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Unstable Ventilatory Control During Sleep After High Spinal Cord Injury: The Contribution Of Chemosensitivity And Hypoventilation

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UNSTABLE VENTILATORY CONTROL DURING SLEEP AFTER HIGH SPINAL CORD INJURY: THE CONTRIBUTION OF CHEMOSENSITIVITY AND HYPOVENTILATION

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DEDICATION

To my loving family, who have always believed in me and taught me to believe in myself.

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I would like to thank my advisor and mentor, Dr. Harry G. Goshgarian. His love of science and research is inspirational and his ability to teach and support his students is second to none. I have been privileged to study and work with him. Dr. Goshgarian has provided me with endless patience, instruction, guidance and support. Without him I would never have been able to obtain my PhD. I am forever in his debt.

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INTRODUCTION

Sleep Disordered Breathing and Spinal Cord Injury

Over the past 3 decades sleep disordered breathing (SDB) in the general population has been the subject of increasing study due to the disorder's negative impact on health and well-being. SDB is associated with excessive daytime sleepiness, impaired cognition and increased morbidity and mortality due to effects on the cardiovascular system, which include: hypertension, coronary artery disease (CAD), pulmonary hypertension, heart failure, atrial fibrillation, deep vein thrombosis (DVT) and increased risk of stroke (Gopalakrishnan and Tak, 2001; Mohsen and Urbano, 2011; Chou et al., 2012; Johansson et al., 2012). Sleep disordered breathing is a general term that encompasses obstructive sleep apnea (OSA), in which patients experience apneas and/or hypopneas as a result of closure or narrowing of the upper airway during sleep, and central sleep apnea (CSA), in which apneas and/or hypopneas results from insufficient descending central ventilatory drive. Patients can also experience a combination of both types of SDB (Gilmartin et al., 2005). The prevalence of sleep apnea syndrome in the general population is estimated to occur in approximately 2-4% of adults per the Wisconsin Sleep Cohort Study (Young et al., 2002; Sean et al., 2005).

An increased incidence of SDB after spinal cord injury (SCI) has been described in the literature over the past decade along with reports of poor sleep quality, sleep fragmentation with frequent arousals and daytime hypersomnolence. However, the

specific features of SDB in this population are poorly described and the underlying mechanisms are not understood. The prevalence of SDB after SCI has been reported as being between 27-77% depending on the source and level of injury (Bonekat et al., 1990; McEvoy et al., 1995; Klefbeck et al., 1998; Burns, 2000; Stockhammer et al., 2002; Berlowitz et al., 2009; Biering-Sorensen et al., 2009; Tran et al., 2009; Sankari et al., 2014). Factors that account for the disparity in reported prevalence of SDB after SCI include: 1) the wide differences in methodologies used to diagnose sleep apnea in this population, which range from hospital-based sleep lab polysomnography (PSG), questionnaires of sleep disturbances (Biering-Sorensen et al., 2000), portable home sleep testing (Leduc et al., 2000), overnight pulse oximetry to measure nocturnal oxygen desaturation and retrospective chart reviews (Burns et al., 2001, Leduc et al.,2007) and 2) the varying criteria and methods used to classify SDB.

Factors that have been positively correlated with OSA in chronic SCI are much the same as those in the general population (obesity, increased neck circumference, age, gender) (Short et al., 1992) with the addition of the time from injury being an important factor as well as injury level (Stockhammer et al., 2002). Correlation with the American Spinal Injury Association (ASIA) impairment scale, which describes the motor and sensory extent of an injury, has been mixed (Burns et al., 2001, 2005; Stockhammer et al., 2002). However, a longitudinal study of SDB within the first year from injury (2 days to 52 weeks) after cervical spinal cord injury (cSCI) in male patients found no correlation between standard predictors of OSA and it's occurrence (Burlowitz et al., 2005) as has been described in chronic cSCI by other investigators (Short et al., 1992). Medications used to control pain and spasticity resulting from injury have been

implicated as possible contributing factors to SDB as well (Biering-Sorensen et al., 2000), although the results of studies exploring the use of baclofen, the most commonly prescribed antispasmotic after SCI, have been mixed (Short et al., 1992; McEvoy et al.,1995; Klefbeck et al., 1998; Burns et al., 2000; Bensmail et al., 2006).

Prior to 2014, the predominant type of SDB identified in SCI patients was OSA (Young et al., 1993; Leduc et al., 2007). However, Sankari and colleagues (2014a) performed in-lab polysomnography (PSG) on 26 chronic (>1 year) SCI subjects (15 cervical, 11 high thoracic) using quantitative measurement of flow and upper airway pressure. The prevalence of SDB (i.e. an apnea hypopnea index >5 events/hour) in cervical SCI subjects was 93% versus 55% in thoracic SCI subjects. A novel finding was that 60% of cervical and 27% of thoracic subjects had CSA and periodic breathing (PB) (Sankari et al., 2014). Figure 1 depicts a representative example of CSA and PB in a cervical SCI subject. Primary CSA occurs in <1% of the general population and is typically found in special populations such as premature neonates, patients with systolic heart failure, those ascending to high altitudes, and opiate users (Javaheri and Dempsey, 2013; Panossian et al., 2009; White et al., 2005). The CSA and PB identified in SCI subjects by Sankari et al. (2014) could not be explained by heart failure or narcotic use.

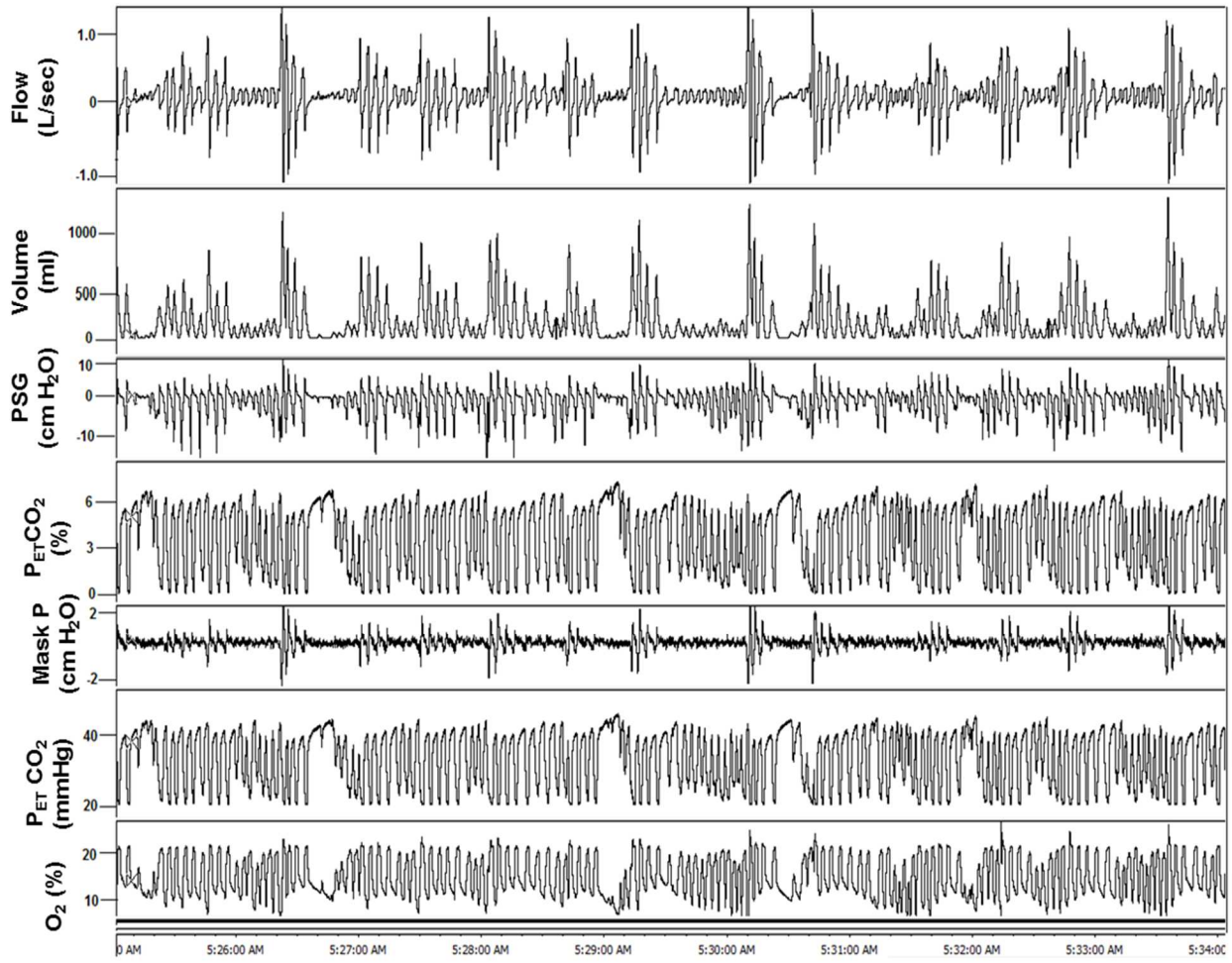

Figure 1. Periodic Breathing in Spinal Cord Injury

A representative polygraph of central sleep apnea and periodic breathing in a C5-7 cervical SCI subject (male, age 37 years, body mass index 28.5 kg/m^2) in Non-REM sleep. Repetitive episodes of hyperpnea and hypopnea/apnea are indicative of periodic breathing. Supraglottic airway pressure signal (PSG) indicates that respiratory effort was greater during hyperpneas, diminished during hypopneas and absent during apnea indicating central sleep apnea. $P_{ET}CO_2$: end tidal CO_2, Mask P: mask pressure.

Ventilation after Spinal Cord Injury

Loss of motor drive to respiratory muscles after cervical and high thoracic SCI results in impairment of respiratory function related to the level and completeness of injury. Injuries below the level of C5, or incomplete injuries typically leave the diaphragm sufficiently innervated to maintain ventilatory homeostasis during wake without the need for mechanical ventilation, although some hypoventilation may occur during wake and more frequently during sleep when higher brain center input to brainstem respiratory control centers is withdrawn (Castriotta and Murthy, 2009). Expiratory muscles (internal intercostals, pectoralis major and abdominal muscles) have the greatest magnitude of impairment compared with diaphragm and inspiratory accessory muscles in lower cervical and high thoracic injuries although varying levels of weakness in diaphragm and inspiratory accessory muscles are often present to some degree in lower cervical injury (Terson de Paleville et al., 2010). The external intercostals (inspiratory muscles) are of particular importance during sleep when their contribution to VT is ~20% in healthy humans (Tabachnik et al., 1981). Therefore, loss of external intercostal drive may contribute to sleep related hypoventilation after SCI (see Figure 2).

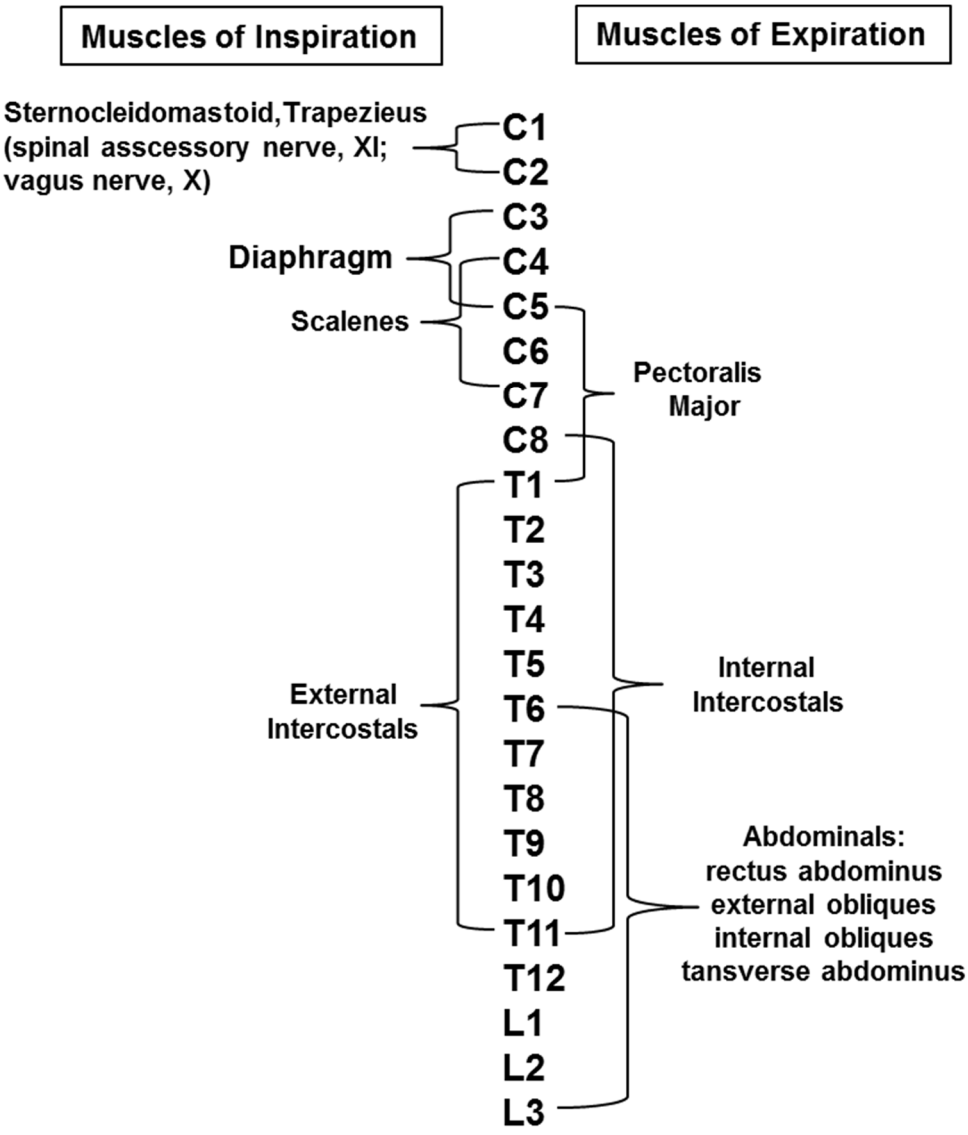

Figure 2. The Muscles of Respiration

The human primary and accessory muscles of respiration are depicted with their level of spinal/cranial innervation and phase of respiratory activity (adapted from Schilero et al., 2009).

Decreased total lung capacity (TLC), forced vital capacity (FVC), forced expiratory volume at 1 second (FEV1), peak expiratory flow (PEF), inspiratory capacity (IC) and expiratory reserve volume (ERV) are typically observed in patients with cSCI (Schilero et al., 2009). In the supine position, FVC and FEV1 are typically higher in SCI patients due to the effect of gravity on abdominal contents (pushing up on the diaphragm) resulting in a greater upward curve in the diaphragm resulting in greater diaphragmatic excursions during inspiration as a result of a more favorable position of the muscle fibers on the length-tension curve. However, residual volume (RV) is smaller in the supine position (Schilero et al., 2009) also due to elevation of the diaphragm as a result of gravity on the abdominal contents. Thus, sleeping in the supine position may be accompanied by a decreased work of breathing in cSCI, but a lower RV may have implications for gas exchange such as lower arterial oxygen saturation (SaO_2) and increased arterial carbon dioxide (Schilero et al., 2009).

Putative Mechanisms for Sleep Disordered Breathing in SCI

Cervical SCI patients have many ventilatory features that may predispose them to SDB, including but not limited to: respiratory muscle weakness, the loss of chest wall afferent input to brainstem respiratory control centers, hypoventilation, abnormal O_2 and CO_2 sensing, positional differences in blood pressure and ventilation, sympathetic nervous system dysregulation and related cardiovascular dysfunction, poor sleep quality, sleep fragmentation and the use of medications for pain and spasticity that may influence respiration during sleep (Teasell et al., 2000; Burns et al., 2001; Biering-Sorensen et al., 2009; Castriotta and Murthy, 2009; Wilson et al., 2010) .

Periodic Breathing

Periodic breathing (PB) during sleep is a type of central sleep disordered breathing in which ventilation waxes and wanes in a cyclical manner with periods of hypopnea and/or apnea with an accompanying rise in end-tidal CO_2 ($P_{ET}CO_2$) resulting in a period of hyperpnea with resultant hypocapnia which then begins the cycle again. These oscillations in respiration are typically accompanied by periods of oxygen desaturation during hypopnea/apnea followed by re-saturation during hyperpnic periods, as well as periods of hyper- and hypocapnia (Eckert et al., 2007). Periods of oxygen desaturation and re-saturation are associated with the production of reactive oxygen species and intracellular inflammatory cascades that are implicated in the morbidity and mortality associated with SDB (Gopalakrishnan and Tak, 2001; Mohsen and Urbano, 2011; Chou et al., 2012; Johansson et al., 2012).

Specific conditions are known to precipitate PB and it can be experimentally induced in the lab using hypoxic conditions. Animal models of PB have shown that the mechanism in neonates (i.e. the lamb) is heightened sensitivity to hypoxia, which destabilizes breathing and increases loop gain or the peripheral chemoreceptor controller loop (Wilkinson et al., 1997). This is also the case in humans during sleep at high altitudes (Nussbaumer-Ochsner et al., 2012). In the premature infant PB is exacerbated by hypoxemia and relieved by administration of O_2, CO_2, or drugs that stimulate respiration (Darnall, 2010).

Periodic breathing can be induced by hypoxia, hypoventilation or respiratory depressants and mitigated by respiratory stimulants (i.e. caffeine) and breathing supplemental O_2 or CO_2. However, CO_2 administration as long-term treatment for CSDB in the home is difficult to control and requires tight servo-control with breath to breath feedback of $P_{ET}CO_2$ (dynamic CO_2 administration) (Badr et al., 1994; Solin and Naughton, 2000; Mebrate et al., 2009) and is currently not being used clinically. In patients with a narrow CO_2 reserve (the difference between eupneic $P_{ET}CO_2$ and the apneic threshold, which is the amount $P_{ET}CO_2$ must be reduced to induce a central apnea) as we have found to be the case in many of our SCI subjects (Sankari et al., 2014b), small perturbations in respiration lead to oscillations in PCO_2 that result in CO_2 levels dipping below the apneic threshold causing central apnea to occur (Yumino and Bradley, 2008).

According to Dempsey and colleagues (2009), the central apnea that commonly follows hyperpnea during periodic breathing appears to depend critically on hypocapnea being sensed by the peripheral chemoreceptors. However, there is close interdependence between central and peripheral chemoreceptors and it is difficult to ascertain the contribution of peripheral vs. central chemosensing when looking at the control of ventilation in the intact, otherwise healthy human (Xie et al., 2009, St Croix et al., 1996).

Periodic breathing also occurs in patients with severe diastolic heart failure (HF). In Cheyne-Stokes respiration (CSR), a form of periodic breathing found in HF in cycles (time from peak to peak of hyperpneas) last approximately 60 seconds. This is in contrast to CSR or PB that is idiopathic or a result of ascent to altitude in which cycles

are approximately 35 seconds in duration. The longer cycle duration in HF is likely due to prolonged circulation time in these individuals as a result of poor cardiac function (Sin and Man, 2003; Yumino and Bradley, 2008; Binggeli et al., 2010). Heart failure patients with CSR are typically hypocapnic during wake and sleep with $PaCO_2$ levels close to their apneic threshold. Approaches to PB in the HF point to a number of factors that can perpetuate unstable breathing cycles and so investigating the underlying mechanism and treatments in HF patients may give us critical insight into the mechanisms that contribute to periodic breathing after high SCI injury. The following are taken from literature that has investigated PB in the HF population:

1) Chronic hypocapnia: Patients with HF and CSR-CSA have low $PaCO_2$ during wake and sleep. The chronic hyperventilation is thought to be from pulmonary vagal irritant stimulation resulting from pulmonary congestion. In healthy individuals at the onset of sleep the apneic threshold increases but in HF $PaCO_2$ does not, so the CO_2 reserve is smaller than in individuals without HF. The CO_2 reserve is a more important factor in breathing instability than the actual baseline PCO_2 (Yumino and Bradley, 2007).

2) Increased central and peripheral chemoreceptor sensitivity as a result of repeated exposure to hypoxia. This results in ventilatory overshoot in response to changes in ventilation during sleep (Javheri, 1999; Solin et al., 2000).

3) Spontaneous arousals during sleep cause stimulation of ventilation from higher brain inputs. The resulting hyperpnea lowers $PaCO_2$, leading to central apnea or hypopnea that perpetuates the periodic breathing cycle (Naughton et al., 1993).

4) Abnormalities in cerebrovascular reactivity to CO_2 lead to lower brain $PaCO_2$. Normal reflex changes in cerebrovascular blood flow in response to changing [H+] serve to counter-regulate ventilation to stabilize breathing in the face of perturbations in $PaCO_2$. The response to a reduction in CO_2 or increased H+ should be to vasoconstriction that results in shielding of central chemoreceptors from transient drops in $PaCO_2$. This phenomenon is called dampening. If cerebrovascular reactivity is reduced, the central chemoreceptors sense small changes in $PaCO_2$ and ventilatory overshoot may occur that perpetuates periodic breathing (Yumino and Bradley, 2008; Xie et al., 2009).

5) Decreased FRC: a large FRC acts as an O_2/CO_2 reservoir that dampens oscillations in $PaO_2/PaCO_2$ during apneas and stabilizes breathing. A low FRC leads to 'under-dampening' (Staniforth et al., 1997). In this case, the peripheral chemoreceptors would be exposed to frequent oscillations in O_2 and CO_2, which may lead to increased chemoreceptor sensitivity. This is unlikely to be the case in SCI subjects who typically have a FRC within normal limits (Stepp et al., 2008).

6) Upper airway instability: The upper airway may collapse during central apneas. If airway resistance increases as ventilation decreases during the hypopnea phase of CSR or PB, hypoventilation may occur. Conversely, if there is decreased airway resistance during the hyperpnea phase, overshoot or hyperventilation is more likely to occur. So, upper airway resistance plays a key role in determining ventilation, as is well known in OSA, but potentially in central sleep disordered breathing as well. In addition, upper airway collapse itself may lead to central apnea as a reflex reaction (Sullivan et al., 1978; Badr et al., 1995). Thus, if upper airway resistance is a main contributor to periodic breathing or central apnea, treatment with CPAP will stabilize the upper airway and stabilize breathing.

7) Hypoxia: As previously mentioned, hypoxia is well known to induce CSR and PB at high altitudes by causing hyperventilation as one attempts to improve PaO2, thus lowering PCO_2 below apneic threshold. This type of CSA can be abolished by administration of O_2, which alleviates the hypoxia, or by CO_2 administration that brings the $PaCO_2$ well above the apneic threshold resulting in a greater CO_2 reserve. A transient drop in PaO_2 occurs after prolonged apneas or hypopneas that can contribute to the magnitude of ventilatory overshoot that follows the apnea/hypopnea. Therefore, even mild hypoxic episodes may perpetuate CSR-CSA (Khoo et al., 1982; Yumino and Bradley, 2008).

Peripheral Chemoreception

The primary peripheral chemoreceptors critical to the control of ventilation in adult humans are the carotid body chemoreceptors. Located bilaterally at the bifurcation of the carotid arteries, the carotid body chemoreceptors function to respond within seconds to changes in PaO_2, $PaCO_2$ and $[H^+]$ to regulate respiration according to metabolic requirements (Forster et al., 1999). Tonic afferent activity of the carotid body is required to maintain adequate ventilation under a wide range of physiologic conditions including wakefulness, NREM sleep and exercise. The activity of the carotid body chemoreceptors plays a critical role in regulating ventilation during NREM sleep when inputs from higher brain centers are quiescent, as they are the "first responders" to correct abnormalities in PaO_2, $PaCO_2$ and [H+] on a breath-by-breath basis. Acute denervation of the carotid bodies results in hypoventilation in awake mammals in experimental preparations and clinically in humans with carotid body pathology that requires their excision (Gautier and Bonora, 1979; Honda et al., 1979; Lowry et al., 1999; Kumar and Prabhakar, 2012).

Oxygen sensing glomus (type I) cells of the carotid body are extensively innervated by fibers of the carotid sinus nerve, a branch of the glossopharyngeal nerve (CN IX) whose cell bodies are located in the petrosal ganglia. The petrosal ganglia are located in the commissural or medial subnuclei of the nucleus tractus solitaries (NTS), which is a major integration center in the brainstem for regulation of cardiac, autonomic and respiratory output (Kumar and Prabhakar, 2012). The primary neurotransmitters released peripherally at the synapse between glomus cells and afferent carotid sinus nerve endings are thought to be acetylcholine and ATP, which are released when the glomus cell is depolarized in response to hypoxia and/or increased [H+] or PCO_2 (Lahiri

and Forster, 2003). James and Nantwi (2006) demonstrated in a rat model of cervical SCI that in carotid body intact animals, administration of a peripherally acting adenosine A_2 receptor agonist (CGS-21680) followed by administration of a centrally acting adenosine A_1 receptor antagonist (DPCPX, previously shown to elicit recovery of phrenic nerve activity after C2 hemi-section), resulted in greater phrenic nerve recovery compared to the recovery initiated in response to the administration of DPCPX alone. However, in carotid body denervated animals the same recovery of phrenic nerve activity in the affected nerve was not elicited. Thus James and Nantwi (2006) proposed that carotid body adenosine A_2 receptors are involved in the excitation of central respiratory centers and are critical in recovery of respiratory function after cervical SCI (James and Nantwi, 2006).

After entering the brainstem with the glossopharyngeal nerve, central afferent axons of carotid sinus neurons first synapse on chemosensitive respiratory network neurons in the caudal nucleus of the NTS to modulate respiration (Teppema and Dahan, 2010). A second synaptic site is an area of the parafacial respiratory group (pFRG), the retrotrapzoid nucleus (RTN). The RTN, located on the ventral medullary surface, is a putative chemosensitive and integrating area containing glutamatergic interneurons expressing the transcription factor Phox2b, a marker of chemosensitive neurons critical in the control respiration, particularly during sleep (Guyenet, 2008; Forester and Smith, 2010). RTN neurons project to the ventral respiratory group (VRG), that act directly on phrenic neurons. Studies over the past decade indicate that the function of the carotid body peripheral chemoreceptors is to *rapidly* modulate the gain of central chemoreceptors, although historically there has been much debate over how the

peripheral and central chemoreceptors interact, whether their interactions are simply additive or hyper-additive (Clement et al., 1995; Forster et al., 2007; Nuding et al., 2009; Xie et al., 2009; Dempsey et al., 2012).

Under conditions of chronic hypoxia (sustained hypoxia [SH]), which is experienced by non-natives residing at high altitudes, the carotid body undergoes acclimatization during which respiratory drive is increased to stimulate ventilation along with a concurrent increase in their sensitivity to O_2 (increased peripheral chemoreceptor gain). Under this chronic condition carotid body will eventually become hypertrophied with increased vascularization and an increase in the number of glomus cells (Lahiri, 2003). Under conditions chronic intermittent hypoxia (CIH), as occurs with SDB, such anatomical changes do not take place, rather a reversible functional plasticity of the carotid bodies occurs, known as sensory long-term facilitation (LTF) (MacFarlane and Mitchell, 2003; Peng et al., 2003; Peng and Prabhakar, 2003).

Augmented sensitivity of the carotid body to O_2 and CO_2 has been implicated as a mechanism by which breathing instability during sleep (CSA or PB) can be elicited and/or sustained (Dunai et. al., 1999; Dempsey, 2004; Eckert et al., 2007). Modeling studies of the respiratory control loop have identified peripheral chemoreflex responses as a key factor in PB (Khoo et al., 1982 and 1991). Individuals with high peripheral chemosensitivity will “over correct” for relatively small perturbations in CO_2 or O_2 with hyperpnea that results in CO_2 falling very near or below the apneic threshold causing apnea/hypopnea, which continues the cycle. The strong relationship between PB and heightened peripheral chemosensitivity suggests a putative mechanism for SDB in SCI.

Conclusions

In conclusion, it is clear that SDB poses a significant threat to vital organ systems that lead to increased morbidity and mortality in the general population. The observation that SDB is more prevalent after SCI compared to spinal cord intact populations makes the study of this disorder of critically important in this population. The discovery of a high percentage of CSDB in high SCI in studies conducted thus far in our lab (approximately 75% in cervical SCI subjects studied) suggests specific mechanisms to focus on, namely the role of sleep-onset hypoventilation and peripheral chemoreceptor sensitivity. Valuable information may be uncovered by targeting and exploring the specific features of chemoreception after high thoracic and cervical SCI that may lead to a greater understanding of the mechanisms involved in the development of SDB in this population. Understanding the key mechanisms will aid in the development of future therapeutic targets. In addition, involvement of hypoventilation in the development of SDB after SCI is a critical mechanism to explore as chronic sleep-related hypoventilation may underlie changes in chemoreflex sensitivity after SCI.

CHAPTER 1

Sleep Onset Hypoventilation in Chronic Spinal Cord Injury

Introduction

Patients with spinal cord injury suffer from poor nocturnal sleep, sleep fragmentation and high prevalence of SDB (Bonekat et al., 1990; Klefbeck et al., 1998; Burns et al., 2000; Berlowitz et al., 2005; Sankari et al., 2014a). However, the underlying mechanisms are not understood. What is clear, is that SDB in chronic SCI poses a significant quality of life issue for this population due to excessive daytime sleepiness, chronic fatigue and cognitive impairment (Ayas et al., 2014; Sankari et al., 2014a; Vaessen et al., 2014), as well as increased risk of cardiovascular morbidity and mortality that accompanies under-diagnosed and untreated SDB (Caples et al., 2007; Marshall et al., 2014).

The prevalence of SDB after SCI has been reported as being between 27-77% (Bonekat et al., 1990; McEvoy et al., 1995; Klefbeck et al., 1998; Burns et al., 2000; Stockhammer et al., 2002; Berlowitz et al., 2005; Biering-Sorenson et al., 2009; Tran et al., 2010; Sankari et al., 2014a and 2014b). In contrast, the prevalence of sleep apnea syndrome in the non-injured population, which is estimated to occur in approximately 2-4% (depending upon age and gender) according to data from the Wisconsin Sleep Cohort Study (Peppard et al., 2013). Work from Sankari and colleagues (2014a) has revealed that three out of four chronic SCI patients have symptomatic SDB, with central SDB noted in cervical SCI and obstructive SDB in thoracic SCI (Sankari et al., 2014a

and 2014b). Furthermore, Sankari et al. (2014b) found that a narrowed CO_2 reserve in patients with cervical SCI was associated with increased steady-state plant gain, which reflects the effectiveness of the respiratory system to eliminate CO_2 for a given alveolar ventilation level. Accordingly, increased steady-state plant gain may promote the development of central apnea upon transition to non-REM sleep when breathing is mainly dependent on chemical stimuli. However, the etiology of increased plant gain and breathing instability during sleep in this SCI population, who may have normal gas exchange during wakefulness, versus able-bodied individuals is not known.

Cervical and high thoracic SCI results in disruption of descending bulbospinal pathways to the muscles of respiration, such as the diaphragm (C3-C5), intercostals (T1-T11) and abdominals (T6-L3) (Zimmer et al., 2008; Schilero et al., 2009). Such disruption results in respiratory muscle weakness or paralysis depending upon level and completeness of injury. Hypoventilation resulting from restrictive ventilatory mechanics, which worsens during sleep, has been proposed as a mechanism for the development of sleep disordered breathing in spinal cord injury (Castriotta and Murthy, 2009).

I hypothesized that Individuals with SCI would develop a greater degree of sleep-related hypoventilation compared to able-bodied controls. To this end, I measured ventilation and upper airway resistance during transitions from alpha (8-12 Hz, wake) to theta (4-7 Hz, stage N1 sleep).

Materials and Methods

Subjects

Protocols were approved by the Human Investigation Committee of the John D. Dingell Veterans Affairs Medical Center and Wayne State University (Detroit, MI) and written informed consent was obtained.

We studied adults (≥18 years old) with chronic SCI and able-bodied participants if they met the inclusion and exclusion criteria. All subjects were instructed not to have alcohol, caffeine products or sedatives on the day of the study.

Inclusion Criteria: Participants with chronic SCI (>6 months post-injury), spanning the spectrum from cervical (cSCI, C4-C7) to thoracic levels (tSCI, T1-T6) (complete and incomplete injuries). Able-bodied subjects (AB) were recruited with similar demographics to the SCI group for age, body mass index (BMI) and gender.

Exclusion Criteria: Participants were excluded from the study for any of the following: (1) pregnant or lactating females; (2) currently ventilator dependent or with tracheostomy tube in place; (3) history of cardiac disease including heart failure, peripheral vascular disease, or stroke; (4) history of head trauma resulting in neurological symptoms or loss of consciousness; (5) advanced lung, liver, or chronic kidney disease; (6) extreme obesity, defined for this protocol as BMI >38 kg/m^2; or (7) other illness that would interfere with completion of the study.

The first visit to the lab consisted of documenting medical history, physical exam that included vital signs, maximal inspiratory and expiratory pressures (MIP and MEP) for SCI individuals, and spirometry to rule out pulmonary disease. The second visit

consisted of an overnight polysomnography (PSG). If the subject had a concern about sleep difficulties, Zolpidem was administered orally 30 minutes prior to beginning of recordings to minimize sleep difficulties. Zolpidem dose was selected based on the subject's age (≥60 years old: 5 mg, <60 years: 10 mg IR or 12.5 mg CR). The number of subjects requiring Zolpidem was similar in both groups (Table 1).

Polysomnography (PSG)

Subjects arrived at the lab between 8:00-9:00 pm to be instrumented and prepared for study. PSG was performed in the supine position using the Comet PSG System (AS40 Model) or the Heritage II PSG System (Grass Technologies, Warwick, RI). Measurements included electrocardiogram (ECG), electroencephalogram (EEG), electrooculograms (EOG) and chin electromyogram (EMG) using the International 10-20 system of electrode placement (EEG: C3-A2 and C4-A2; EOG: O-A2). Subjects wore a nasal mask connected to a pneumotachometer (Hans Rudolph, Model 3700A, Shawnee, KS) that measured airflow. Tidal Volume (V_T) was determined via integration of the pneumotachometer flow signal. End-tidal carbon dioxide ($P_{ET}CO_2$) and end-tidal oxygen ($P_{ET}O_2$) levels were measured with CO_2 and O_2 gas analyzers (Vacumed Model 17515 and 17518 respectively, Ventura, CA). Supraglottic airway pressure was measured with a pressure tipped catheter (Millar Instruments, Houston, TX) placed through one nostril and extending down into the hypopharynx at least 2 cm caudal to the visible base of the tongue and superior to the epiglottis. Pulse oximetry was measured via ear probe (Biox 3740, Datex-Ohmeda Inc, Madison, WI). Subjects were recorded while breathing spontaneously on room air. Ventilation data from the

pneumotachometer, supraglottic catheter, pulse oximeter and gas analyzers were digitized and analyzed using a PowerLab Data Acquisition System (Model 16SP, ADInstruments Inc., Colorado Springs, CO).

Data Analysis

PSGs were scored using American Academy of Sleep Medicine (AASM) 2012 recommended criteria (Berry et al., 2012). Supraglottic pressure and respiratory inductance plethesmography (RIP) bands (Respitrace, model 200, Nims inc., Miami Beach, FL) were used to differentiate between obstructive and central apneas. In order to analyze state-specific changes in ventilation, transitions between wake and sleep were identified first. Sleep stage scorers were blinded to ventilation. Blinding of ventilation signals was accomplished by covering the portion of the screen that contained ventilation signals. Two independent sleep scorers identified and verified agreement of wake to sleep transitions. Occipital EEG signals were used to determine the predominance of alpha (resting wakefulness with eyes closed, 8-12 Hz) vs. theta (stage N1 sleep, 4-7 Hz) waves without K complexes or sleep spindles.

After identification of 3 separate alpha to theta transitions, ventilation data were obtained and analyzed from time-matched segments (Figure 3). In the case that 3 transition segments could not be identified for the subject, two transitions were used. In the SCI group, we analyzed 47 transitions (137 and 134 breaths in alpha and theta respectively). In the AB group, we analyzed 40 transitions (112 and 108 breaths in

alpha and theta respectively). Ventilation data obtained consisted of breath-by-breath minute ventilation (V_E), tidal volume (V_T), respiratory frequency (f), inspiratory time (Ti), expiratory time (Te), total cycle time (Ttot), oxygen saturation (SaO_2), $P_{ET}CO_2$ and $P_{ET}O_2$. Inspiratory R_{UA} was calculated at the linear portion of the pressure-flow relationship using the pressure-flow loops (supraglottic pressure and airflow).

Alpha to theta transitions were only analyzed if there were at least two thirty-second epochs of wake preceding the transition so that brief arousals from sleep with corresponding hyperpneas were not used. We did not analyze any transitions from theta to alpha (arousals from sleep). For each subject, all ventilatory parameters for alpha breaths were grouped and averaged as well as theta breaths. Comparisons were then made between groups and conditions (alpha vs. theta).

To verify the accuracy of visual scoring of segments selected for analysis, we performed spectral analysis using Fast Fourier Transform (FFT) method (MATLAB, Math Works inc., Natick, MA) on EEG segments selected for alpha and theta analysis in 4 subjects. We found > 90% agreement between visual scoring and spectral analysis and thus proceeded with visual classification (Trinder et al., 1992; Yang et al., 2012).

In breaths where significant hypopnea or apnea occurred, $P_{ET}CO_2$ signals were invalid in some breaths due to insufficient flow to capture end-tidal plateau. In such cases, the $P_{ET}CO_2$ values were eliminated (not factored into average $P_{ET}CO_2$ for that segment). Thus, only breaths with reliable signals were used for $P_{ET}CO_2$ analysis, but the V_E, V_T and f for hypopneas were valid and were used in analysis. When apnea occurred, the total time of the apnea was considered as part of the Te of the breath before apnea,

thus V_E for that breath reflected the overall decrease in ventilation as a result of the apnea.

Three secondary analyses were performed: 1) Sleep onset changes in ventilation were compared between cervical and thoracic SCI subjects to determine the effect on injury level on ventilation. 2) To determine the potential confounding effect of SDB on sleep onset ventilatory changes, we compared sleep onset changes in ventilation in 8 cervical SCI individuals and 8 able-bodied controls with SDB. 3) To determine the potential contribution of intercostal muscle atonia on sleep onset changes in ventilation, we analyzed the transition from non-REM to REM sleep in a subset of subjects in each of the following groups who had REM sleep: cervical SCI (n=3), thoracic SCI (n=2), and able-bodied subjects (n=3).

Statistical Analysis

Two-way repeated measures ANOVA (Sigma Plot 12.1) was performed to determine within group (e.g. alpha to theta changes in cSCI) and between group differences in ventilatory parameters and upper airway resistance between the two conditions: alpha and theta EEG frequencies. When appropriate, post-hoc pair-wise multiple comparisons were made using the Student-Newman-Keuls method. When data were not normally distributed, appropriate non-parametric analysis was employed. T-tests were used to compare all demographic data between AB and SCI. All data are reported as mean ± SD and significance was set at $p<0.05$.

Results

Sleep onset ventilatory changes

We studied 18 subjects with SCI and 17 AB with similar demographics (Table 1). In figure 3 we show an illustration of tidal volume (V_T) decrease during the transition from alpha to theta in a representative cSCI subject. The effect of wake to sleep transition on ventilation in a representative AB subject is shown in figure 4. Group changes in V_T and V_E during sleep-onset transitions are detailed in figure 5 (panels A and B). Sleep onset was associated with significant decrease in V_T and V_E in the SCI but not the AB subjects. However, there was no significant change in R_{UA} with sleep onset in either group (Figure 6).

The effect of sleep onset on ventilatory parameters in SCI and AB groups is summarized in Table 2. Sleep onset in the SCI group was associated with decreased Ti while lengthening Te, reduced duty cycle (Ti/Ttot) and unchanged respiratory frequency and Ttot. In contrast, the AB group demonstrated increased respiratory frequency at sleep onset with no significant changes in Ti, Te, or Ttot.

Table 1. Subject Characteristics

	SCI	Able-Bodied	p value
N	18	17	NS
Age (years)	42.4±17.1	42.9±13.9	NS
BMI (kg/m^2)	26.3±4.8	27.8±5.4	NS
Gender (M/F)	11/7	8/9	NS
NC (cm)	38.5±3.0	37.1±3.7	NS
AHI (events/hr)	22.9±22.4	7.3±8.8	p<0.05
AHI (>5 events/hr)	12	8	--
Zolpidem (Y/N)	9/9	8/7	NS
Injury Level (cervical/thoracic)	10/8	--	--
MIP (% predicted)	87.2±29.4	--	--
MEP (% predicted)	42.3±15.8	--	--
FVC (% predicted)	71.5±17.0	86.5±26.5	NS
FEV 1(% predicted)	76.4±16.7	87.6±18.4	NS
FEV1/FVC	80.5±7.0	77.6±9.6	NS

All data mean ± SD. BMI: body mass index, NC: neck circumference, AHI: apnea hypopnea index, MIP: maximal inspiratory pressure, MEP: maximal expiratory pressure, FVC: forced vital capacity, FEV1: forced expiratory volume at 1 second, FEV1/FVC: the ratio of FEV1 to FVC, NS: not significant.

Table 2. Effect of Sleep Onset on Respiratory Cycle Timing and Chemical Stimuli

	SCI		Able-Bodied	
	Alpha	Theta	Alpha	Theta
Frequency (breaths/min)	15.3±3.1	14.9±3.9	15.3±2.5	16.2±2.9‡
Ti (sec)	1.8±0.4	1.7±0.3‡	1.8±0.3	1.7±0.4
Te (sec)	2.4±0.7	3.0±1.6‡	2.3±0.5	2.2±0.5*
Ti/Ttot	0.44±0.05	0.40±0.08‡	0.43±0.05	0.44±0.07
SaO_2 (%)	96.1±1.4	95.8±1.7	96.2±1.0	96.2±0.9
$P_{ET}CO_2$ (mmHg)	38.9±2.7	40.6±3.4‡	39.5±3.2	39.9±3.2
$P_{ET}O_2$ (mmHg)	94.1±7.1	91.2±8.3‡	99.4±5.4	98.9±6.1

All data mean ± S.D. SCI n=18, AB n=17. * between-group (SCI vs. Able-Bodied) difference $p<0.05$, ‡ within-group difference (alpha vs. theta) $p<0.05$. Ti: inspiratory time, Te: expiratory time, Ti/Ttot: ratio of ti to total cycle time.

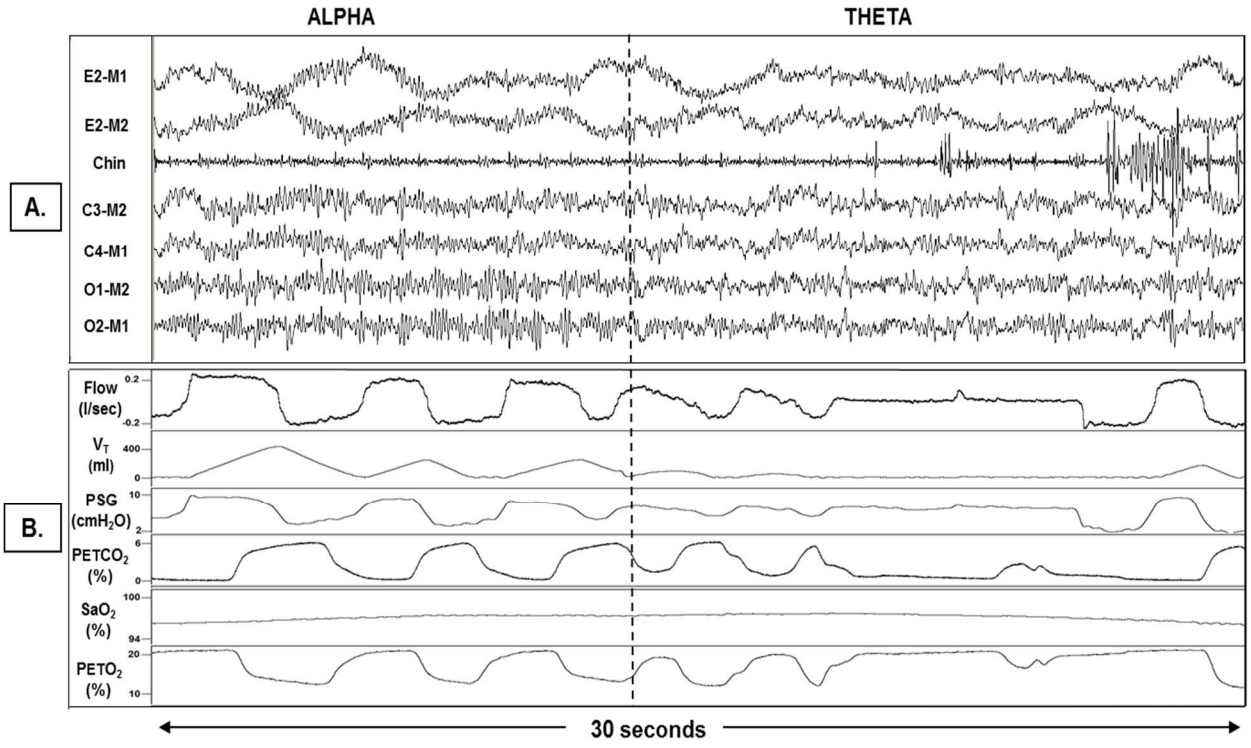

Figure 3: Transition from Alpha to Theta in Cervical Spinal Cord Injury

Panel A: A representative polygraph with EEG and EMG recordings in a 36 year old chronic cervical (C6, incomplete injury) SCI individual (male, BMI 28.2 kg/m^2) during the transition from wake (alpha) to N1 sleep (theta). E: eye, M: mastoid ground, C: central, O: occipital. Panel B: A 30 seconds polygraph with ventilation recording, time matched to the EEG data in panel A. Note the reduction in flow and tidal volume with sleep-onset evident in panel B. V_T: tidal volume; PSG: supraglottic pressure; $P_{ET}CO_2$: end-tidal CO_2; $P_{ET}O_2$: end-tidal O_2.

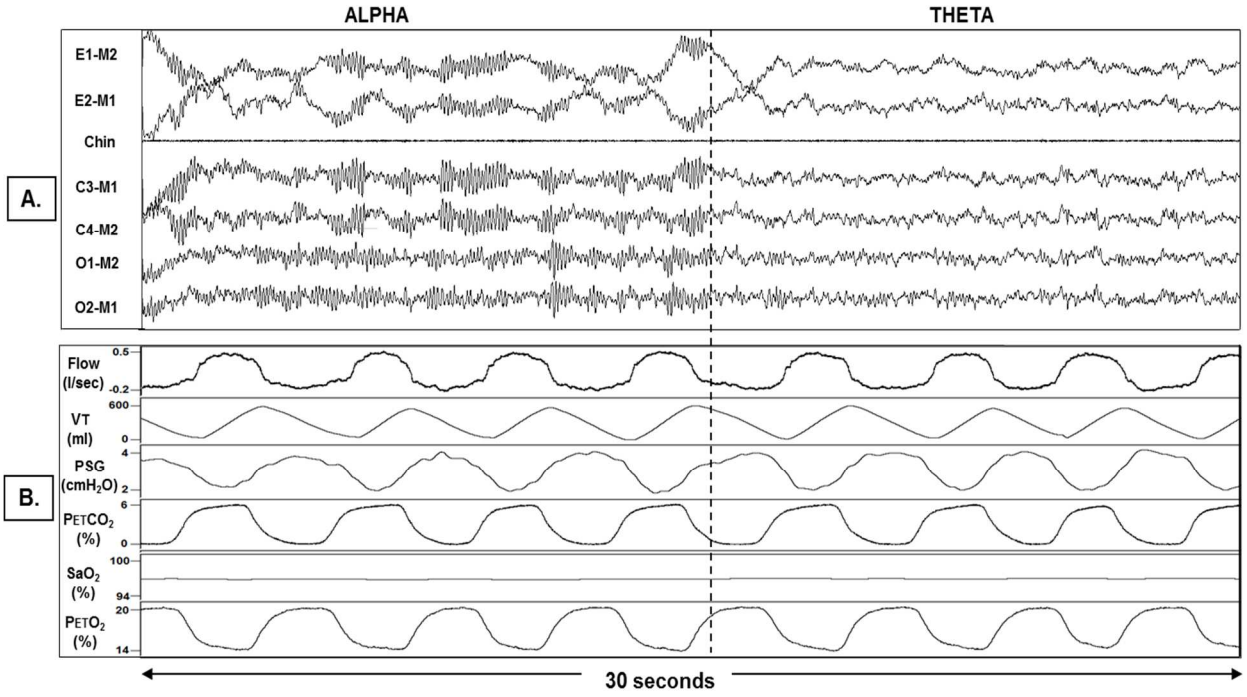

Figure 4: Transition from Alpha to Theta in an Able-Bodied Subject

Panel A: A representative polygraph with EEG and EMG recordings in a 55 year old able-bodied subject (male, BMI 27 kg/m^2) during the transition from wake (alpha) to N1 sleep (theta). E: eye, M: mastoid ground, C: central, O: occipital. Panel B: A 30 second polygraph of ventilation, time matched to the EEG data in panel A. V_T: tidal volume; PSG: supraglottic pressure; $P_{ET}CO_2$: end-tidal CO_2; $P_{ET}O_2$: end-tidal O_2.

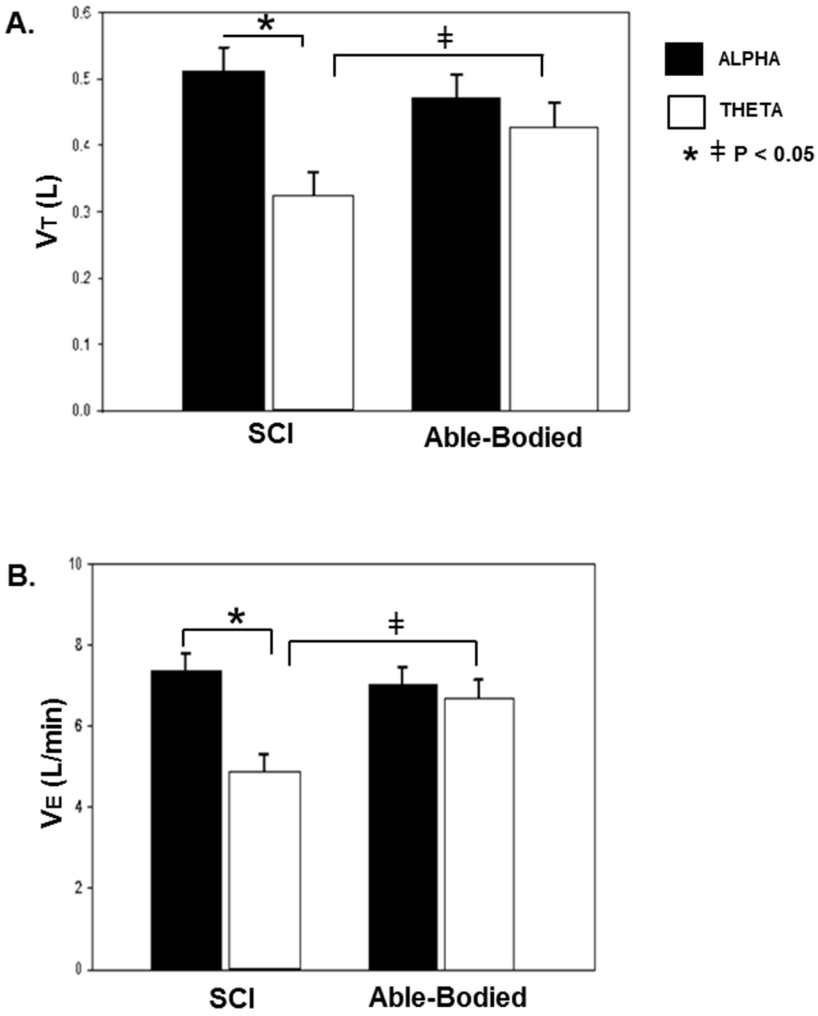

Figure 5: Effect of Sleep Onset on Ventilation

Panel A illustrates a greater decrease in tidal volume (V_T) with sleep onset in SCI compared to able-bodied subjects. Panel B illustrates the greater decrease minute ventilation (V_E) at sleep onset in SCI group compared with able-bodied group.

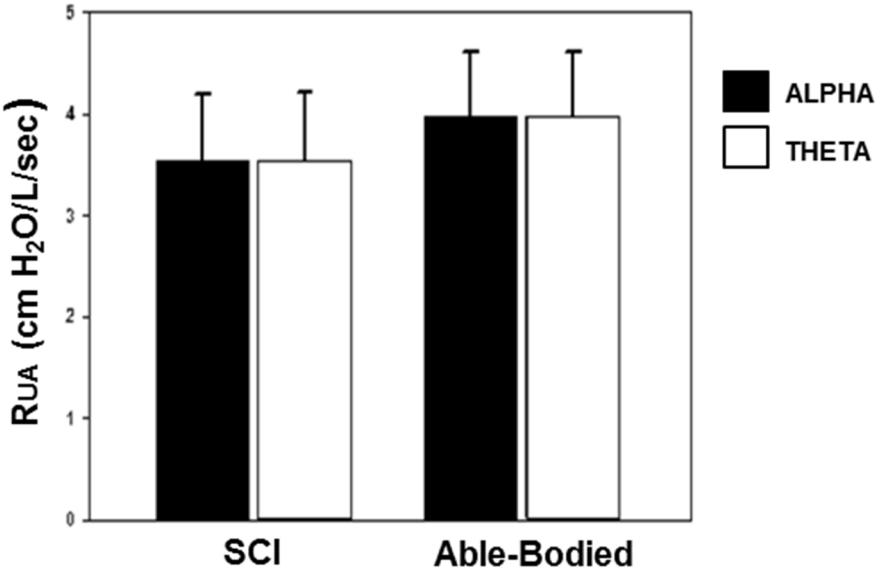

Figure 6: Effect of Sleep Onset on Upper Airway Resistance

There was no significant change in upper airway resistance at sleep onset in SCI or able-bodied subjects. Between-group (SCI vs. Able-bodied) difference p = 0.624; within group (SCI-alpha vs. theta, Able-bodied-alpha vs. theta) difference p= 0.954.

Comparison of cervical vs. thoracic SCI:

To determine if spinal injury level influenced sleep onset ventilation, we compared the ventilatory parameters detailed above between cSCI (n=10) and tSCI (n=8) subjects. Alpha to theta transitions were associated with significantly decreased V_T in cSCI subjects (alpha: 576.0±256.3 mL, theta: 293.1±105.8 mL, $p<0.05$) but not in tSCI (alpha: 445.2±100.9 mL, theta: 364.6±90.7 mL, p=ns). Similarly, V_E was significantly reduced in cSCI (alpha: 7.8±2.6 L, theta: 4.3±1.6 L, $P<0.05$) compared to tSCI (alpha: 6.9±1.5 L, theta: 5.6±1.4 L, $p=0.08$). Thus, injury level has a significant impact on sleep onset hypoventilation. Further results of cSCI vs. tSCI analyses are detailed in Table 3.

Effect of sleep disordered breathing on sleep onset hypoventilation in cervical SCI and able-bodied subjects

To determine the potential contribution of SDB to sleep-onset hypoventilation in the cSCI group; we compared 8 cSCI individuals and 8 AB subjects with SDB as defined by an AHI ≥ 5 events/hour. Sleep onset was associated with decreased V_T and V_E in participants with cSCI and SDB, but not in AB subjects with SDB (Table 4). Changes in other ventilatory parameters are detailed in Table 4. Thus, cSCI with SDB have a significantly greater reduction in ventilation with sleep onset compared to AB subjects with SDB.

Table 3. Cervical vs. Thoracic SCI: Sleep Onset Ventilation

	cSCI		tSCI	
	Alpha	Theta	Alpha	Theta
Frequency (breaths/min)	14.7±3.3	14.2±4.5	15.9±3.0	15.7±2.9
Ti (sec)	1.9±0.4	1.7±0.4‡	1.7±0.3	1.6±0.3
Te (sec)	2.5±0.7	3.51±2.0	2.3±0.7	2.4±0.6
Ti/Ttot	0.44±0.04	0.38±0.09‡	0.43±0.06	0.42±0.06
SaO_2 (%)	95.8±1.3	95.1±1.6	96.6±1.4	96.7±1.4*
$P_{ET}CO_2$ (mmHg)	38.9±3.2	41.3±4.1‡	38.9±1.7	39.5±1.8
$P_{ET}O_2$ (mmHg)	94.3±4.8	90.6±6.8‡	93.8±10.1	92.0±10.6
R_{UA} ($cmH_2O/L/sec$)	3.61±1.7	3.5±1.9	3.5±1.4	3.6±1.7

All data mean ± S.D. cSCI: cervical SCI; tSCI: thoracic SCI. cSCI n=10, tSCI n=8. * between-group (cervical vs. thoracic) difference $P<0.05$, ‡ within-group difference (alpha vs. theta) $P<0.05$. Ti: inspiratory time, Te: expiratory time, Ti/Ttot: ratio of Ti to total cycle time, R_{UA}: upper airway resistance.

Table 4. Cervical SCI vs. Able-Bodied Subjects: Effect of Sleep Disordered Breathing on Sleep Onset Ventilation

	cSCI		Able-Bodied	
	Alpha	Theta	Alpha	Theta
Frequency (breaths/min)	14.4±3.5	13.9±5.1	15.7±2.3	17.3±3.2
V_E (L/min)	7.4±2.6	4.4±1.8‡	6.6±2.4	6.1±2.1
VT (L)	0.58±.26	0.30±0.12‡	0.43±0.18	0.37±0.16
Ti (sec)	2.0±0.5	1.8±0.4‡	1.7±0.1	1.5±0.2
Te (sec)	2.6±0.7	3.8±2.1‡	2.2±0.6	2.1±0.7*
Ti/Ttot	0.44±0.04	0.37±0.09‡	0.43±0.06	0.44±0.07
SaO_2 (%)	95.8±1.4	95.1±1.8	96.0±1.3	96.1±1.1
$P_{ET}CO_2$ (mmHg)	39.3±3.1	41.6±3.4‡	39.6±2.6	39.8±2.2
$P_{ET}O_2$ (mmHg)	94.6±5.0	91.4±6.5‡	101.1±4.2	100.4±5.9*
R_{UA} (cmH_2O/L/sec)	3.4±1.3	3.2±1.7	4.3±2.9	4.8±5.2

All data mean ± SD. cSCI: cervical SCI; cSCI n= 8, able-bodied (AB) n=8. * between-group (cSCI vs. AB) difference $p<0.05$, ‡ within-group difference (alpha vs. theta) $p<0.05$. V_E: minute ventilation, V_T: tidal volume, Ti: inspiratory time, Te: expiratory time, Ti/Ttot: ratio of Ti to total cycle time, R_{UA}: upper airway resistance.

Comparison of ventilation with REM onset in cervical and thoracic SCI and able-bodied subjects

To ascertain the relative contribution of the loss of intercostal muscle activity on sleep onset hypoventilation, we compared the ventilatory changes during non-REM to REM transitions. We reasoned that the magnitude of non-REM to REM ventilatory changes would be attenuated in individuals with cSCI because of the loss of intercostal muscle activity. There was a paucity of REM sleep in all subjects, owing to the high level of instrumentation in our protocols. We identified non-REM to REM transitions in a sub-set of 3 cSCI individuals, 2 tSCI subjects and 3 AB subjects. A single transition from non-REM to REM sleep was analyzed for each subject (3 transitions in cSCI and AB, 2 transitions in tSCI group). The 10 non-REM breaths immediately prior to REM onset were analyzed for tidal volume in each group, as well as for the first 10 breaths of REM sleep. Any breaths associated with an arousal were not included in the analysis. The onset of REM was determined using the AASM 2007 guidelines (Iber et al., 2007). REM segments exhibited the following features: low-amplitude, mixed-frequency EEG activity, absence of K complexes and sleep spindles and decrease in chin tone with rapid eye movements following within 1 epoch as determinants of the exact point of REM onset.

Figures 7 and 8 depict representative polygraphs of EEG frequencies and respiration during the transition from non-REM to REM sleep in a cSCI and AB subject, respectively. Able-bodied subjects had a larger drop in ventilation with REM onset than SCI subjects (Table 5).

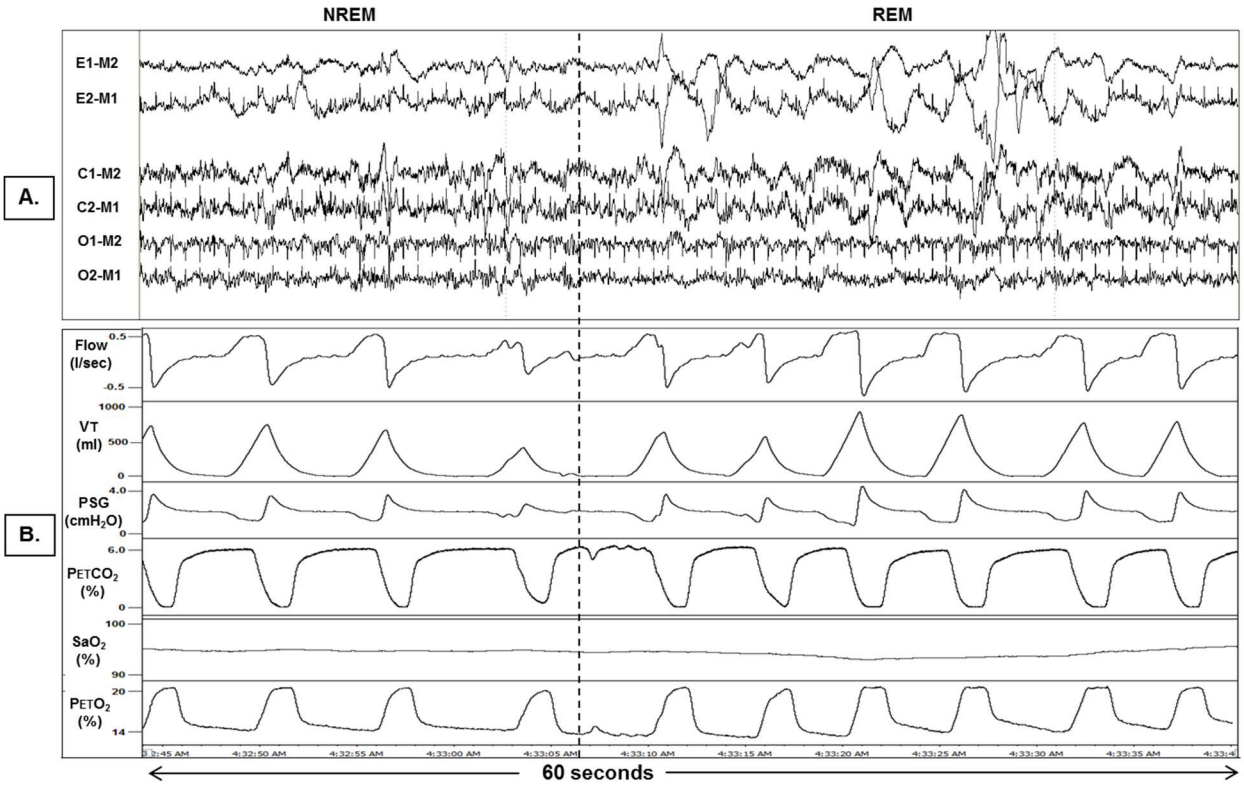

Figure7. Ventilation during Transition from Non-REM to REM Sleep in Spinal Cord Injury

Panel A: A 30 second polygraph with EEG and EMG recording in a cervical (C7, complete injury) SCI individual (28 year old male, BMI 31.0 kg/m^2) during the transition from NREM to REM sleep. E: eye, M: mastoid ground, C: central, O: occipital. Panel B: A 30 seconds polygraph with ventilation recording, time matched to the EEG data in panel A. Note the absence of a large drop in flow and tidal volume coinciding with REM onset in this cervical SCI subject compared to an able-bodied subject in Figure 8.

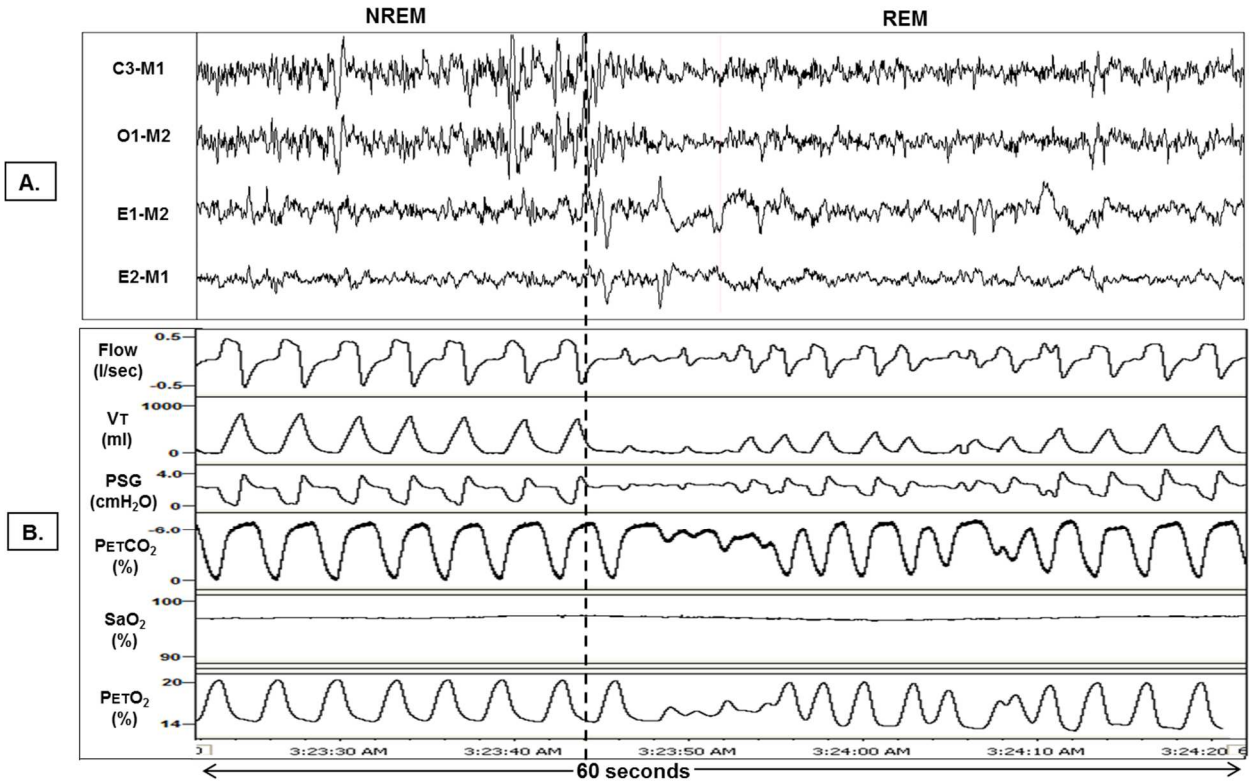

Figure 8. Ventilation during Transition from Non-REM to REM Sleep in an Able-bodied Subject

Panel A: A 30 second polygraph with EEG and EMG recording in an able-bodied control subject (68 year old male, BMI 23.6 kg/m^2) during the transition from NREM to REM sleep. E: eye, M: mastoid ground, C: central, O: occipital. Panel B: 30 A seconds polygraph of ventilation recording, time matched to the EEG data in panel A. Note the large drop in flow and tidal volume with REM onset in this able-bodied subject.

Table 5. Tidal Volume During Non-REM to REM Transitions

	Non-REM	REM
cSCI		
1c	763.8±93.2	710.6±155.4
2c	295.5±56.5	269.2±106.8
3c	232.5±40.9	290.4±81.6
tSCI		
1t	279.9±47.2	212.6±128.4
2t	249.3±93.6	247.3±51.3
Able-Bodied		
1AB	448.7±42.5	322.9±148.0
2AB	434.0±22.4	394.3±71.1
3AB	470.8±26.9	153.4±85.3

cSCI: cervical SCI; tSCI: thoracic SCI; Tidal Volume (mL). Non-REM: average of the 10 breaths of non-REM sleep just prior to REM onset for each subject ± S.D. REM: average of the first 10 breaths in REM sleep for each subject ± S.D. Each subject is denoted by a number followed by c for cervical SCI, t for thoracic SCI or AB for able-bodied.

Discussion

Summary of findings

The purpose of this study was to identify the ventilatory changes at sleep onset during the transition from alpha to theta EEG frequencies. The major findings of this study were: 1) Sleep onset was associated with hypoventilation in individuals with chronic SCI compared to able-bodied subjects. 2) Sleep onset hypoventilation was predominantly present in participants with cervical but not thoracic SCI, and 3) There was no change in upper airway resistance in either group.

The effect of sleep onset on ventilation and timing in spinal cord injury

The current study demonstrated that the loss of the wakefulness stimulus to breathe was associated with decreased V_E and increased $P_{ET}CO_2$ in individuals with SCI but not in able-bodied participants. Decreased ventilation was not due to increased upper airway resistance. Thus, altered upper airway mechanics were not responsible for the sleep onset hypoventilation in individuals with SCI.

Evidence in the literature suggests that sleep onset is associated with transient breathing instability and a decrease in ventilation, even in healthy individuals (Douglas et al., 1982; Colrain et al., 1987; Trinder et al., 1992; Dunai et al., 1999). Trinder and colleagues reported a 19% drop in V_T during alpha to theta transitions in healthy adult males and a 13% decrease in females (Trinder et al., 1992). Our study did not consider

gender differences, but we found no significant (<10%) decrease in V_T in theta compared with alpha in the AB group; the reasons for the difference in findings is currently unclear.

Putative Mechanisms of Sleep Onset Hypoventilation in SCI

The major finding in our study was a greater drop in V_T at sleep onset in SCI compared to able-bodied individuals. Moreover, V_T drop was related to the level of injury and was greater in cervical SCI, who have significant ventilatory instability in comparison to able-bodied subjects (Sankari et al., 2014a). Consistent with previous work (Kay et al., 1996), we found that upper airway resistance does not explain the decrease in ventilation at sleep onset. We therefore suggest that other factors that may contribute to ventilatory instability during sleep onset such as state-related fluctuations in the drive to the primary respiratory muscles and variability in compensatory mechanisms.

We considered several possible mechanisms of sleep-onset hypoventilation in SCI subjects. First, chest wall deformities, such as in mid- and high thoracic SCI, might contribute to increased mechanical loading and reduced lung volume leading to hypoventilation (Castriotta and Murthy, 2009). Second, reduced central ventilatory drive may cause hypoventilation in subjects with SCI, especially cervical SCI (Manning et al., 1992). There is evidence from animal studies that central ventilatory drive may be diminished in cervical SCI (Zimmer and Goshgarian, 2007). This is unlikely in our study as there was no difference in ventilation or $P_{ET}CO_2$ during wakefulness in SCI vs. able-

bodied control subjects. Whether SCI is associated with a more pronounced sleep-related decrease in central ventilatory motor output cannot be determined from our data.

Third, sleep onset hypoventilation may be influenced by peripheral chemoreceptor activity. A previous study suggested an interaction between chemical stimuli and state effects on ventilation during sleep-onset (Dunai et al., 1999). Specifically, individuals with increased peripheral chemoreceptor activity displayed amplified state-related changes in ventilation and subsequent dampening following hyperoxic exposure. However, sleep onset hypoventilation was more pronounced in cervical SCI subjects even when compared to able-bodied individuals with SDB, a group that is known to have augmented peripheral chemoreceptor activity.

Finally, individuals with spinal cord injury are more susceptible to sleep onset hypoventilation than healthy individuals, owing to the denervation of some, or all, intercostal muscles (8, 20, 29). Evidence in the literature suggests that rib cage contribution to V_T increases by 20% during non-REM sleep relative to wakefulness (28). Therefore, we interpret accentuated sleep-onset hypoventilation in individuals with tetraplegia as secondary to loss of intercostal muscle activity. The attenuation of non-REM to REM hypoventilation in subjects with cervical spine injury supports this interpretation. However, our study precludes drawing firm conclusions regarding the relative contribution of the loss of intercostal muscle activity versus increased peripheral chemoreceptor activity on sleep-onset hypoventilation. In summary, our study demonstrated an augmented sleep-related hypoventilation in patients with SCI, mostly in subjects with cervical SCI and without significant changes in upper airway resistance.

Physiologic Implications

Sleep-onset hypoventilation may contribute to sleep-related breathing instability. Individuals with SCI can maintain stable sleep and alveolar ventilation despite modest hypoventilation. However, significant hypoventilation may occur in individuals with a high spinal level of SCI and abnormal respiratory mechanics or those who use CNS suppressing medications. Sankari and colleagues have previously shown that tetraplegia is a risk factor for central apnea owing to increased plant gain (Sankari et al., 2014b). Therefore, sleep onset hypoventilation may increase the propensity to develop central apnea by increasing plant gain. The ensuing sleep fragmentation may lead to ventilatory overshoot and recurrent episodes of apnea/hypopnea alternating with hyperpnea (Douglas et al., 1982; Douglas, 1992; Badr, 2005; Eckert et al., 2007; Yumino and Bradley, 2008; Nemati et al., 2011). Therefore, sleep-onset hypoventilation may promote the development of recurrent central apnea and sleep-related breathing instability in subjects with SCI.

Conclusions

We have shown the occurrence of significant sleep-onset hypoventilation in patients with chronic SCI compared to able-bodied subjects. The magnitude of sleep onset hypoventilation is not associated with increased upper airway resistance and is related to the level of SCI. Diminished neuromuscular output owing to intercostal muscle paralysis could play a role in the development of sleep apnea post-injury to the cervical spine.

CHAPTER 2

The Role of Chemoreflexes in Breathing Instability and Sleep Disordered Breathing in Chronic Spinal Cord Injury

Introduction

The incidence of spinal cord injury (SCI) is estimated to be 12,500 new cases each year in the United States alone. Approximately 59% of cases result in tetraplegia (NSCISC Facts and Figures at a Glance, 2014). While less than 10% of SCI patients will require mechanical ventilatory support beyond 1 year of injury, respiratory complications (i.e. pneumonia) remains the primary cause of death after SCI (NSCISC 2013 Report). Patients with cervical (cSCI) and high thoracic (tSCI) injuries are at the greatest risk for respiratory-related complications due to impairment of neural outflow to critical respiratory muscles.

Sleep disordered breathing (SDB) is a major cause of morbidity and impaired quality of life in patients with SCI. Recent reports found that more than half of SCI patients developed SDB in their first year post-injury (62% have AHI >10 event/hour). In fact, SCI may be an independent risk factor for the development of SDB (Sankari et al, 2014b). Berlowitz and colleagues (2005) followed a cohort of cSCI patients from the acute to the chronic phase of injury and reported that at 2 weeks post-injury 60% had SDB (apnea hypopnea index >10) , the incidence rose to 83% by week 13, and fell to

62% by 52 weeks (Berlowitz et al., 2005). Other studies published over the past 2 decades by various investigators have placed the prevalence of SDB to be between 27-77% (Star et al., 1988; Bonekat et al., 1990; Short et al, 1992; Levi et al., 1995; McEvoy et al., 1995; Klefbeck et al., 1998; Burns et al., 2000; Stockhammer et al., 2002; Biering-Sorenson et al., 2009; Sankari et al., 2014a).

The specific type of SDB experienced after SCI is a matter of debate. The majority of studies places SDB in SCI under the classification of obstructive sleep apnea (OSA). However, Sankari et. al. (Sankari et al., 2014a) studied a group of 26 consecutively enrolled chronic SCI subjects (15 cSCI, 11 tSCI) and reported that approximately 93% of cervical and 55% of thoracic SCI patients had symptomatic SDB as defined by an apnea hypopnea index (AHI) >5 events per hour and excessive daytime sleepiness. Another significant finding of this study was that the majority of cSCI had central sleep disordered breathing (CSDB) and periodic breathing (PB) (60%) in the absence of heart disease and irrespective of narcotic use, compared to tSCI subjects who primarily exhibited OSA (18% had CSA and 27% had PB) (Sankari et al, 2014a). Central SDB occurs in <1% of the general adult population and is typically found in patients with systolic heart failure, those ascending to high altitudes, and opiate users (White et al., 2005; Panossian et al., 2009), although some studies report that the incidence of CSA increases with age (Wellman et al., 2007). Central sleep apnea can also occur concomitantly with OSA (Morgenthaler et al., 2007; Salloum et al., 2010; Chowdhuri et al, 2012). One reason for the common classification of SDB in SCI as OSA may be due to the insensitivity of diagnostic methods to differentiate between central and obstructive events (Yumino and Bradley, 2008). Sankari and colleagues

used quantitative flow via pneumotachometer and a pressure tipped airway catheter which allows for greater accuracy in diagnosing types of events compared to methods commonly used by other investigators (Sankari et al., 2014a).

The mechanisms underlying the increase in CSDB and PB in chronic SCI are not understood. In SCI, one proposed mechanism for CSDB and PB is sleep-related hypoventilation (Castriotta and Murthy, 2009). Our lab has observed that patients with SCI, particularly cSCI, demonstrate significant sleep onset hypoventilation compared with subjects with tSCI and able-bodied controls (Bascom, unpublished observations, See Chapter 1). Sleep onset hypoventilation leads to frequent arousals from sleep, significant fluctuations in end-tidal CO_2 and O_2 ($P_{ET}CO_2$, $P_{ET}O_2$, respectively), increased plant gain, and a narrowed CO_2 reserve, all of which set the stage for breathing instability (Dempsey, 2004). Trinder and colleagues (1993) reported a positive correlation between able-bodied subjects who had a large magnitude of sleep onset hypoventilation (during the transition from wake to stable non-REM sleep) and breathing instability and the degree of respiratory instability during stable sleep (Trinder et al., 1993). Based on the findings in by Trinder and colleages (1993), Dunai and colleagues (1996) postulated that both state-related fluctuations in ventilation and chemical influences (i.e. chemoreflexes) may work together during sleep to create breathing instability and induce perturbations. Delays in feedback to the peripheral chemoreceptors, that are inherent in the respiratory feedback loop, may accentuate state-related instability (Dunai et al., 1996). To test this hypothesis Dunai and colleagues (1996) identified 2 groups of able-bodied subjects with low and high peripheral chemoreceptor drive and examined state-related fluctuations in ventilation

during sleep onset. They reported that subjects with high peripheral chemoreceptor drive exhibited a significantly greater magnitude sleep onset ventilatory instability and hypoventilation than subjects with low peripheral chemoreceptor drive. Furthermore, this hypoventilation was mitigated with hyperoxia administration. They concluded that heightened peripheral chemoreceptor activity can contribute to SDB (Dunai et al, 1996 and 1999).

Indeed, altered chemoresponsiveness has been implicated in CSDB and breathing instability by several investigators (Khoo et al., 1982; Dempsey 2004; Eckert et al., 2007; Yumino and Bradley, 2008) and may be an important mechanism in the development of SDB in chronic SCI. High chemoreflex sensitivity leads to ventilatory overshoot in response to relatively small perturbations, destabilizes breathing, and predisposes individuals to CSDB and PB (Dempsey, 2004). The peripheral chemoreceptors serve to respond rapidly to adjust ventilation in response to changes in O_2 and CO_2 on a breath-by-breath basis and have been proposed as a key component of the ventilatory feedback loop for producing breathing instability in the face of feedback delays (Khoo et al., 1982; Longobardo et al., 1982; Younes, 1989).

There have been no studies reported that specifically focused on peripheral chemoreceptor responsiveness in SCI. The majority of studies have reported blunted hypercapnic ventilatory response (HCVR) (Kelling et al., 1985; McCool et al, 1988; Manning et al., 1992), which is a measure of peripheral *and* central chemosensitivity, while other investigators have found no significant difference in sensitivity to steady-state hypoxia and hypercapnia compared with able-bodied controls (Pokorski et al., 1990 and 1995; Ben-Dov et al., 2009). Since peripheral chemoreceptors respond very

rapidly, on a breath-by-breath basis, chemical stimuli that are rapidly administered and brief would best take advantage of their specific time response characteristics in order to avoid involving the slower responding central chemoreflexes (Black et al.,1967; Dutton et al.,1967; Gelfand and Lamberton , 1973).

Brief hyperoxia has long been established as having an inhibitory effect on the carotid body, causing a short-lived decrease in ventilation which has been used as a measure of the contribution of the carotid bodies to eupneic breathing (Dejours, 1962; Daristotle et al., 1991; Cutz et al., 1997; Gautier 2003). The use of hyperoxia to assess peripheral chemoreceptor tonic drive has well established validity, is safe and well tolerated by subjects.

McClean and colleagues (1988) utilized a similar method of targeting carotid body response to CO_2 by administering a single breath of CO_2 gas via inhalation, and measuring the ventilatory response to this brief stimuli (McClean et al., 1988, Martinez, 2008). The response to brief CO_2 administration is a transient (within 10 seconds) increase in tidal volume (V_T). The time course of this response suggests it is a function of peripheral chemoreceptor, as opposed to central chemoreceptors that would require up to 60 seconds to manifest (Black and Torrance, 1966; Dutton et al., 1967, Gray, 1968; Gelfand and Lamberto, 1973). The combination of these two interventions (brief hyperoxia and hypercapnia) would allow for the assessment of carotid body chemoreflex responses to O_2 and CO_2 stimuli.

The hypotheses proposed in my studies are: 1) subjects with chronic SCI will have a higher peripheral chemoreceptor contribution to eupneic breathing, as evidenced

by a greater magnitude of decrease in ventilation in response to transient hyperoxia, and 2) subject with SCI will have a heightened ventilatory response to transient hypercapnia compared to able-bodied subjects. In order to test these hypotheses, I administered rapid, but brief chemical stimuli to determine the ventilatory response to O_2 and CO_2 in both groups of subjects.

Materials and Methods

Protocols were approved by the Human Investigation Committee of the John D. Dingell Veterans Affairs Medical Center and Wayne State University (Detroit, MI) and written informed consent was obtained.

We studied adults (≥18 years old) with chronic SCI and able-bodied participants if they met the inclusion and exclusion criteria. All subjects were instructed not to have alcohol, caffeine products or sedatives on the day of the study.

Inclusion Criteria- participants with chronic SCI (>6 months post-injury), spanning the spectrum from cervical (cSCI, C4-C7) to thoracic levels (tSCI, T1-T6) (complete and incomplete injuries). Able-bodied subjects (AB) were recruited with similar demographics to the SCI group for age, body mass index (BMI) and gender.

Exclusion criteria-Participants were excluded from the study for any of the following: (1) pregnant or lactating females; (2) currently ventilator dependent or with tracheostomy tube in place; (3) history of cardiac disease including heart failure, peripheral vascular disease, or stroke; (4) history of head trauma resulting in neurological symptoms or loss of consciousness; (5) advanced lung, liver, or chronic

kidney disease; (6) extreme obesity, defined for this protocol as BMI >38 kg/m^2; or (7) other illness that would interfere with completion of the study.

Subjects first underwent overnight in-lab polysomnography (PSG) to determine the presence or absence of sleep disordered breathing (apnea hypopnea index, events/hour; AHI) (Comet PSG System, AS40 Model or the Heritage II PSG System; Grass Technologies, Warwick, RI). PSG studies were scored according to American Academy of Sleep Medicine (AASM) 2012 recommended criteria (Berry et al., 2012).

On a separate visit, subjects arrived at the lab between 10 am-4 pm for study. Studies were performed in the supine position during wakefulness. Instrumentation included electrocardiogram (ECG), electroencephalogram (EEG), electrooculograms (EOG) and chin electromyograms (EMG) using the International 10-20 system of electrode placement (EEG: C3-A2 and C4-A2; EOG: O-A2). Subjects wore a nasal mask connected to a pneumotachometer (Hans Rudolph, Model 3700A, Shawnee, KS) that measured airflow. Tidal Volume (V_T) was determined via integration of the pneumotachometer flow signal. End-tidal carbon dioxide ($P_{ET}CO_2$), end-tidal oxygen ($P_{ET}O_2$) levels and inspired O_2 levels (FiO_2) were measured with CO_2 and O_2 gas analyzers (Vacumed Model 17515 and 17518 respectively, Ventura, CA). Pulse oximetry was measured by an ear probe (Biox 3740, Datex-Ohmeda Inc, Madison, WI). Respiratory effort was measured by respiratory inductance plethesmography (RIP) belts placed on the chest and abdomen (Q-RIP, Braebon Medical Corp., Ogdensburg, NY). Ventilation data from the pneumotachometer, pulse oximeter and gas analyzers were digitized and analyzed using a PowerLab Data Acquisition System (Model 16SP,

ADInstruments Inc., Colorado Springs, CO). Electroencephalograph, EMG, EOG, ECG, and respiratory effort were recorded and analyzed via the Comet PSG system (AS40 amplifier) or Heritage II system (Grass Technologies, Warwick, RI). EEG was used to verify that subjects remained in stable wakefulness during interventions. Medical adhesive tape was placed over subject's lips to help prevent mouth breathing. Spontaneous ventilation was recorded for a minimum of 15 minutes prior to any intervention. Subjects were instructed that CO_2 or O_2 would intermittently be administered through the nasal mask for a short period of time, in random order, and that they were to breathe through their nose. If mouth breathing occurred, tape was replaced and the subject was again instructed not to breathe through their mouth. The order of interventions (hyperoxia vs. single breath CO_2) was randomized to eliminate order effect.

Hyperoxia Methods

Oxygen was bled into a port on the mask through O_2 tubing attached to a gas tank containing 100% O_2. Flow was quickly increased to 12-15 L/min until inspired O_2 values reached ≥ 50% in the mask. O_2 administration was continued for 1 minute followed by 5 minutes of room air breathing between trials. Trials were repeated 3 times. A representative example of a hyperoxia test in a cSCI subject is depicted in Figure 9 and an AB subject in Figure 10.

Analysis consisted of comparing the average of 10 baseline room air breaths immediately preceding hyperoxia with the nadir V_T breath within the first 30 seconds of hyperoxia. Results of 3 trials per subject were averaged. In one instance in a cSCI

subject, only 2 reproducible trials were obtained, so in this case the average of 2 trials was used. Ventilation (minute ventilation [V_E], V_T, and frequency) for the nadir breath during hyperoxia was expressed as a percentage of the baseline room air ventilation. Nadir hyperoxia ventilation was compared between SCI and AB subjects. In addition,

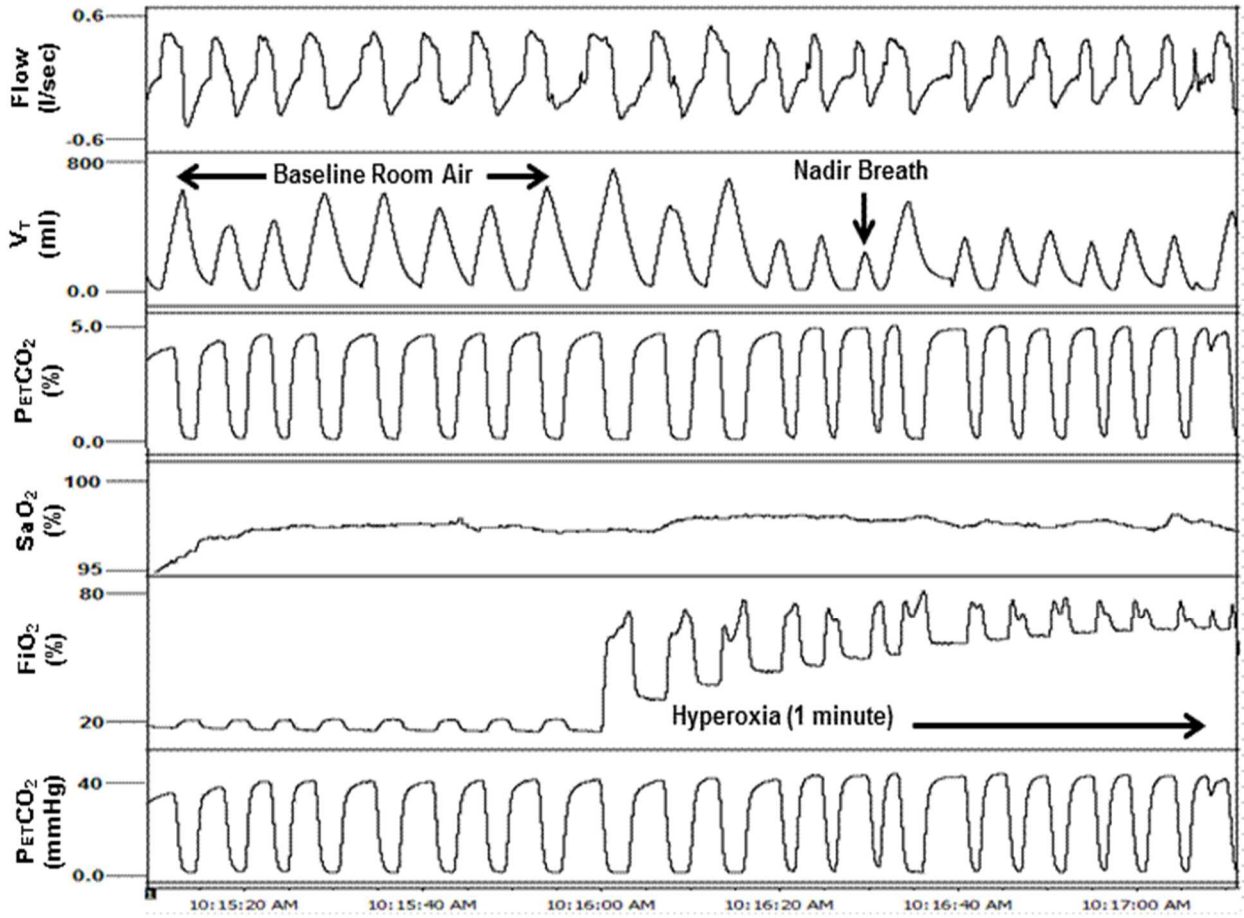

Figure 9: Effect of Transient Hyperoxia on Ventilation in Spinal Cord Injury

A representative polygraph of a 1 minute hyperoxia trial in a 38 year old male cervical SCI subject (BMI 28.2 kg/m^2). Baseline ventilation on room air preceding hyperoxia is followed by a striking decrease in tidal volume during hyperoxia administration. V_T: tidal volume, $P_{ET}CO_2$: end-tidal CO_2, $P_{ET}O_2$: end-tidal O_2, SaO_2: oxygen saturation; FiO_2: concentration of inspired O_2.

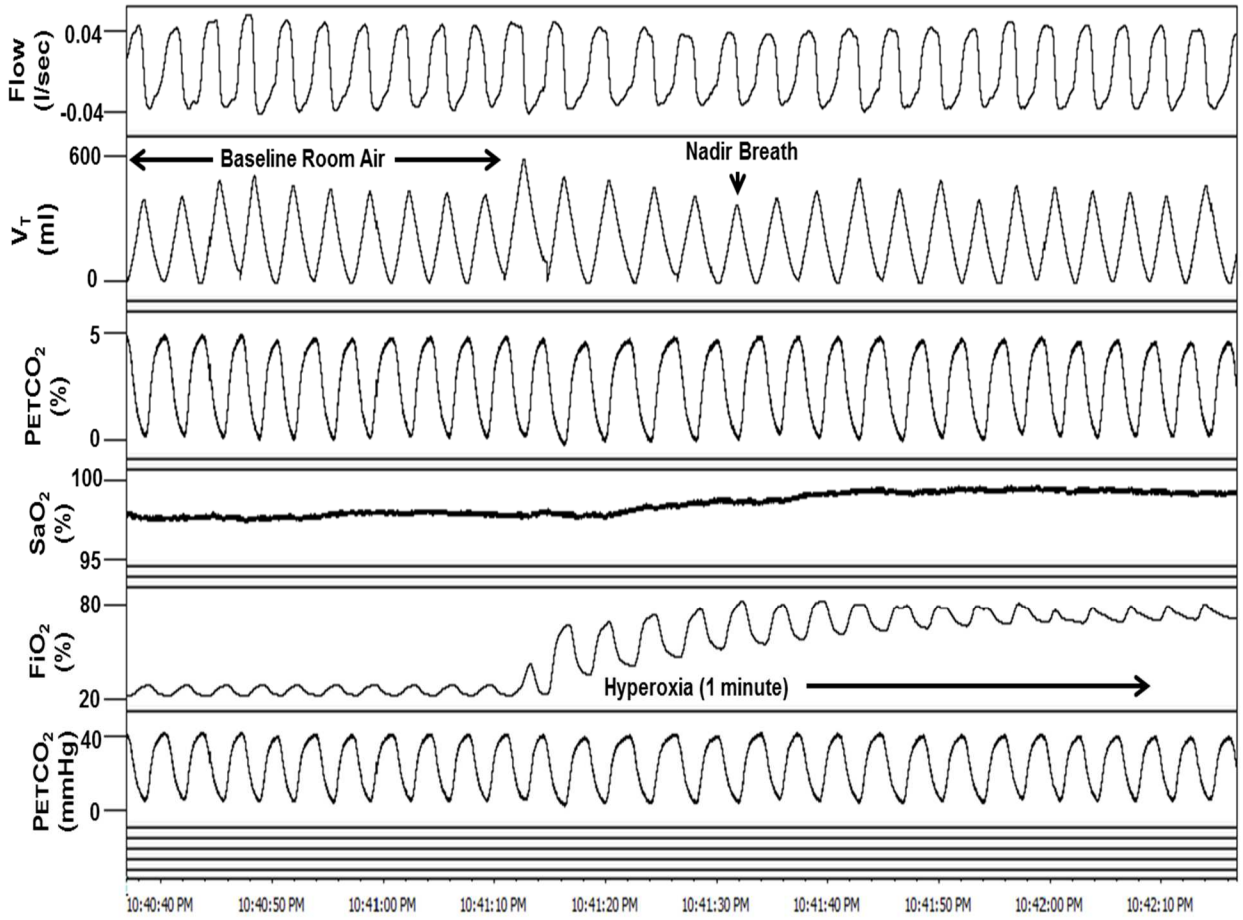

Figure 10: Effect of Transient Hyperoxia on Ventilation in an Able-Bodied Subject

A representative polygraph of a 1 minute hyperoxia trial in a 56 year old male able-bodied subject (BMI 25.8 kg/m^2). Baseline ventilation on room air preceding hyperoxia is followed by a smaller decrease in tidal volume during hyperoxia administration compared to that in Figure 1. V_T: tidal volume, $P_{ET}CO_2$: end-tidal CO_2, $P_{ET}O_2$: end-tidal O_2, SaO_2: oxygen saturation; FiO_2: concentration of inspired O_2.

average time (in seconds) to nadir breath during hyperoxia and average inspired % O_2 (FiO_2) were calculated and compared between groups.

A sub-analysis was performed to compare the response to transient hyperoxia in cervical vs. thoracic SCI subjects to determine if spinal injury level influences the response. In addition, analyses were performed for all subjects to determine if AHI correlates with the ventilatory response to hyperoxia.

Single Breath CO_2 Methods

Single breath CO_2 tests ($SBCO_2$) using the instrumentation and conditions described above were performed. While breathing room air, CO_2 was bled into a port on the mask via small bore tubing connected to a gas blender (Model PMR4, Orangeburg, NY), which was in turn connected to a gas tank containing 40% CO_2 balanced with N_2. Gas flow was adjusted to reach a target of 8-10% inspired CO_2 for a single breath. Flow was adjusted to administer approximately 8-10% inspired CO_2 during expiration of one breath to load the circuit and continued until peak inspiration of the following breath before being abruptly terminated. Figures 11 and 12 demonstrate trials of $SBCO_2$ in a cSCI and AB subject, respectively. Trials were preformed 3 times with 2-3 minutes between trials.

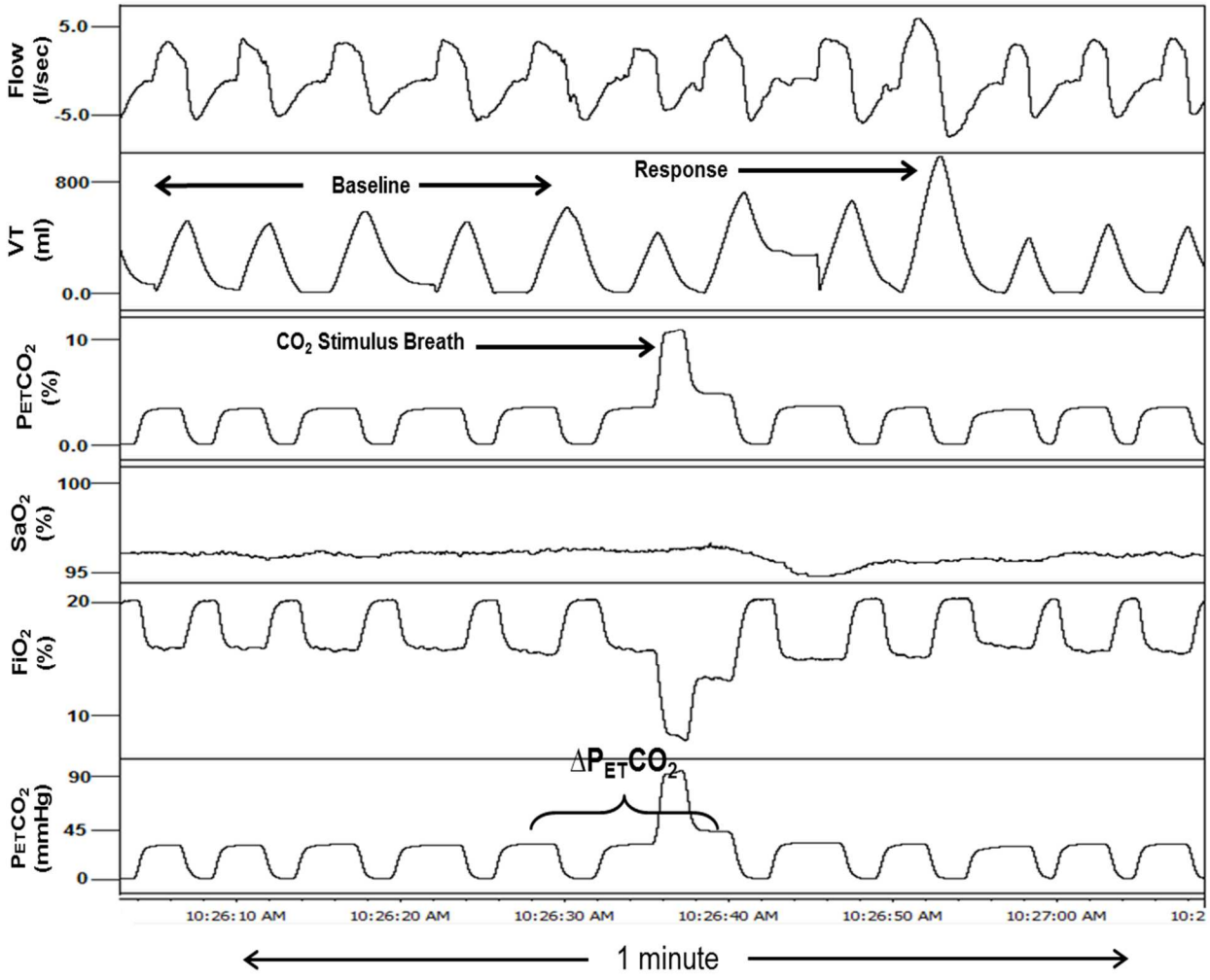

Figure 11: Effect of Transient Hypercapnia on Ventilation in Spinal Cord Injury

A representative polygraph of a single breath CO_2 test in a 28 year old male cervical SCI subject (BMI: 31.5 kg/m^2). Baseline room air ventilation is compared with the response breath (largest V_T within 5 breaths of CO_2) after administration of 1 breath of CO_2 ("CO_2 stimulus breath"). Note the large increase in tidal volume in the response breath after CO_2 administration in a SCI subject compared to the response breath in Figure 12, an able-bodied subject. V_T: tidal volume, $P_{ET}CO_2$: end-tidal CO_2, $P_{ET}O_2$: end-tidal O_2, SaO_2: oxygen saturation; FiO_2: concentration of inspired O_2. $\Delta P_{ET}CO_2$:$P_{ET}CO_2$ after CO_2 administration minus the average $P_{ET}CO_2$ for baseline room air breaths prior to test.

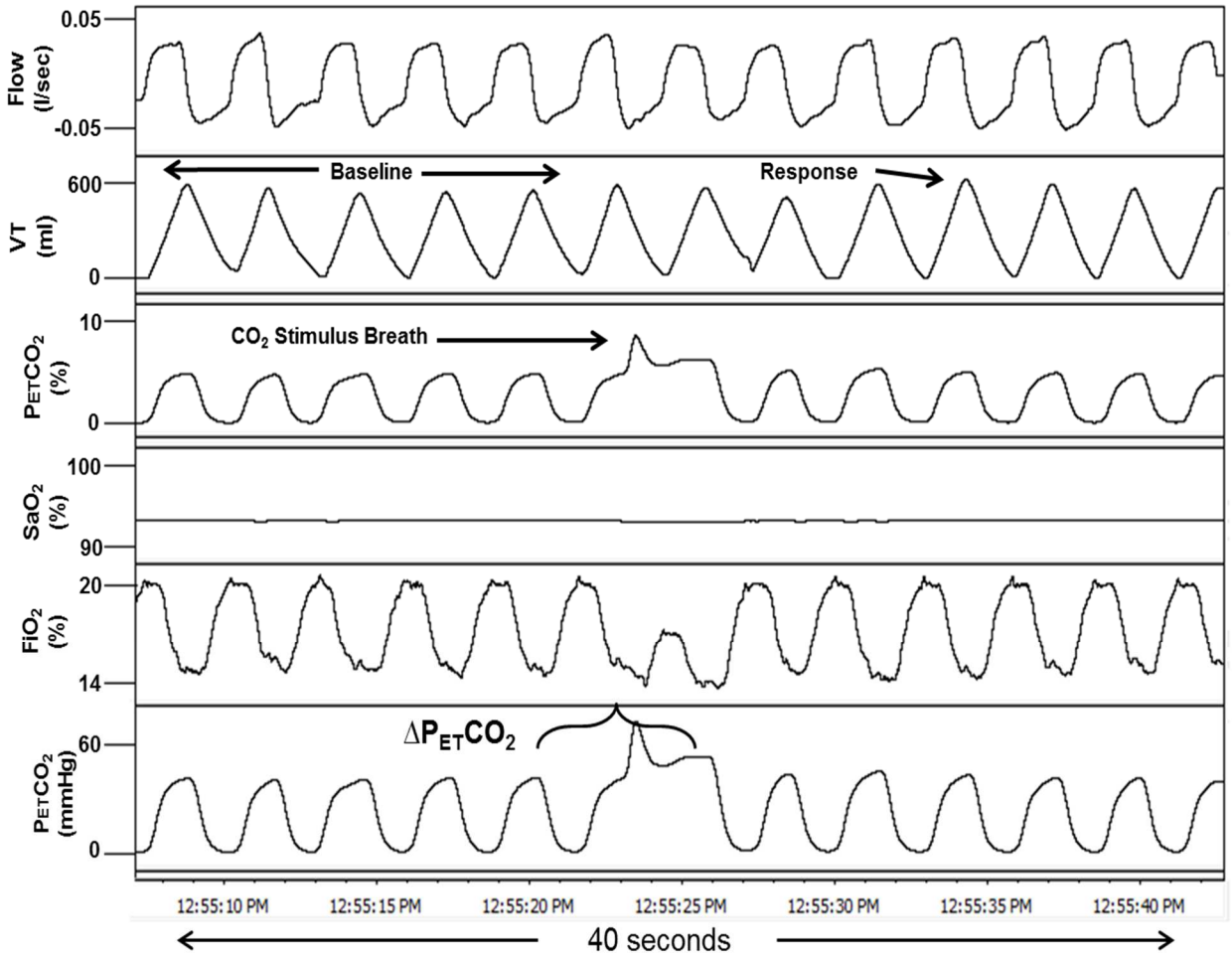

Figure 12: Effect of Transient Hypercapnia on Ventilation in an Able-bodied Subject

A representative polygraph of a single breath CO_2 test in a 30 year old male able-bodied subject (BMI: 22.9 kg/m^2). Baseline room air ventilation is compared with the response breath after administration of 1 breath of CO_2 ("CO_2 stimulus breath"). Note the relatively small increase in tidal volume in the response breath (largest V_T within 5 breaths of CO_2) after CO_2 administration of the able-bodied subject in this figure, compared to the response in Figure 11, a SCI subject. V_T: tidal volume, $P_{ET}CO_2$: end-tidal CO_2, $P_{ET}O_2$: end-tidal O_2, SaO_2: oxygen saturation; FiO_2: concentration of inspired O_2. $\Delta P_{ET}CO_2$:$P_{ET}CO_2$ after CO_2 administration minus the average $P_{ET}CO_2$ for baseline room air breaths prior to test.

Analysis consisted of averaging the 5 breaths of baseline room air ventilation (V_E and $P_{ET}CO_2$) immediately prior to CO_2 administration for comparison with the "response breath", which was taken as the largest breath based on V_T within the first 5 breaths after administration. The chemoreflex response to a single breath of CO_2 was calculated as $\Delta V_E/\Delta CO_2$ (L/min/mmHg). In addition, the V_T and V_E of the response breath was expressed as a percentage of the average V_T and V_E, respectively, for baseline room air breaths. Time from CO_2 breath to response breath was calculated in seconds, as well as average inspired CO_2. Results were averaged for 3 trials in each subject and outcomes were compared between SCI and AB groups.

A sub-analysis was performed to compare the response to a single breath of CO_2 in cervical vs. thoracic SCI subjects to determine if spinal injury level influences the response. In addition, analysis was for all subjects to determine if AHI correlates with $SBCO_2$ response.

Statistical Analysis

T-tests were used to compare outcome measures and demographic data between the SCI and AB groups, or between cSCI and tSCI, when data was normally distributed. If data was not normally distributed, non-parametric tests were employed (SigmaPlot 12.1). Pearson Product Moment Correlation was performed to determine the relationship between AHI and ventilatory response to chemical stimuli. All data are reported as mean ± SD and significance was set at $p<0.05$.

Results

Hyperoxia in SCI vs. Able-bodied Subjects

Fifteen subjects with chronic SCI and 15 AB subjects with similar demographics (Table 6) were studied to determine the ventilatory response to hyperoxia. Figure 13 illustrates that SCI subjects had a significant decrease in V_T (63.4±21.7 % baseline) and V_E (63.1±23.0 % baseline) with hyperoxia compared to AB subjects (V_T: 87.1±14.3 % baseline, V_E: 91.38±15.1 % baseline), while frequency was not different in either group. The time from initiation of hyperoxia to the nadir breath was similar in both groups (SCI: 20.2±3.6 sec. AB: 18.3±5.4 sec, p=0.26) as was the average FiO_2 administered for all hyperoxia trials (SCI: 73.0±11.5% vs. AB: 71.2±8.7%, p=0.63). There was no significant correlation between AHI and V_E% baseline (r=-0.28) in SCI and AB (n=30). Subjects with SCI had a greater magnitude of inhibition of peripheral chemoreceptors in response to brief hyperoxia than able-bodied subjects.

Table 6. Subject Characteristics

	Hyperoxia		Single Breath CO_2	
	SCI	Able-Bodied	SCI	Able-Bodied
N	*15*	*15*	12	*12*
Age *(*years*)*	40.7±13.4	41.3±18.3	39.8±13.2	*41.3*±16.6
*BMI (*kg/m2*)*	25.8±5.4	27.4±4.1	27.0±5.1	*26.9*±4.1
*G*ender *(*M/F*)*	11/4	10/5	9/3	*9/3*
*AHI (*events/hour*)*	20.0±17.4	11.7±18.2	21.2±19.3	*12.7*±20.3
Injury level (cervical/thoracic)	8/7	—	6/6	—

All data Mean ±*SD.* BMI: Body Mass Index*,* AHI: Apnea Hypopnea Index. No significant difference in age, BMI, gender or AHI ($p > 0.05$).

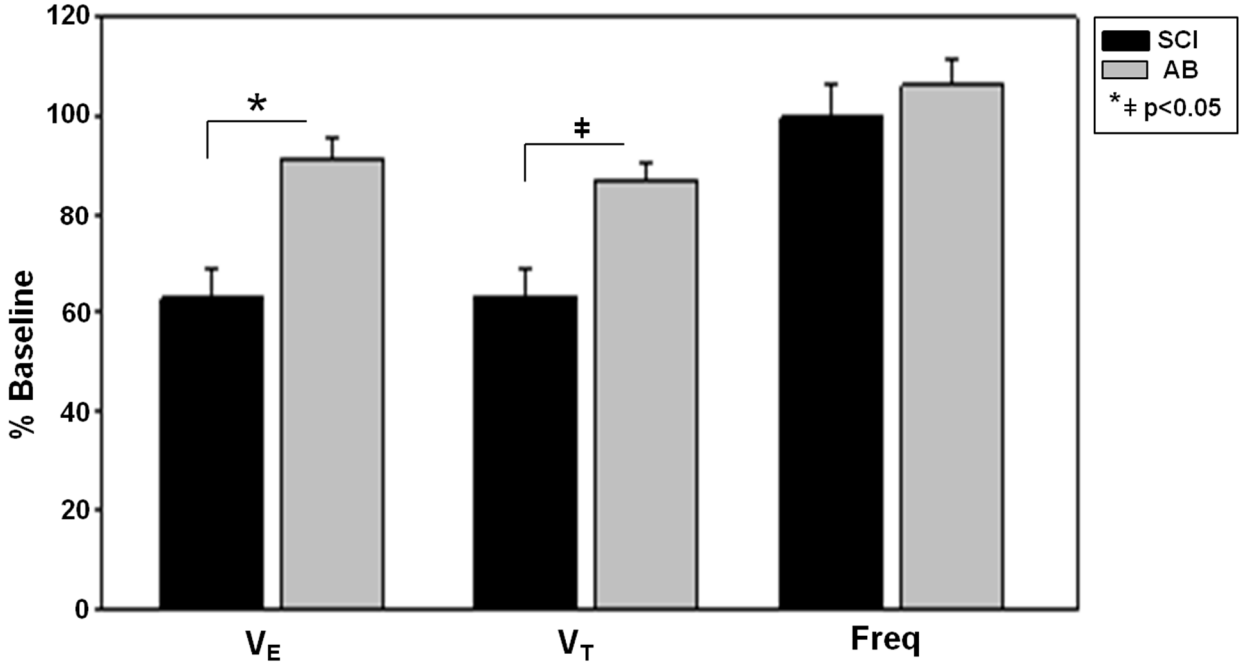

Figure 13: Comparison of Response to Hyperoxia in Spinal Cord Injury and Able-bodied Subjects

Minute ventilation (V_E), tidal volume (V_T) and frequency (Freq) during hyperoxia administration in spinal cord injury (SCI) and able-bodied (AB) subjects are expressed as a percentage of baseline room air ventilation prior to intervention. SCI subjects had a significant decrease in V_E and V_T with hyperoxia (*p<0.001, ‡ p=0.001) compared to AB subjects. There was no significant change in frequency in either group (p=0.43).

Hyperoxia in Cervical vs. Thoracic SCI Subjects

To determine the effect of spinal injury level on ventilatory response to transient hyperoxia, cSCI (n=8) vs. tSCI (n=7) subjects were compared (Table 7). There was no significant difference in the nadir breath V_T or V_E or frequency expressed as a % of baseline ventilation during hyperoxia trials. Results are detailed in Table 8. There was no difference in time to response to hyperoxia (cSCI: 21.4±3.8 sec, tSCI: 18.7±30. sec; p=0.8), or average trial FiO_2 (cSCI: 72.9±9.2%, tSCI: 73.2±14.4%; p=0.96). There was no significant correlation between AHI and $SBCO_2$ response in SCI subjects (n=15) (*r*=-0.40). Thus, injury level had no effect on ventilatory response to brief hyperoxia.

Table 7. Cervical and Thoracic SCI Subject Characteristics

	Hyperoxia		Single Breath CO_2	
	Cervical	Thoracic	Cervical	Thoracic
N	*8*	*7*	6	*6*
Age *(years)*	42.5±13.0	38.7±14.7	40.5±11.0	*39.0*±16.1
*BMI (*kg/m2*)*	24.4±5.7	27.4±4.9	26.2±5.4	*27.8*±5.2
*G*ender *(*M/F*)*	7/1	4/3	5/1	*4/2*
*AHI (*events/hour*)*	29.0±16.5	9.6±12.4*	33.2±17.0	*9.2*±13.6‡

All data Mean ±*SD.* BMI: Body Mass Index, AHI: Apnea Hypopnea Index. No significant difference in age, or gender. (p >0.05). AHI significantly higher in cervical vs. thoracic SCI subjects (* p=0.02, ǂ p=0.03).

Table 8. Cervical vs. Thoracic SCI Chemoresponse Ventilation

	Hyperoxia			Single Breath CO_2		
	Cervical	Thoracic	p value	Cervical	Thoracic	p value
N	*8*	*7*		6	*6*	
V_E *(% BASELINE)*	54.5±21.3	72.9±22.1	0.13	172.9±40.3	*153.3*±23.2	*0.33*
V_T *(% BASELINE)*	55.8±20.1	72.1±21.5	0.15	208.9±52.9	*157.6*±30.4	*0.07*
FREQUENCY (% BASELINE)	99.9±33.6	99.1±13.0	1.0	—	—	
$SBCO_2$ *(L/MMHG)*	—	—		0.82±0.48	*0.74*±0.39	*0.75*

All data Mean ±*SD*. $SBCO_2$: Ventilatory Response to a single breath of CO_2 ($\Delta V_E/\Delta CO_2$). No significant difference in any chemoreflex responses between cervical and thoracic SCI subjects.

Single Breath CO_2 in SCI vs. Able-bodied Subjects

Twelve subjects with chronic SCI and 12 AB subjects with similar demographics (Table 6) were studied to determine the response to a single breath of hypercapnia. The ventilatory response to $SBCO_2$ ($\Delta V_E/\Delta CO_2$) was significantly higher in the SCI group compared with the AB group, as detailed in Figure 14. Tidal volume for the response breath after CO_2 administration, expressed as a percentage of baseline room air ventilation, was also significantly increased in SCI compared to AB subjects (183.2±49.1 % vs. 125.7±13.6%, respectively, $p<0.05$). Similarly, V_E increased to a greater degree in SCI subjects compared to AB subjects (163.1±33.0 % vs. 118.5±4.8%, respectively, $p<0.05$). The average inspired CO_2 for $SBCO_2$ trials was not different between SCI and AB groups (8.7±1.6% vs. 8.0±1.3%, respectively, $p=0.24$) nor was the time from CO_2 administration to the response breath (SCI: 10.6±4.2 sec; AB: 11.9±4.1 sec, $p=0.44$). No significant correlation was found between AHI and $SBCO_2$ response in SCI and AB subjects ($n=24$) ($r=0.27$).

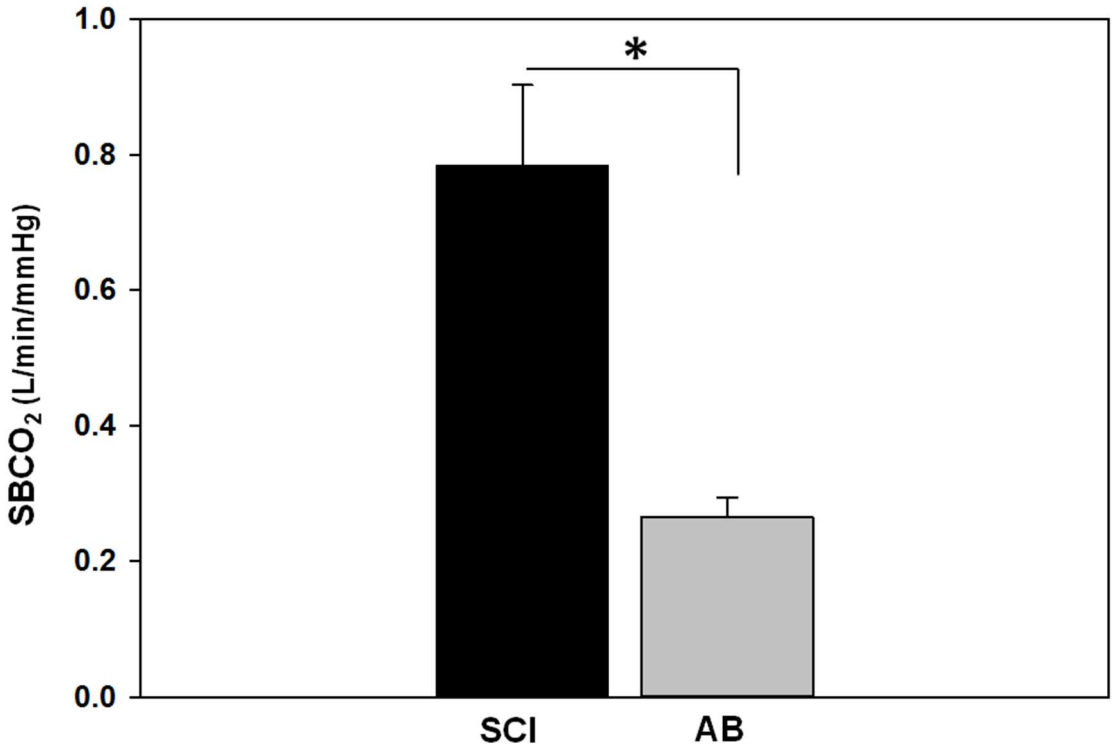

Figure 14: Comparison of Response to Brief Hypercapnia in Spinal Cord Injury vs. Able-bodied Subjects

The ventilatory response to a single breath of CO_2 ($SBCO_2$ L/min/mmHg) was significantly higher in SCI subjects compared to able-bodied subjects (AB) (*$p<0.001$).

Single Breath CO_2 in Cervical vs. Thoracic SCI Subjects

In order to determine the contribution of spinal injury level to the response to a single breath of CO_2, cervical (n=6) vs. thoracic (n=6) SCI subject's responses were compared. Table 7 describes the characteristics of the cervical and thoracic SCI groups. There was no difference in $SBCO_2$ ($\Delta V_E/\Delta CO_2$) response between cSCI and tSCI subjects (Table 8). Similarly, no difference was found in V_E or V_T expressed as a percentage of baseline ventilation between injury levels (Table 8). In addition, no difference was found in time from inspiration of CO_2 to response between cSCI (12.0±5.6 sec) and tSCI subjects (9.2±1.8 sec; p=0.28). The inspired CO_2 level for trials were not different between groups (cSCI: 8.9±1.4%, tSCI: 9.2±1.8; p=0.59). There was no significant correlation between AHI and $SBCO_2$ response in SCI subjects (n=12) (r=0.27).

Discussion

The purpose of this study was to determine the peripheral chemoreflex responses to brief hyperoxia and hypercapnia in chronic SCI and AB subjects. The major findings of the study were: 1) Subjects with SCI have a greater magnitude of reduction in ventilation in response to brief hyperoxia than AB. 2) SCI participants had a greater ventilatory response to a single breath of CO_2 than AB. 3) There was no difference in peripheral chemoreceptor response to brief hyperoxia or hypercapnia in cervical vs. thoracic SCI. 4) Peripheral chemoresponsiveness was not significantly correlated with AHI in SCI and AB subjects.

Peripheral chemoreceptor response to transient hyperoxia

The carotid body supplies a tonic excitatory input to central respiratory centers and contributes to eupneic ventilatory drive. Peripheral chemoreceptors are of great importance in maintaining ventilatory homeostasis during non-REM sleep when wakefulness inputs from higher brain centers are absent (Forester, 2000). The receptors have been postulated to be involved in the development of SDB (Khoo et al., 1982; Dempsey, 2004; Eckert et al., 2007; Yumino and Bradley, 2008). In this study I utilized brief hyperoxia to suppress the tonic drive of the carotid body to assess the putative contribution the carotid body to eupneic ventilation in SCI and AB subjects.

Hyperoxia has been reported to reduce ventilation transiently in adults by ~10% thus unmasking the oxygen-led drive to breathe, mediated by the peripheral chemoreceptors (Dejours, 1962; Gautier, 2006). In the current study, AB subjects had a decline in V_E of ~9% with hyperoxia, in agreement of historical data, compared to SCI subjects with ~37% decline. From this I conclude that SCI subjects have an increased peripheral chemoreceptor gain and a heightened reliance on the carotid body for maintenance of eupneic ventilation.

Peripheral chemoreceptor response to brief hypercapnia

Studies of chemosensitivity to CO_2 in an animal model, utilizing an isolated perfusion of the carotid body, found that central chemoreceptors respond in a slower fashion to CO_2 stimulation compared to the carotid body (30.9 sec vs. 19.6 sec, respectively). Therefore, the carotid body contributes significantly to the ventilatory

response to brief oscillations in CO_2 (Smith and Dempsey, 2006) such as were administered in the current study. The methods used to determine the ventilatory effect of transient hypercapnia in this study utilize the response characteristic unique to peripheral chemoreceptors.

McClean et al. (1988) using similar methods to determine the response to transient CO_2 in able-bodied adults (26 males and 26 females) found the average response to be ~0.34 L/min/mmHg (no significant gender or age effect was found) whereas able-bodied subjects in the current study had an average response of ~0.26 L/min/mmHg (vs. SCI with ~0.78 L/min/mmHg) (McClean et al., 1988). One reason for the slightly higher response in the McClean et al. study may be their use of 13% inspired CO_2 while the current study utilized 8-9% CO_2. However, the time response in both studies were consistent, with an average of 10-12 seconds from CO_2 administration to response.

During PB and CSDB, as is frequently found in cSCI, individuals are exposed to frequent cycles of hypopnea and hyperpnea, and corresponding oscillations in O_2 and CO_2. If the carotid body has enhanced sensitivity to these rapid changes, as was found in SCI subjects in the current study, ventilatory overshoot and undershoot are likely to occur and perpetuate or worsen breathing instability (Smith and Dempsey, 2006).

Putative Mechanisms

This study demonstrated increased peripheral chemoreceptor activity and responsiveness in patients with SCI during wakefulness. I considered several potential mechanisms including age, gender, SDB and chronic intermittent hypoxia. There was

no difference in the age or gender distribution of the two groups. Similarly, both groups included patients with SDB. Chronic SCI patients are likely to suffer from repetitive episodes hypoxia over several years. Chronic intermittent hypoxia (CIH, intermittent hypoxia: IH), especially during sleep, results in the development of sensory long-term facilitation (LTF), which manifests as increased peripheral chemoreceptor activity AND enhanced propensity to develop LTF following acute episodic hypoxia (Peng, 2003a and 2003b; Prabhakar, 2011). Interestingly, patients with SCI demonstrate both features. Tester and colleagues (2014) demonstrated that chronic SCI subjects (cervical and thoracic), after being exposed to eight 2-minute episodes of IH, exhibited LTF for 30 minutes. Sensory LTF, secondary to CIH is a time-dependent phenomenon that is completely reversible over time following re-oxygenation. Sensory LTF does not depend on the severity of hypoxia used for IH conditioning and is not species specific. Enhanced LTF and increased peripheral chemoreceptor activity is consistent with CIH-induced sensory LTF (Figure 15) (Peng, 2003a; Prabhakar, 2011).

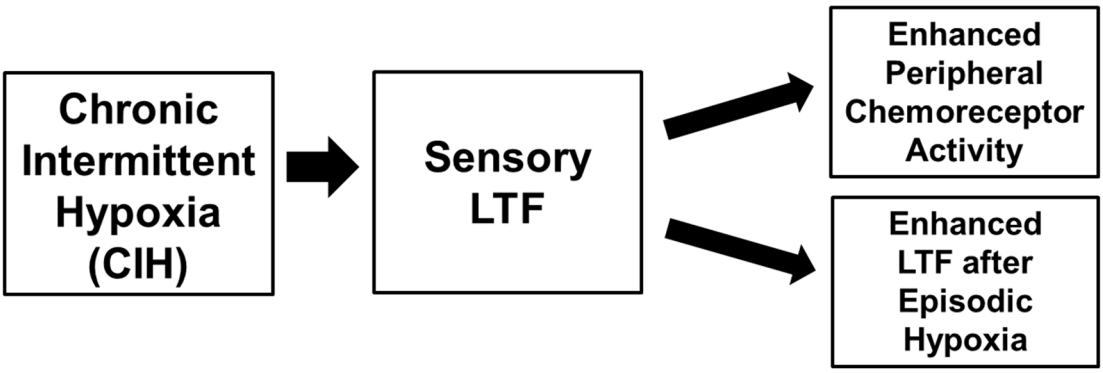

Figure 15. Chronic Intermittent Hypoxia and Sensory Long-Term Facilitation

A schematic representation of the process by which chronic intermittent hypoxia leads to long-term facilitation and enhancement of chemoreflex response to O_2 and CO_2 fluctuations. This is a proposed mechanism for breathing instability and sleep disordered breathing in chronic SCI.

Future studies to test peripheral chemoreflex responsiveness in chronic SCI subjects before and after treatment with CPAP, or an alternative treatment, to eliminate the CIH associated with hypoventilation and SDB, would allow investigators to test the hypothesis that CIH is a key mechanism in the development of augmented peripheral chemosensitivity in SCI.

FINAL CONCLUSIONS

Summary of Results

The results of my first aim (chapter 1) indicate that subjects with chronic cervical SCI subjects experience a greater magnitude of sleep onset hypoventilation (significant reduction in V_E, V_T, and increase in $P_{ET}CO_2$) compared to able-bodied subjects, which cannot be explained by upper airway mechanics (no significant change in upper airway resistance was found in either group). I have postulated that putative mechanisms include: 1) loss of intercostal innervation resulting in reduced respiratory pump musculature, 2) increased peripheral chemoreceptor gain which has been shown to cause increased breathing instability with sleep onset (Dunai et al., 1999) or, 3) decreased central respiratory drive (Manning et al., 1992).

The major findings of my second aim (chapter 2) are that subjects with chronic cervical and high thoracic SCI have a greater reduction in ventilation in response to brief hyperoxia compared to able-bodied subjects, and a heightened response to a single breath of CO_2, both indicators of increased peripheral chemoresponsiveness. In addition, peripheral chemoresponsiveness was not significantly correlated with AHI in SCI and AB subjects. From these results I conclude that SCI subjects have an increased peripheral chemoreceptor gain and a heightened reliance on the carotid body for maintenance of eupneic ventilation.

Clinical Significance

Both hypoventilation (Castriotta and Murthy, 2009) and high peripheral chemoreceptor gain (Dempsey, 2004) have been implicated in the development of SDB in the general population. SDB is associated with excessive daytime sleepiness, impaired cognition and increased morbidity and mortality due to effects on the cardiovascular system, which include: hypertension, coronary artery disease (CAD), pulmonary hypertension, heart failure, cardiac arrythmias and increased risk of stroke (Gopalakrishnan and Tak, 2001; Mohsen and Urbano, 2011; Chou et al., 2012; Johansson et al., 2012). The high prevalence of SDB in the SCI population makes the study of this disorder of critical importance. Investigating the underlying mechanisms, and contributing factors, as was done in the studies I have reported, will help in the development of targeted treatment that can mitigate the negative health effects of this disorder as well as improve quality of life for SCI patients.

Future Directions

Future studies to elucidate the mechanism of hypoventilation with sleep onset in SCI may include: 1) assessment of intercostal and diaphragm EMG during sleep onset to determine the contribution of respiratory muscles to over-all ventilation in SCI vs. able-bodied subject, 2) assessment of sleep onset ventilation with and without hyperoxia (as in Dunai et al., 1999), 3) assessment of peripheral chemoreceptor reflex response before and after treatment to alleviate sleep related hypoxia, 4) administration of P0.1 measurements during wake and sleep in SCI and able-bodied subjects to

assess central ventilatory drive, or 5) a trial of central respiratory stimulants (such as acetazolamide or theophylline) to determine if SDB improves when central drive in increased in SCI. While much is still left to learn about the etiology of SDB after SCI, the current studies have indicated 2 important contributing factors (sleep onset hypoventilation and high peripheral chemoreceptor gain) that point the way to future investigations.

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ABSTRACT

UNSTABLE VENTILATORY CONTROL DURING SLEEP AFTER HIGH SPINAL CORD INJURY: THE CONTRIBUTION OF CHEMOSENSITIVITY AND HYPOVENTILATION

by

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A high prevalence of sleep-disordered breathing (SDB) after spinal cord injury (SCI) has been reported in the literature; however, the underlying mechanisms are not well understood. My studies had 2 aims: 1) to determine the effect of the withdrawal of the wakefulness drive to breathe on the degree of hypoventilation in SCI patients and able-bodied controls and 2) to determine the response of the peripheral chemoreceptors to brief hyperoxia (60 seconds of >60% FiO_2) and hypercapnia (a single breath of elevated CO_2). I studied subjects with chronic cervical and high thoracic SCI and matched able-bodied subjects. For the first aim subjects underwent polysomnography, which included quantitative measurement of ventilation, timing, and upper airway resistance (R_{UA}) on a breath-by-breath basis during transitions from wake to stage N1 sleep. Compared to able-bodied controls, SCI subjects had a significantly greater reduction in tidal volume during the transition from wake to N1sleep (from 0.51±0.21 L to 0.32±0.10 L vs. 0.47±0.13 L to 0.43±0.12 L; respectively, $p<0.05$). Moreover, end-tidal CO_2 and O_2 were

significantly altered from wake to sleep in SCI (38.9±2.7 vs. 40.6±3.4 mmHg; 94.1±7.1 vs. 91.2±8.3 mmHg; respectively, $p<0.05$), but not in able-bodied controls (39.5±3.2 vs. 39.9±3.2 mmHg; 99.4±5.4 vs. 98.9±6.1 mmHg; respectively, p=ns). R_{UA} was not significantly altered in either group. In aim 2 SCI subjects had a greater reduction in ventilation with hyperoxia administration (63.9±23.0 % of baseline V_E) compared to able-bodied subjects (91.4±15.1 % of baseline V_E, $p<0.05$) and a higher ventilatory response to a single breath of CO_2 (SCI: 0.78±0.4 L/min/mmHg vs. able-bodied: 0.26±0.1 L/min/mmHg, $p<0.05$). In conclusion, individuals with SCI experience hypoventilation at sleep onset, which cannot be explained by upper airway mechanics and a high peripheral chemoreflex response to O_2 and CO_2. Sleep onset hypoventilation and high peripheral chemoresponsiveness may contribute to the development SDB in the SCI population.

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1995-2000	Western Michigan University, Bachelors of Science
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PUBLICATIONS

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