lung volume or PG differences are independently associated with OSA.

In contrast to dynamic LG studies, steady state chemoreflex studies that have compared OSA patients to body mass index (BMI) matched control participants consistently show OSA patients exhibit abnormalities in chemoreflex control which increase LG. OSA patients exhibit an increased ventilatory response to hypoxia (Narkiewicz et al., 1999c) and increased CO₂ sensitivity below eupnoea due to a reduced eupnoiec end-tidal CO₂ (P_{ET}CO₂) and CO₂ reserve (Salloum et al., 2010). Consequently, OSA patients exhibit an increased dynamic ventilatory response to combined hypercapnic hypoxia, which potentially exacerbates post-obstruction hypocapnia and upper airway hypotonia (Younes et al., 2007). These abnormalities in chemoreflex control normalize with continuous positive airway pressure (CPAP) treatment (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006), suggesting that elevated CG is largely an induced feature of OSA that is reversible with treatment.

Thus, although LG is widely accepted as being a contributory factor in OSA pathophysiology (White, 2005, Eckert and Malhotra, 2008), the data to help clarify if elevated LG is independently associated with OSA, or whether it is obesity dependent remain conflicting. Further conflicting data and debate surrounds the mechanisms most likely causing elevated LG in OSA (CG versus PG) and whether these are inherent or induced traits (Orr et al., 2014, Younes, 2014). While there is evidence from steady-state chemoreflex studies showing CPAP can reduce CG, whether CPAP reduces dynamic LG has also not been investigated. The primary
purpose of this study was therefore to compare ventilatory control parameters and lung volume in newly diagnosed and untreated male OSA patients to those of age, height, weight and BMI matched non-OSA males, and to investigate the effects of 6 weeks of CPAP treatment on PG, CG and overall LG assessed using a pseudorandom binary stimulation (PRBS) CO$_2$ test (Khoo et al., 1995). A secondary aim was to explore potential predictors of LG disturbances and OSA severity, including lung volume and related anthropomorphic measures.

4.3 Methods

4.3.1 Participants

Ten men with newly diagnosed and previously untreated severe OSA (AHI > 30/hr), and ten healthy non-OSA men matched for age, height and BMI gave written informed consent and participated in the study. All participants were recruited from the Adelaide Institute for Sleep Health following diagnostic polysomnography to either confirm or exclude OSA, and to exclude participants with any other sleep disorder. All participants were non-smokers (had never smoked or had quit smoking a minimum of 6 months prior to commencement in the study) and demonstrated normal lung function while seated, with forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) $\geq$80% predicted (JLab software version 4.53; Compactlab, Jaeger, Wuerzburg, Germany). None of the participants were born or had lived at high altitude, had any known heart, lung or kidney disease, diabetes or any other major health problem. Patients with mild depressive disorders were included as long as their medication and dosage did not change throughout the study. However, none were taking any medications with
sedative or myorelaxant properties, or with effects on cardiac or respiratory control. The study was approved by the Southern Adelaide Clinical Human Research and Ethics Committee.

4.3.2 Measurements and Protocol

Measurements were obtained in OSA patients prior to commencing CPAP treatment (baseline) and then repeated approximately two and six weeks after commencing CPAP, with equivalent measurements and time-points in control participants who received no intervening treatment. OSA patients received treatment as usual with conventional CPAP. CPAP machine data were downloaded after 6 weeks of treatment to determine CPAP usage in fortnightly intervals.

Participants arrived in the laboratory at 6pm after being asked to abstain from eating for the preceding 4 hours, and to have avoided caffeine, alcohol and strenuous exercise throughout the day of the study. Upon arrival, height, weight, blood pressure, and abdominal girth were measured. Supine abdominal height was also assessed as an additional measure of central obesity on the basis of previous reports showing strong relationships with intra-gastric pressure (Lambert et al., 2005), which our group has previously found to predict upper airway closing pressure in OSA (Stadler et al., 2009).

Subjects then lay supine with their nose clipped for measurements of supine functional residual capacity (FRC) using a helium dilution re-breathing method via a mouth-piece (CareFusion Jaeger Masterscreen PFT GMBH Hockberg, Germany system). To avoid potential effects of body fluid shifts on lung volume, participants
lay supine for 10 min prior to the first measurement, with a further 10 min between each replicate measurement to ensure helium washout from the lungs between measurements. FRC was determined as the mean of three replicate measures within 200 mL.

Electroencephalograms (C4-A1 and C3-A2) and left and right electrooculograms were monitored throughout the remaining protocol in order to confirm wakefulness (Compumedics, E-Series, Melbourne Australia). The electrocardiogram, arterial oxyhemoglobin saturation (SaO₂, via ear pulse oximetry, POET II model 602-3; Criticare Systems, Waukesha, WI) and P_{ET}CO₂ (Capnostream20, Oridion, Israel), pre-calibrated before each experiment against a known standard (8% CO₂, alpha-standard gas, BOC gases Australia), were also monitored continuously. Participants were fitted with ear phones and listened to quiet music to minimise external stimuli influencing breathing.

Each participant wore a nasal mask (Gel mask, Respironics, Murrysville, PA) and mouth tape (with easily removable tabs) to ensure nasal breathing. The mask was fitted with a two way non-rebreathing valve (series 2600, Hans Rudolph, Kansas City, MO) with a pneumotachograph (PT16, Jaeger, Germany), calibrated via replicate 1-L syringe manoeuvres before and after each experiment, and attached to the inspiratory side of the breathing valve for quantitative inspiratory flow and volume measurements. The common outlet port of a Gatlin-shaped 4 inlet port valve system (series 2440C, Hans Rudolph) was connected to the pneumotachograph via 1 m of tubing (Hans Rudolph, 35mm Clean-bore) for delivery of inspiratory gases. A computer controlled solenoid system that monitored
the airflow signal controlled rapid pneumatic balloon inflation and deflation to allow only one inlet port to be open at a time, and ensured that all switches between ports were conducted during expiration. Three of the inlet ports were open to room air while the fourth was connected to a foil bag (300L, Scholle Industries, Adelaide, Australia) connected via 3-way stop-cocks to enable closure of the bag and manual switching between the bag and the inlet port. The bag was filled with a mixture of medical grade CO₂ blended with medical air at flow rates adjusted to achieve 4% CO₂ on the pre-calibrated CO₂ analyser. The bag was then partially filled and flushed to discard dead-space, and then filled to near capacity immediately prior to the experiment.

4.3.3 Pseudorandom binary stimulation (PRBS) protocol
Participants lay supine and were instructed to keep their eyes open, to stay still and to breathe normally. Following a 10 min baseline to record stable breathing on room air the PRBS protocol commenced. This consisted of a sequence of 63 breaths alternating between room air and 4% CO₂ in a pre-determined pseudorandom sequence spanning the full range of switching frequencies of between 1 and 6 consecutive breaths of CO₂ (Khoo et al., 1995). The same sequence was repeated a total of 8 times without interruption between adjacent sequences.

4.3.4 Data analysis and statistical procedures
Inspiratory, expiratory and total breath times were determined from the flow signal, which was digitally integrated to measure inspiratory tidal volume and minute ventilation breath-by-breath. Breath-by-breath measurements of inspiratory and
expiratory (end-tidal) partial pressure of CO₂ were determined from the nadir and peak in mask CO₂ respectively after adjusting for gas sampling delay.

The first pseudorandom binary stimulation sequence and any sequence containing breaths contaminated by movement artefact, swallows, sighs or periods of sleep were excluded from analysis (Khoo, 2000b). CG, PG, overall LG, feedback delay and other parameters of a simplified model of CO₂ chemoreflex control of breathing were estimated by fitting breath by breath measures of minute ventilation (V₁), inspiratory CO₂ (PICO₂) and PETCO₂ during the PRBS test to the CO₂ chemoreflex control model of breathing described by Khoo (Khoo, 2000b), with a minor modification to allow estimation of overall LG. The main outcomes of this analysis were gain parameters estimated at 1 and 2 cycle/minute (i.e. CG₁, CG₂, PG₁, PG₂, LG₁ and LG₂) and the model-based estimated impulse response of the system over multiple breaths following a sudden change in CO₂.

Age, height, BMI, FEV₁ and FVC were compared between groups using Student’s independent samples t-tests. Linear mixed model analysis was used to examine visit, group and interaction effects in measures repeated over time at each visit (abdominal girth, supine abdominal height, weight, mean arterial pressure (MAP) and CG, PG and LG). Pearson’s product-moment correlation coefficient was used to explore the strength of linear correlations between abdominal girth, supine abdominal height, FEV₁, FVC, FRC and AHI. PRBS derived CO₂ impulse responses were compared between groups and visits using linear mixed model analysis. CPAP data were assessed in 2 week blocks to assess CPAP compliance over time. Significant main or interaction effects were examined using Bonferroni
adjusted post-hoc analyses where appropriate. All statistical analysis was performed using SPSS (IBM SPSS Statistics Version 22). All data are presented as means ± SEM. P-values <0.05 were considered statistically significant.

4.4 Results

4.4.1 Subjects

Following consent several participants did not complete the full protocol; one OSA patient was prescribed mandibular advancement splint treatment instead of CPAP and was excluded. One OSA patient and another control participant (with an AHI of 4 /hr) had already been commenced on CPAP before baseline measurements could be collected. A further OSA participant was lost to follow up prior to baseline and another control participant hyperventilated and was unable to breathe normally while wearing the nasal mask. Consequently baseline data from 7 OSA participants and 8 non-OSA controls were available for analysis. The characteristics of these participants are presented in Table 4.1. One further OSA participant was subsequently lost to follow-up, and some equipment and other problems led to some further missing FRC and other measurements.

Consistent with case-matching between groups, there were no statistically significant differences in age, height, weight or BMI between OSA patients and non-OSA controls (Table 4.1). Both FEV1 (p = 0.021) and FVC (p = 0.015) were significantly lower in the OSA compared to non-OSA group. Two OSA patients and three control participants were past smokers, but there were no differences in FEV1 or FVC between ex- and non-smokers when participants were grouped according
to smoking history instead of OSA status. There were no significant differences between groups, visits or group by visit interaction effects in weight, mean arterial pressure or FRC (Table 4.2). There was also no significant difference across visits in supine abdominal height or abdominal circumference. When comparing all participants, OSA patients had a significantly greater supine abdominal height (29.2 ± 1.5 cm versus 24.2 ± 1.3 cm, group p = 0.03). However, re-analysis restricted to only the 4 OSA patients and their case-matched control participants did not show a significant difference between groups in supine abdominal height. There was also a trend towards a lower supine FRC in the OSA patient group (group by visit interaction effect p = 0.078).
Table 4.1. Characteristics of OSA patients and matched controls.

<table>
<thead>
<tr>
<th></th>
<th>OSA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>46.0 ± 3.2</td>
<td>46.0 ± 4.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.3 ± 1.5</td>
<td>179.2 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.6 ± 1.2</td>
<td>30.2 ± 0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>102.7 ± 4.5</td>
<td>97.1 ± 4.5</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>92.2 ± 1.7 *</td>
<td>102.9 ± 3.5 *</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>93.4 ± 3.1 *</td>
<td>106.6 ± 3.6 *</td>
</tr>
<tr>
<td>AHI</td>
<td>55.3 ± 6.4 *</td>
<td>5.2 ± 0.9 *</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Anthropometric characteristics of OSA patients (n = 7) and non-OSA controls (n = 8). Body mass index (BMI), percent predicted forced expiratory volume in the first second (FEV1), percent predicted forced vital capacity (FVC), and total sleep apnoea hypopnoea index (AHI). * indicates a significant difference between groups (p < 0.05).
Table 4.2. Measurements repeated at each visit in OSA patients and matched controls.

<table>
<thead>
<tr>
<th>OSA</th>
<th>B</th>
<th>2wks</th>
<th>6wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC (L)</td>
<td>1.8 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>AG (cm)</td>
<td>111.4 ± 4.8</td>
<td>113.5 ± 7.5</td>
<td>115.9 ± 5.0</td>
</tr>
<tr>
<td>SAH (cm) *</td>
<td>27.8 ± 1.3</td>
<td>28.9 ± 3.1</td>
<td>29.0 ± 2.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>102.7 ± 4.5</td>
<td>103.3 ± 5.3</td>
<td>105.2 ± 5.7</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101.9 ± 5.6</td>
<td>99.8 ± 3.4</td>
<td>101.1 ± 4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>B</th>
<th>2wks</th>
<th>6wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC (L)</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>AG (cm)</td>
<td>105.7 ± 2.2</td>
<td>106.5 ± 2.4</td>
<td>104.5 ± 2.7</td>
</tr>
<tr>
<td>SAH (cm) *</td>
<td>24.2 ± 0.4</td>
<td>24.1 ± 0.7</td>
<td>24.2 ± 0.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>97.1 ± 4.5</td>
<td>96.7 ± 4.5</td>
<td>97.1 ± 4.5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>94.3 ± 2.9</td>
<td>94.1 ± 2.0</td>
<td>97.8 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM in OSA patients and non-OSA controls at baseline (B), two weeks (2wks) and six weeks (6wks). Supine functional residual capacity (FRC), abdominal girth (AG), supine abdominal height (SAH), weight and mean arterial pressure (MAP). The number of participants contributing to each measure is shown in parenthesis. * indicates a significant difference between groups (p < 0.05).

4.4.2 CPAP data

CPAP usage on all days, days used, and the percentage of days with ≥4 hours use was consistent with normal clinical use, but significantly declined over the 6 week period of study (Fig. 4.1). However, there was no significant effect of visit on the percentage of days used (84.0 ± 5.4%), time spent in large leak (5.5 ± 3.4 mins), residual AHI (3.8 ± 0.6 events/hr) or CPAP pressure (12.3 ± 1.1 cmH2O).

4.4.3 Loop Gain

LG2 was significantly higher in OSA patients (0.25 ± 0.02 versus 0.17 ± 0.02, p = 0.045, Fig 4.2) and there was a trend for higher LG1 in OSA patients (0.60 ± 0.05...
versus $0.44 \pm 0.05$, $p = 0.056$), but there were no other significant between group differences, effects of CPAP treatment (visit), or group by visit interaction effects in LG, CG or PG.

**Figure 4.1. CPAP usage across the 6 weeks of treatment.**
p values indicate main effect of visits. * indicates significant post-hoc contrast versus 2 weeks ($p = 0.003$). Values are mean ± SEM, n= 6.

**Figure 4.2. Loop gain parameters in OSA and control participants at each visit.**
Loop gain (LG2), controller gain (CG2) and plant gain (PG2) calculated at 2 cycles/min measured at baseline (B), and after two and six weeks of CPAP use in OSA patients (black) and in age and body-habitus matched controls without CPAP treatment (white). Values are mean ± SEM, n= 7 OSA, 8 non-OSA.
The estimated impulse response to a sudden change in CO₂ showed a significant group by breath interaction effect ($p < 0.001$, Fig. 4.3 shows all visits combined), with a greater peak response in OSA patients compared to controls ($0.127 ± 0.024$ versus $0.095 ± 0.016$ L/min/mmHg CO₂, $p<0.001$) and lower ventilatory response from breaths 4 to 11, but no other group, visit or group by visit interaction effects.

Figure 4.3. Estimated impulse responses to a sudden change in CO₂.
Data showing OSA patients (black) and matched controls (grey) when collapsed across visits. * indicates significant post-hoc contrasts between groups ($p < 0.05$). Values are mean ± SEM, n= 7 OSA, 8 non-OSA.
4.4.4 Correlations

There was a trend for a positive correlation between supine abdominal height and AHI (Fig. 4.4) but no other correlations between AHI and FRC, or any other anthropomorphic or LG measure.

![Figure 4.4. Positive correlation between abdominal (Abdo) supine height and AHI for OSA and matched controls.](image)

$R^2 = 0.3596$

$p = 0.051$
4.5 Discussion

The main findings of this study were a higher LG in male OSA patients compared to an age and body habitus matched control group of males, and an altered dynamic response to normoxic CO₂ consistent with less stable CO₂ chemoreflex control in OSA patients. Contrary to previous reports (Loewen et al., 2009; Salloum et al., 2010), this study found no evidence to support a change in chemo-reflex control over 6 weeks of CPAP use. There was also no significant difference between groups or treatment effect in CG, PG or FRC, which could potentially reflect type II error. A trend towards a positive correlation between supine abdominal height and AHI in the OSA patients agree with previous findings, which suggest that central obesity may importantly contribute to elevated LG and increased airway collapsibility to promote OSA (Hudgel et al., 1998; Stadler et al., 2009; Aihara et al., 2011; Sundar et al., 2011; Sands et al., 2014).

The lack of difference between groups in CG and PG, and a lack of treatment effect in CG may be due to limitations of the PRBS of CO₂ technique. However, all currently available techniques to assess dynamic LG have some limitations. Proportional assist ventilation and CPAP pressure drop techniques rely on measurements conducted during sleep and cannot separately measure CG and PG components of overall LG (Younes et al., 2001; Sands et al., 2014). New methods utilizing spontaneous respiratory disturbances during a diagnostic sleep study in OSA patients have not yet been tested in non-OSA controls (Terrill et al., 2014). Thus we elected to use PRBS using 4% CO₂ during wakefulness as this is currently the only available method to allow CG, PG and overall LG comparisons between OSA patients and non-OSA controls. However, as this method is
conducted in normoxia, the contribution of increased hypoxic sensitivity to CG in OSA is almost certainly underestimated. Future studies may benefit from new methods incorporating hypoxic chemoreflex assessment into dynamic LG modelling, in order to better evaluate CG contributions to elevated LG in OSA patients.

Although CPAP use during the final fortnight of treatment remained above what would often be considered to be clinically acceptable compliance (≥4 hrs use on >65% of nights) (Gay et al., 2006), it is possible that treatment effects require greater compliance. However, CPAP usage was similar to previous reports showing a reduction in chemosensitivity with CPAP use. Spicuzza et al. found that hypoxic ventilatory responsiveness decreased following 1 month of CPAP of approximately 6 hrs per night (Spicuzza et al., 2006). Salloum et al. found 1 month of CPAP reduced CO₂ responsiveness below eupnoea in a similar sized group, but did not report CPAP compliance (Salloum et al., 2010). More importantly, in the current study CG was not found to be elevated and thus abnormal in the OSA patients prior to treatment, such that CPAP would not be expected to have reduced LG via amelioration of abnormal CG.

Obesity is known to reduce FRC (Quanjer et al., 1993b) and would be expected to increase PG. We are not aware of any previously published data comparing lung volumes in OSA patients to non-OSA participants matched for factors known to alter lung volume (i.e; age, weight, height and BMI). We anticipated that case-control matching of morphologic traits expected to influence lung volume would eliminate FRC and PG differences to allow direct comparison of CG and potential
treatment effects between groups. LG was elevated in OSA patients in the absence of other conditions such as hypertension or heart disease with the potential to confound circulation time and mixing gain effects (Lanfranchi et al., 2003; Stanchina et al., 2007). Thus, elevated LG in the OSA patients without differences between groups in CG, PG and/or FRC could reflect type II error. Further studies are needed in larger cohorts to examine lung volume and PG versus CG effects in OSA compared to matched controls in more detail.

IH induces lasting neuroplastic changes at multiple locations within the ventilatory neural network. Acute intermittent hypoxia consisting of several short bursts can induce LTF within motor neuron cell bodies such as those of the phrenic or hypoglossal nerves (Bach & Mitchell, 1996). Although IH does not increase the sensitivity to CO₂ (Mateika et al., 2004; Peng & Prabhakar, 2004; Khodadadeh et al., 2006; Pialoux et al., 2009), phrenic LTF increases ventilatory drive for the same level of chemical stimulation (Mitchell et al., 2001). When rats and humans are exposed to IH to induce ventilatory LTF and then returned to a poikilocapnic environment, ventilatory feedback reduces P_{ET}CO₂ and constrains the expression of LTF (Olson et al., 2001; Harris et al., 2006). In humans this reduces the CO₂ reserve below eupnoea and also increases CG below eupnoea (Chowdhuri et al., 2010). Chronic intermittent hypoxia in rats, designed to mimic OSA with 8 hr daily exposures for several days to weeks, has been shown to induce neuroplasticity at the carotid bodies to increase both basal neural discharge and hypoxic sensitivity (Peng et al., 2001; Peng et al., 2003). In humans even acute IH increases hypoxic sensitivity while chronic exposure to IH enhances this response (Harris et al., 2006; Lee et al., 2009). Consequently, the combination of ventilatory LTF and
sensitization of the carotid bodies hypoxic sensitivity potentially increases the magnitude of ventilatory response to combined hypercapnic hypoxia, thus increasing CG and LG. In both animals and humans these forms of neuroplasticity are reversible, with neural activity and ventilatory responses returning to baseline following several days of re-exposure to room air (Peng et al., 2003; Peng & Prabhakar, 2004; Pialoux et al., 2009; Brugniaux et al., 2011).

OSA patients exhibit the same abnormalities in chemoreflex control that are seen following exposure to experimental IH, and these effects are also reversible with CPAP treatment. Salloum et al. compared steady-state CG and PG between OSA patients and age, sex and BMI matched controls and found that OSA patients had an elevated CG below eupnoea, due to a reduced eupnoeic P_{ET}CO_2 and CO_2 reserve, which decreased following one month of CPAP, but with no differences or CPAP effects on PG (Salloum et al., 2010). i.e. LG disturbances in OSA patients appeared to decrease following CPAP purely via a reduction in CG. Above eupnoea OSA patients also exhibit an elevated ventilatory response to normocapnic hypoxia (Narkiewicz et al., 1999) and hypercapnic hypoxia (Younes et al., 2007), both of which normalize with CPAP use (Spicuzza et al., 2006; Loewen et al., 2009). We therefore hypothesized that secondary to repeated exposure to IH and neuroplasticity effects, OSA patients would exhibit higher LG than age and body habitus matched controls due primarily to an elevated CG, and that this would normalize following 6 weeks of CPAP treatment. Whilst OSA patients did show higher LG, CG was not different between groups and showed no change over the course of CPAP treatment.
The estimated impulse response to a sudden change in CO₂ in this study concords with the findings of Hudgel et al. who used a similar CO₂ PRBS test and found a greater peak and faster post-peak recovery in the closed-loop response (reflecting the combined CG and PG feedback behavior of the system), but no difference in the open-loop response (reflecting CG behavior without feedback effects) in obese OSA patients compared to normal-weight non-OSA controls (Hudgel et al., 1998). Although lung volume was not measured, given the absence of weight and BMI matching the authors speculated that less stable control in the closed-loop response in OSA patients may have been secondary to reduced lung volume in obese patients versus lean controls (Hudgel et al., 1998). In the present study supine lung volume was measured and patients were closely matched for age, sex, height, weight and BMI to controls; all factors known to affect lung volume (Quanjer et al., 1993a). This was specifically designed to control for potential obesity effects on lung volume likely to influence PG. Despite case-control matching on traditional anthropomorphic measures of obesity, OSA patients exhibited reduced lung function (FEV1 and FVC) and greater supine-abdominal height, suggesting more centrally distributed adiposity, which showed a trend for a positive correlation with AHI, compared to non-OSA controls. Although there was no significant difference between groups in PG, these findings combined with those of Hudgel et al. (Hudgel et al., 1998) support that central obesity mediated effects on PG may contribute to higher LG in OSA patients. These are important findings given that PG abnormalities are likely to be less amenable to treatments other than weight loss or CPAP, compared to CG disturbances reflecting neuroplasticity in ventilatory control that may be more amenable to novel treatments.
We are aware of only one other recent study that has evaluated LG in obese OSA patients compared to obese non-OSA controls (Sands et al., 2014). Sands et al. found that LG was higher in obese individuals (both OSA and non-OSA) versus healthy weight non-OSA controls. However, there was no significant difference in LG between obese OSA patients and obese non-OSA controls, further supporting that obesity effects likely play a key role in elevated LG (Sands et al., 2014). The findings of the current study further suggest that differing body weight distribution effects may importantly contribute to both increased LG and increased airway collapsibility in OSA.

The literature therefore suggests two potential mechanisms underlie chemical control instability in OSA, which differ depending on measurement methods. Steady-state chemoreflex studies suggest that abnormal CG, due to IH induced neuroplasticity, underlies elevated LG in OSA. In contrast, dynamic LG studies suggest obesity driven differences in lung volume and PG, rather than CG, contributes to elevated LG in OSA. These discrepancies likely reflect limitations in both methods. Chemoreflex tests only assess steady-state responses and do not assess feedback mechanisms and therefore are not able to accurately quantify PG. However, these methods are better able to assess the full range of chemoreflex activity (i.e. hypo- to hyper-ventilation) and the interaction of both O\textsubscript{2} and CO\textsubscript{2}. While dynamic LG methods assess feedback mechanisms and PG, they are typically conducted with normoxia or hyperoxia, and thus primarily assess the CO\textsubscript{2} CG contribution to LG (Lai & Bruce, 1997; Younes et al., 2001; Sands et al., 2011; Wellman et al., 2013), which is not reported to be independently elevated in association with OSA (Narkiewicz et al., 1999; Sin et al., 2000) or affected by
CPAP use (Spicuzza et al., 2006; Foster et al., 2009). Thus LG methods likely underestimate the contribution of abnormal CG in OSA, which, at least above eupnoea, appears to be primarily driven by increased sensitivity of the peripheral chemoreceptors to hypoxia (Narkiewicz et al., 1999; Younes et al., 2007). This critical limitation of dynamic LG measurements may explain the lack of difference between groups and lack of treatment effect in CG in this study. Further work examining potentially important hypoxic chemoreflex drive contributions to CG and LG is required to better understand the differential role of CG and PG disturbances in OSA pathogenesis, and the impact of treatments designed to normalise LG.

FRC is an important determinant of end-tidal CO₂ and the degree to which tidal volume dilutes alveolar gas, and is thus an important factor influencing PG. Obesity reduces lung volume via compression of the thoracic cavity (Stadler et al., 2010) and should therefore increase PG. Reduced lung volume also appears to increase upper airway collapsibility by increasing upper airway transmural pressure (Kairaitis et al., 2007; Stadler et al., 2009; Stadler et al., 2010). Consequently, obesity may promote OSA via reducing lung volume and increasing PG, and also via anatomical interactions increasing airway collapsibility. Despite being matched for height, weight and BMI, OSA patients had a significantly greater supine abdominal height, but not abdominal girth when standing compared to non-OSA controls. There was also a strong positive correlation between supine abdominal height and AHI in the OSA patients. These findings support that differential weight distribution associated with central adiposity potentially contributes to OSA via increased LG and increased airway collapsibility effects.
Reduced FEV1 and FVC in OSA patients did not appear to be explained by any systematic difference in smoking history and might predominantly reflect body mass distribution influences on lung function. However, inflammatory airways diseases characterised by airflow obstruction, such as asthma and chronic obstructive pulmonary disease, commonly coexist with OSA (Shaya et al., 2009) so these findings could reflect common risk factors, lung volume effects of obesity, or possibly IH induced oxidative stress (McNicholas, 2009) known to exacerbate obstructive airway disease (Shaya et al., 2009) and potentially cause airway damage via IH induced inflammation (Aihara et al., 2011; Sundar et al., 2011).

In conclusion, this study supports that male OSA patients have a higher LG than age, weight, height and BMI matched male non-OSA controls. However, there were no differences in CG, PG or FRC and no effect of CPAP treatment. OSA patients exhibited differences in lung function and weight distribution, which may differentially affect lung volume, PG and airway collapsibility compared to age and body habitus matched non-OSA participants. Further studies in larger cohorts are needed to examine these mechanisms in more detail. Several previous studies comparing ventilatory control in OSA patients versus non-OSA participants have suggested that augmented chemoreflex sensitivity in OSA patients is induced by OSA and is reversible with treatment (Narkiewicz et al., 1999; Spicuzza et al., 2006; Younes et al., 2007; Loewen et al., 2009; Salloum et al., 2010), while studies more specifically examining LG suggest that obesity, lung volume and PG effects may dominate elevated LG in OSA patients (Hudgel et al., 1998; Sands et al., 2014). These apparent discrepancies may reflect important differences between steady-state chemoreflex tests versus more dynamic LG tests. As LG
methodologies to date have used normoxia or hyperoxia, these methods likely underestimate the contribution of abnormal hypoxic CG to LG in OSA patients. This limitation may explain the lack of difference between groups and lack of treatment effect in CG in this study. Further work incorporating hypoxic CG into LG measures is required to better understand the contribution of CG versus PG abnormalities in OSA patients, and potential effects of CPAP treatment. In addition, larger studies designed to further examine the contribution of obesity and lung volume effects on PG versus CG disturbances in OSA patients are also needed.
SUMMARY AND CONCLUSIONS

Ventilatory chemical control instability, quantified as elevated LG, is widely accepted to be a contributory factor in OSA pathophysiology (Khoo, 2000a, Longobardo et al., 2008, White, 2005). Key evidence to support this theory includes that stabilising ventilatory control and reducing LG reduces AHI (Edwards et al., 2012, Wellman et al., 2008), and that inducing instability in chemoreflex control induces obstructive apnoeas in normally healthy non-apnoiec participants (Onal et al., 1986, Sankri-Tarbichi et al., 2009). However at the time of commencing the studies contained in this thesis, there were no published studies comparing dynamic LG between OSA and body-habitus matched controls. Although Hudgel and colleagues found ventilatory control to be less stable in OSA versus non-OSA participants, this study did not control for factors such as age, height or weight known to alter lung volume and ventilatory control, nor was LG quantified (Hudgel et al., 1998). The authors postulated that reduced lung volume, and consequently increased plant gain, due to obesity was the predominant factor reducing ventilatory control stability in the OSA patients compared to the lean controls. However there was no published literature comparing lung volumes in OSA patients and body-habitus matched controls. Therefore it was unknown whether differences in lung volumes or plant gain were independently associated with OSA. Nor was it certain whether elevated LG is independently associated with OSA, or whether it is secondary to obesity.
The strongest evidence to suggest LG is abnormally elevated in OSA patients independent of weight comes from steady-state chemoreflex measures between OSA patients and matched controls. These studies show OSA patients exhibit abnormalities in chemoreflex control that increase CG, which without a concomitant opposing change in PG would also increase LG (Narkiewicz et al., 1999c, Salloum et al., 2010, Younes et al., 2007). These abnormalities ameliorate with CPAP treatment and therefore are clearly not inherent but induced by OSA itself (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006). Additionally, IH induced ventilatory neuroplasticity induces the same reversible changes in chemoreflex control (Chowdhuri et al., 2010, Mateika et al., 2004, Lee et al., 2009), strongly suggesting IH during OSA induces elevated CG and therefore LG. However, despite this evidence (and the lack of evidence to suggest elevated LG is otherwise independently associated with OSA), debate remains concerning whether elevated LG is an inherent or possibly induced trait in OSA patients (Orr et al., 2014, Younes, 2014).

IH induces sensitisation of the HVR and also vLTF in both humans and animals (Lee et al., 2009, Peng and Prabhakar, 2004, Pialoux et al., 2009b, Brugniaux et al., 2011). An increase in the HVR is accepted as destabilising as it reflects an increase in CG. In contrast, both hLTF and vLTF have been proposed as potentially stabilising neuroplastic mechanisms (Mahamed and Mitchell, 2007, Mateika and Syed, 2013). However, as vLTF is an increase in the ventilatory response to the same level of chemical stimuli (Mitchell et al., 2001a), and an increase in LG is defined as an increase in the ratio of the ventilatory response to the disturbance
which elicited it (Wellman et al., 2008), by definition vLTF must also reflect an increase in LG. It has also been shown that although IH does not change the sensitivity of the hypercapnic ventilatory response in animals or humans (Mateika et al., 2004, Khodadad et al., 2006, Pialoux et al., 2009b, Peng and Prabhakar, 2004), following the induction of vLTF, when CO₂ is not controlled, ventilatory feedback reduces P_{ETCO₂} (Harris et al., 2006, Olson et al., 1982). In humans this reduces the CO₂ reserve and increases controller gain below eupnoea (Chowdhuri et al., 2010). Therefore we propose, as detailed in Chapter 1 (Deacon and Catcheside, 2014), that it is the expression of vLTF which results in these destabilising changes to chemoreflex control that are seen both following experimental IH (Chowdhuri et al., 2010) and also in untreated OSA patients (Salloum et al., 2010).

Although increased HVR and LTF of ventilation, EMG_{gg} and reduced upper airway resistance have been documented in humans following acute IH, protocols are typically conducted under sustained hypercapnia or isocapnia (Chowdhuri et al., 2010, Harris et al., 2006, Lee et al., 2009, Pierchala et al., 2008). However it is known that the level of concomitant CO₂ can critically modulate the effects of IH (Waters and Tinworth, 2003). It has been theorised that the combination of both sustained hypercapnia and concomitant intermittent hypercapnia during IH may facilitate the induction of LTF (Harris et al., 2006, Mahamed and Mitchell, 2008). Intermittent hypercapnia can also induce long-term phrenic depression via α₂-adrenergic pathways, and this has been proposed to act in opposition to serotonergic facilitatory pathways elicited by IH (Bach and Mitchell, 1998, Kinkead
et al., 2001). This raises uncertainty regarding the relevance of results from IH studies conducted in poikilocapnia, isocapnia or hypercapnia to OSA pathophysiology, and highlights the need for studies of IHH to more accurately reflect stimuli experienced in OSA.

Given considerable uncertainty regarding the potential role of IHH induced neuroplasticity in the pathophysiology of OSA, the studies detailed in this thesis aimed to investigate whether IHH, designed to mimic the blood gas perturbations experienced in OSA, also induces vLTF and changes to chemoreflex control as has been reported following both isocapnic and hypercapnic IH in humans. Whether these neuroplastic changes increase LG, whether LG is different between OSA patients and body-habitus matched non-OSA participants, and whether LG differences reflect lung volume and PG or CG abnormalities was also investigated. Lastly, based on the evidence that CPAP ameliorates destabilising abnormalities in chemoreflex control in OSA patients, whether CPAP reduces LG was also investigated.

The lack of neuroplasticity in healthy males following IHH both during sleep (study 1, Chapter 2) and wake (study 2, Chapter 3) does not support an additive effect of combined IHH. Several past IH studies and one other study of IHH in humans have also failed to elicit vLTF (Diep et al., 2007, Jordan et al., 2002, McEvoy et al., 1996, Aboubakr et al., 2001, Rowley et al., 2007). This has led some to doubt that LTF or ventilatory neuroplasticity in general occurs in humans. Although LTF was not induced in these studies, this does not support the hypothesis that LTF and
neuroplasticity does not occur in humans. All we are able to discern from these findings, is that this particular protocol of IHH and the selected sample sizes were not sufficient to demonstrate the induction of neuroplasticity in humans. This does not preclude the possibility that a different protocol of IHH and/or larger sample size could demonstrate neuroplasticity in humans, or that IHH experienced during cyclical airway obstruction may induce neuroplasticity in OSA patients.

Where in the past the central nervous system was thought to be largely fixed and relatively incapable of change in form or function in adults, neuroplasticity is now understood to be a fundamental mechanism by which the adult central nervous system can adapt to changing demands (Kolb and Muhammad, 2014, Azmitia, 1999, Chakraborty et al., 2007). LTF of ventilation or ventilatory motor neurons has been demonstrated in several adult animals including dogs (Cao et al., 1992), cats (Fregosi and Mitchell, 1994), rats (Olson et al., 2001), goats (Turner and Mitchell, 1997) and in evolutionarily more distant avian species (Mitchell et al., 2001c). Thus, vLTF appears to be highly conserved along the evolutionary line and there is no evidence to suggest this fundamental adaptive mechanism of the central nervous system would not also be conserved in humans. In addition to the studies from different laboratories that have reported sensitisation of the HVR and vLTF following IH in humans (Brugniaux et al., 2011, Pialoux et al., 2009a, Pialoux et al., 2009b, Pierchala et al., 2008), there is also considerable evidence that abnormalities in chemoreflex (Leuenberger et al., 2007), ventilatory (Peng et al., 2003, Prabhakar et al., 2007b), sympathetic and cardiovascular neural control (Leuenberger et al., 2005, Narkiewicz et al., 1998, Narkiewicz and Somers, 1999,
Zoccal and Machado, 2011) in OSA patients are due to IH induced neuroplasticity. Thus, although it is not possible to determine from studies 1 and 2 why IHH did not induce neuroplasticity, strong evidence supports that IH induced neuroplasticity can occur in humans and is a prominent treatment reversible feature in OSA patients. Failure to demonstrate robust neuroplastic changes in these studies may therefore reflect a particular feature of this protocol.

In both Chapter 2 and 3 (studies 1 and 2) possible confounders are discussed in detail. These include arousal state, body position, number, intensity and duration of hypoxic exposures, returning to room air breathing both during and following IH and concomitant hypercapnia during IH. Studies 1 and 2 were conducted during sleep and wake, respectively, therefore arousal state is not likely to explain the lack of IHH-induced effects. Studies 1 and 2 were conducted in the lateral and supine position, respectively, which also seems to preclude body position as a key confounder. Although the protocol used in studies 1 and 2 consisted of 24 episodes compared to 15 episodes in a protocol previously reported to induce vLTF by Pierchala and colleagues (Pierchala et al., 2008), their protocol was otherwise very similar to that used in study 1 (and study 2 except for consciousness state). Both study 1 and the protocol by Pierchala and colleagues were conducted in 12 healthy non-OSA males during sleep, used similar duration gas exposures (30 s versus approximately 1 min, respectively), reduced SaO₂ to a similar degree (86.8 % versus 86.1 %, respectively), the time between gas exposures was similar (2 min versus approximately 114 s, respectively) and followed a similar protocol to return participants to room air breathing between gas episodes and during recovery. The
main difference between studies is that Pierchala and colleagues supplemented CO₂ during hypoxia only to maintain isocapnia (Pierchala et al., 2008), whereas in study 1 the addition of 3% CO₂ with hypoxia produced mild hypercapnia, raising $P_{ET}CO₂$ approximately 2 mmHg above control.

Few studies have investigated either intermittent hypercapnia or IHH. However, evidence that severe intermittent hypercapnia can induce long-term depression of phrenic neural drive (Bach and Mitchell, 1998) which may oppose facilitatory IH pathways (Kinkead et al., 2001) is supported by a study in piglets reporting that whether IHH resulted in ventilatory facilitation, depression or no net effect was dependant on the cycle duration of gas exposures (Waters and Tinworth, 2003). Considering this, and that the main difference between the protocol used in study 1 and that of Pierchalla et al. (Pierchala et al., 2008) was the level of concomitant CO₂ during IH, it appears likely that the timing of IHH may have prevented the induction of neuroplasticity, not IHH per se. Therefore although study 1 and 2 did not induce neuroplasticity as hypothesised, these results are still important as they suggest a critical dependence of concomitant CO₂ levels during IH and the need for further studies in both animals and humans using IHH to more accurately reproduce gas disturbance stimuli experienced during OSA. These findings also raise the possibility that both ventilatory facilitation and depression may occur in different patients or at different times within an individual.

The first published study of vLTF in awake humans was by Harris et al. in 2006 which used IH with a background of hypercapnia (5 mmHg above eupnoea) (Harris
et al., 2006). Based on these data Mateika’s group proposed that sustained hypercapnia is necessary to induce vLTF in humans during wakefulness (Mateika and Syed, 2013). However, the results of that study show that following the induction of vLTF, when CO₂ is not controlled, the increased V₁ results in a reduction of PₑₚCO₂ which then constrains V₁ and the expression of vLTF (Harris et al., 2006). This is supported by similar results in awake unanaesthetised rats (Olson et al., 2001) and also by another study from Mateika’s laboratory in humans during sleep, where vLTF during poikilocapnia coincided with reduced PₑₚCO₂ and CO₂ reserve and increased controller gain below eupnoea (Chowdhuri et al., 2010). This concords with the results of an earlier study by Mateika et al. in 2004 of poikilocapnic IH during wakefulness, which did not report vLTF, but a reduction of PₑₚCO₂ and an increase in the ventilatory response to hypoxia only when CO₂ was raised above the chemoreflex threshold (Mateika et al., 2004). In combination, these results suggest that hypercapnia is not necessary to induce vLTF during wakefulness. These findings only support that hypocapnia inhibits ventilatory output following IH and the induction of vLTF as it does under control conditions, and that the prevention of hypocapnia following IH due to ventilatory feedback is necessary to see the full expression of vLTF.

Study 2 was based on the hypothesis that although vLTF would not likely be expressed during room air breathing in awake humans (defined as an increase in V₁, although room air breathing PₑₚCO₂ would be expected to be reduced), it should be expressed during hypercapnia. Therefore, vLTF should increase the ventilatory response during a CO₂ PRBS test resulting in an increase in CG and
LG. However, studies 1 and 2 (Chapter 2 and 3) were run concurrently so it was not known that the selected IHH protocol did not induce neuroplasticity during sleep before study 2 was already completed. Further work is still needed to further test the original hypothesis.

Study 3 (Chapter 4) is the first study to compare dynamic LG in OSA patients and non-OSA controls matched for age, sex, weight, height and BMI and it is the first study to confirm that dynamic LG is elevated in OSA patients independent of weight. Contrary to these findings, another recent study comparing LG in obese OSA patients with both lean and age, sex and BMI matched non-OSA participants, found LG was not related to the presence of OSA but to obesity (Sands et al., 2014). This is the only other published study comparing dynamic LG in OSA patients and BMI matched non-OSA participants. Why the two studies show different results is not certain however may relate to different methods used to quantify LG.

The study by Sands et al. did not investigate the causal mechanism of elevated LG (Sands et al., 2014). Although in study 3 FRC, PG and CG were measured, the lack of difference between groups meant it was also not possible to determine the mechanism causing elevated LG in the OSA patient group. As LG is the product of CG and PG, one or both must be elevated for LG to be elevated. For this reason the lack of difference in CG or PG between groups appears most likely to reflect a type II error. Further studies with larger cohorts will be important to investigate the mechanisms contributing to elevated LG in OSA patients.
The lack of CPAP treatment effects on LG in OSA patients does not support the hypothesis that IH induces treatment reversible neuroplastic changes to chemoreflex control which increase CG and overall LG. This was surprising given considerable evidence from steady-state chemoreflex tests and amelioration of treatment emergent central sleep apnoea to support that OSA patients exhibit treatment reversible destabilising abnormalities in chemoreflex control (Loewen et al., 2009, Salloum et al., 2010, Spicuzza et al., 2006, Corcoran et al., 2009, Guilleminault and Cummiskey, 1982). On the other hand, the studies of Hudgel et al. (Hudgel et al., 1998) and Sands et al. (Sands et al., 2014) suggest that obesity and plant gain are the main contributors to elevated LG in OSA, not CG. This disparity between steady-state chemoreflex studies and dynamic LG studies raises some doubts about the ability of dynamic LG methods to determine CG contributions previously shown to be abnormal in OSA to overall LG.

Currently, CO₂ based PRBS techniques are the only established dynamic LG methods available to quantify CG and PG components of overall LG. However, CG estimated by these methods reflects only the dynamic CO₂ response above eupnoea, where steady-state chemoreflex tests report no differences in CO₂ sensitivity above eupnoea between OSA patients and non-OSA controls, nor CPAP treatment effects (Foster et al., 2009, Spicuzza et al., 2006, Sin et al., 2000, Narkiewicz et al., 1999c). Also, PRBS does not evaluate the hypocapnic ventilatory response or the hypoxic CG contribution to hypercapnic hypoxic CG, both of which are reported to be elevated in OSA patients and reversed with CPAP treatment.
(Loewen et al., 2009, Narkiewicz et al., 1999c, Spicuzza et al., 2006, Younes et al., 2007). Additionally, given data are baseline adjusted and only the slope of the CO₂ response is used in PRBS model fits, if vLTF were present causing a leftward shift along the metabolic hyperbola with no change in sensitivity, although the magnitude of the ventilatory response to the same level of CO₂ would be elevated (thus increasing LG defined as an increase in the \( \Delta \) ventilatory response / \( \Delta \) disturbance), CG as assessed by the PRBS to CO₂ would not be different. Oversimplification of models underlying dynamic LG estimates may substantially limit their utility and sensitivity to detect both CO₂ set-point (i.e. eupnea and therefore the magnitude of a ventilatory response independent of changes to slope) and more interactive O₂ and CO₂ related abnormalities in chemoreflex control. Although methods such as CPAP pressure drops are calculated from the magnitude of ventilatory response as opposed to the slope of chemoreflex response, and would likely incorporate a hypoxic CG contribution to the overall ventilatory response, the degree of hypoxia would be mild and the ventilatory response would still be dominated by CO₂ CG. Given these methods are incapable of separating CG and PG components, they are also not suitable for testing the hypothesis that IH induced neuroplasticity increases CG, or that CPAP reduces CG. These limitations may explain why dynamic LG studies suggest obesity and lung volume as the driving influence increasing LG in OSA patients and not CG. As dynamic LG methods do not accurately assess the aspects of chemoreflex control known to be abnormal and treatment reversible in OSA patients, and primarily assess PG contributions to overall LG. These considerations may also explain the lack of treatment effect on LG in study 3, as lung volume (and therefore PG) off CPAP
would not be expected to be affected by several weeks of CPAP treatment and showed no difference following 6 weeks of CPAP treatment.

In study 3, despite comparable weight and abdominal girth when standing between groups, OSA patients exhibited a higher supine abdominal height which positively correlated with AHI. This supports previous work showing that abdominal compression increases airway collapsibility in OSA patients (Stadler et al., 2009). The findings of study 3 also suggest that for the same degree of obesity, OSA patients distribute weight differently (more ventrally and centrally), which may contribute to increased airway collapse. OSA patients also exhibited reduced lung function (both FEV1 and FVC), which could partly influence PG. However as there was no difference between groups in PG or FRC it is not possible to determine from this study how central adiposity or reduced lung function contribute to LG.

In conclusion, the studies detailed in this thesis provide the first evidence that LG is elevated in OSA patients independent of weight and BMI. These findings also support previous work that central adiposity contributes to airway collapse in OSA patients, and extends this work to show that for a comparable degree of obesity, OSA patients appear to distribute weight differently which may negatively impact on airway collapsibility through both LG and airway collapsibility effects. Although FRC and PG were not different between groups, this could well reflect type II error. The positive correlation between supine abdominal height and AHI supports the theory that LG is elevated in OSA patients partly due to central adiposity dependant reductions in lung volume and increased PG. However, there was no evidence to
support the primary hypothesis that IHH induces ventilatory neuroplasticity in healthy males. In addition, as neuroplasticity was not induced it was not possible to determine whether these neuroplastic mechanisms increase LG. Despite these negative findings, these studies highlight the critical importance of concomitant CO₂ levels during IH on the induction of neuroplasticity. Future research in humans and animals is needed to further investigate neuroplastic mechanisms in OSA pathophysiology using IHH stimuli most relevant to human OSA. Although LG was found to be higher in OSA patients versus matched controls, the lack of CPAP effects on both LG and CG was surprising considering the evidence for treatment reversible chemoreflex abnormalities and ventilatory control instability in OSA patients. This likely reflects limitations in currently available dynamic LG methods which may not adequately evaluate the chemoreflex control abnormalities known to be present in untreated OSA patients. Thus, dynamic LG methods appear to primarily assess PG contributions to overall LG, which would not be expected to be affected by CPAP treatment. This may explain the disparity between steady-state chemoreflex studies and dynamic LG studies in OSA patients, and the lack of CPAP treatment effects in study 3. Further studies incorporating hypoxic CG contributions to overall LG and also the absolute magnitude of the ventilatory response to a chemical disturbance, as opposed to only the slope of the response, need to be incorporated into future LG models to better evaluate the role of neuroplasticity induced chemoreflex abnormalities contributing to elevated LG in OSA patients. This is necessary to more rigorously evaluate treatment avenues to reduce LG; a current focus within the field aiming to advance novel OSA treatments.
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