MECHANISMS IN THE PERCEPTUAL AND RESPIRATORY-RELATED EVOKED
POTENTIAL RESPONSE TO INSPIRATORY LOADS

By

WEIYING ZHAO

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2001
This dissertation is dedicated to my homeland, China, and to my dear parents and husband.
ACKNOWLEDGMENTS

First and foremost, I would like to express my gratitude toward my advisor, Dr. Daniel Martin, and my committee member Dr. Paul Davenport, for giving me the opportunity to work with them. Their enthusiastic support, great guidance, and fruitful discussions on many aspects of this dissertation are invaluable. My full appreciation also goes to the rest of my committee, Dr. Scott Powers and Dr. Orit Shechtman, for constantly providing me with valuable comments and suggestions during my dissertation work.

I would also like to thank some friends for their encouragement and support. They are Chienhui Huang, Weirong Zhang, and Toni Chirara etc.. Their involvement in my dissertation work has been very helpful. I will always cherish the fun and joy we have shared together.

Very special thanks also go to all the subjects who participated in my dissertation research experiments. It is their cooperativeness, patience and support that made this dissertation project successful.

Finally, I would like to thank my husband, Youzhong Liu; my mother, Shuqiu Tong; my father, Binjian Zhao; and my brother, Weixiong Zhao, for their encouragement and care during my graduate work.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTERS</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2 REVIEW OF RELATED WORK</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Ventilatory Responses to Mechanical Loads</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Types of Loads</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Ventilatory Responses to Added External Loads</td>
<td>5</td>
</tr>
<tr>
<td>2.1.3 Summary</td>
<td>7</td>
</tr>
<tr>
<td>2.2 Perception of Mechanical Loads</td>
<td>8</td>
</tr>
<tr>
<td>2.2.1 Psychophysical Methods</td>
<td>9</td>
</tr>
<tr>
<td>2.2.1.1 Load detection: threshold measurements</td>
<td>9</td>
</tr>
<tr>
<td>2.2.1.2 Magnitude estimation: scaling methods</td>
<td>10</td>
</tr>
<tr>
<td>2.2.1.3 Magnitude production</td>
<td>12</td>
</tr>
<tr>
<td>2.2.2 Perception of Added Mechanical Loads</td>
<td>13</td>
</tr>
<tr>
<td>2.2.2.1 Load detection</td>
<td>13</td>
</tr>
<tr>
<td>2.2.2.2 Ability to discriminate between added resistive and elastic loads</td>
<td>15</td>
</tr>
<tr>
<td>2.2.2.3 Magnitude estimation</td>
<td>16</td>
</tr>
<tr>
<td>2.2.3 Potential Sites and Neural Mechanisms Subserving Load Perception</td>
<td>18</td>
</tr>
<tr>
<td>2.2.3.1 Peripheral afferent mechanisms</td>
<td>19</td>
</tr>
<tr>
<td>2.2.3.2 Respiratory motor command corollary discharge</td>
<td>26</td>
</tr>
<tr>
<td>2.2.4 Summary</td>
<td>27</td>
</tr>
<tr>
<td>2.3 Respiratory-Related Evoked Potential (RREP)</td>
<td>28</td>
</tr>
<tr>
<td>2.3.1 RREP Methodology</td>
<td>29</td>
</tr>
<tr>
<td>2.3.2 RREP Components</td>
<td>32</td>
</tr>
<tr>
<td>2.3.2.1 Early-latency components</td>
<td>33</td>
</tr>
<tr>
<td>2.3.2.2 Late-latency components</td>
<td>35</td>
</tr>
<tr>
<td>2.3.3 Afferent Mechanism of RREP</td>
<td>38</td>
</tr>
<tr>
<td>2.3.3.1 Afferent activation of cerebral cortex and anatomical pathways</td>
<td>38</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4-1</td>
<td>Medications Listed by Double Lung Transplant Patients</td>
</tr>
<tr>
<td>4-2</td>
<td>Demographic Characteristics of Subjects</td>
</tr>
<tr>
<td>4-4</td>
<td>Latencies of RREP</td>
</tr>
<tr>
<td>4-5</td>
<td>Amplitudes of RREP</td>
</tr>
<tr>
<td>4-6</td>
<td>Contrast Analysis for RREP Amplitudes in Different Scalp Regions</td>
</tr>
<tr>
<td>4-7</td>
<td>Mouth Occlusion Pressure</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3-1</td>
<td>Load Detection Apparatus</td>
</tr>
<tr>
<td>3-2</td>
<td>Magnitude Estimation Apparatus</td>
</tr>
<tr>
<td>3-3</td>
<td>Apparatus for RREP</td>
</tr>
<tr>
<td>3-4</td>
<td>Internaltion 10/20 system scalp position</td>
</tr>
<tr>
<td>4-1</td>
<td>Detection Threshold</td>
</tr>
<tr>
<td>4-2</td>
<td>Weber Fraction</td>
</tr>
<tr>
<td>4-3</td>
<td>Detection Latency during Resistive Loading</td>
</tr>
<tr>
<td>4-4</td>
<td>Detection Percent during Resistive Loading</td>
</tr>
<tr>
<td>4-5</td>
<td>Peak Mouth Pressure during Load Detection</td>
</tr>
<tr>
<td>4-6</td>
<td>Peak Volume during Load Detection</td>
</tr>
<tr>
<td>4-7</td>
<td>Peak Air Flow during Load Detection</td>
</tr>
<tr>
<td>4-8</td>
<td>Inspiratory Duration during Load Detection</td>
</tr>
<tr>
<td>4-9</td>
<td>Expiratory Duration during Load Detection</td>
</tr>
<tr>
<td>4-10</td>
<td>Time to Peak Air Flow during Load Detection</td>
</tr>
<tr>
<td>4-11</td>
<td>Breathing Frequency during Load Detection</td>
</tr>
<tr>
<td>4-12</td>
<td>Minute Ventilation during Load Detection</td>
</tr>
<tr>
<td>4-13</td>
<td>Handgrip Response to Resistive Load</td>
</tr>
<tr>
<td>4-14</td>
<td>Handgrip Response to Mouth Pressure during Resistive Loading</td>
</tr>
<tr>
<td>4-15</td>
<td>Signals of RREP during Control Trial in a Double Lung Transplant Patient</td>
</tr>
</tbody>
</table>
4-16 Signals of RREP during Control Trial in a Normal Subject.................................71

4-17 Latency of P3 (* indicates significant trial effect (attend vs ignore, p < 0.05). ** indicates significant group effect (DLT vs NOR, p < 0.05))............................................................74

4-18 Central Process Time (* indicates significant trial effect (attend vs ignore, p < 0.05). ** indicates significant group effect (DLT vs NOR, p < 0.05)) ...............................................74

4-19 Mouth Occlusion Pressure (Grand Average) Trace During Attend Trial.............76

4-20 Mouth Occlusion Pressure (Grand Average) Trace During Ignore Trial ..............77

5-1 Breathing Pattern During Resistive Load Detection in a Normal Subject...............86

5-2 Breathing Pattern during Resistive Load Detection in a Double Lung Transplant Subject..................................................................................................................87

5-3 Breathing Pattern and Handgrip Response during Magnitude Estimation in a Normal Subject..................................................................................................................93

5-4 Breathing Pattern and Handgrip Response during Magnitude Estimation in a Double Lung Transplant Patient ..........................................................................................96

5-5 Relationship between Weber Fraction and Slopes of Magnitude Estimation ............99

5-6 Response of P3 in a Double Lung Transplant Recipient during Attend Trial ...........105

5-7 Response of P3 in a Normal Subject during Attend Trial ........................................105
Human beings are able to have conscious awareness of some aspects of their breathing. The perception of respiratory mechanical events, including load detection and magnitude estimation, has been studied extensively by using psychophysical methods. Recently, the respiratory-related evoked potential (RREP) has been used to provide a unique way to investigate the neural activities associated with breathing against mechanical loads. However, the afferent pathways mediating load perception and RREP are still largely unknown. The major goal of this study was to investigate the role of lung vagal afferents in perceptual and RREP response to inspiratory loads.

Using human double-lung transplantation as a model of pulmonary denervation, we studied breathing pattern, load detection and magnitude estimation during breathing against inspiratory resistive loads and RREP in response to inspiratory occlusions. Two groups of subjects, double lung transplant recipients (DLT, n = 10) and matched normal subjects (NOR, n = 12), completed this experiment. Results demonstrated that the
breathing patterns during unloaded and loaded breathing were similar in both groups. Handgrip responses, as well as the slope of logHG%-logPm and logHG%-logR were also comparable in the two groups during the magnitude estimation experiment. However, the detection threshold and Weber Fraction were significantly elevated in the DLT group as compared with the NOR group (2.91 ± 0.5 cmH₂O/L/s vs 1.55 ± 0.3 cmH₂O/L/s, and 0.50 ± 0.1 vs 0.30 ± 0.1, respectively). Moreover, despite a similar early-latency RREP response, DLT had significantly delayed and attenuated P3 response. These results suggest that lung vagal afferents are involved in cognitive processing of respiratory stimuli during breathing against mechanical loads, but are not essential to load perception and RREP response. Respiratory sensation related to loaded breathing may be due to multiple and simultaneous sensory input.
CHAPTER 1
INTRODUCTION

Human beings are able to consciously control their breathing and have conscious awareness of some aspects of their breathing. The fact that the sensations are appreciated at a conscious level means that there are pathways for information to be transduced from peripheral receptors and transmitted to the cerebral cortex. However, the afferent mechanisms and pathways involved in load perception and how the information is processed in the higher brain center are still uncertain.

Many types of respiratory sensations have been studied, including chest tightness, sense of effort, air hunger, etc.. Among them, the most extensively studied are the sensations associated with breathing against external mechanical loads. The perception of respiratory mechanical events is dependent on two processes (48): load detection and magnitude estimation. These have been studied by using psychophysical methods in both humans and animals (44, 83, 84, 144, 150). Although it is thought that the afferents that signal load perception are primary located in the respiratory muscle pump and are related to the motor drive (156), the role of other potential afferent mechanisms, like lung afferent mechanisms, remains unknown.

The respiratory-related evoked potential (RREP) was first recorded over the somatosensory region of the cortex during inspiratory occlusion in adults (45). Respiratory-related evoked potential provides a unique way to investigate the neural mechanisms mediating respiratory load perception. However, the afferents eliciting this evoked potential are still unknown. Vagal nerve stimulation has been reported to elicit
evoked potentials in the cerebral cortex of cats (6, 7, 91, 131) and monkeys (104). So it is possible that respiratory-related afferent signals from the lung are responsible for a portion of the RREP.

The major goal of this research is to determine the role of the lung vagal afferent system in perceptual and RREP responses to inspiratory loads. Lung transplantation recipients provide a good model to clarify the role of lung and lower airway receptors in respiratory sensation since all the afferent traffic from receptors located distal to the surgical anastomosis are interrupted. In this dissertation, load detection, magnitude estimation, and both early and late components of the RREP in double lung transplant patients were compared with matched normal subjects. The results of this study will provide new information on the relationship between the selected afferent respiratory mechanoreceptor populations and mechanical load perception, and the component peaks of the RREP.
2.1 Ventilatory Responses to Mechanical Loads

Ventilation of the lung is a mechanical process (48). Respiratory muscles produce the pumping force to move air in and out of the lung. Any acute changes in the mechanical load, either from intrinsic mechanical changes, or from application of extrinsic mechanical load, will alter this mechanical process and result in a load compensation response. Load compensation is usually studied through the use of external mechanical loads. This section will first introduce different types of external loads and their relationship with intrinsic load change in some disease models, and then review the literature that related to the ventilatory responses to external loads.

2.1.1 Types of Loads

An extrinsic resistive load is applied by breathing through a breathing circuit which consists of a rigid tube with a series of side ports and interposing filters between these ports (154). The filters can be made of filter paper, stainless steel wire screen or sintered metal filters. The filter material and the manifold design should provide pressure-flow characteristics that are linear over the range of the flow rate expected in any study (156). For resistive loads, the pressure generated by respiratory muscles is a function of flow rate. For a given load, the respiratory muscles have to generate more pressure if the airflow rate is increased. During the breathing cycle, the load is greatest at mid-inspiration when flow is the largest.
The elastic load circuits consist of a series of airtight, rigid containers (drums or boxes) of various known volumes (150, 154). The smaller the volume is, the greater the elastance will be. Elastic loads alter the pressure-volume relationships. For each given load, the opposing pressure is a function of the volume. Thus, during a breathing cycle, the pressure generated by inspiratory muscles is greatest at end-inspiration when the inspiratory volume reaches the peak.

Unlike resistive and elastic loads, the pressure generated by respiratory muscles to oppose a threshold load is independent of airflow or volume. This is because of the use of a valve in the breathing circuit. The valve requires a certain pressure to remain open (148). When it opens, it offers little extrinsic resistive or elastic opposition to air movement. Once pressure decreases below the threshold level, the valve closes. Therefore, such a device provides a constant load to inspiratory or expiratory muscles (depending on the phase in which they are applied).

Airway occlusion, also known as a static load (71), is another type of load. The inspiratory port of the breathing circuit is connected with a balloon occlusion assembly (118) or a T-shaped occlusion valve (71). Change in mouth pressure is the result of inspiration against a closed airway applied at the onset of the breath (45), or during the inspiratory phase (inspiration interruption method) (42), or at mid-inspiration (118). Occlusion of the airway imposes an infinitive load on the respiratory system because there is no airflow and volume changes despite the pressure generated by respiratory muscles.

From a clinical perspective, resistive loads closely mimic the increased airway resistance and flow limitation experienced in patients with asthma or chronic obstructive
pulmonary disease (COPD) (21, 71). Elastic loads mimic the effects of lung stiffness experienced by patients with restrictive lung disease (21, 71). An external inspiratory threshold load is very similar to what happens with dynamic hyperinflation. In the latter case, inspiratory muscles must generate enough pressure to counteract the elastic recoil at end-expiration before flow begins. This positive elastic recoil is termed as intrinsic positive end-expiratory pressure (PEEPi) (120). Extrinsic mechanical loads are similar to intrinsic loads to elicit ventilatory and perceptual responses. Therefore they remain a valid tool to study ventilatory and perceptual responses to mechanical loads. However, caution still should be taken when using external mechanical loads to mimic respiratory disease.

2.1.2 Ventilatory Responses to Added External Loads

The ventilatory responses to external mechanical loads are dependent on factors such as species, anesthetic state, duration of the loads, and, most importantly, the type of loads (148). It was demonstrated that minute ventilation decreased when breathing against a resistive load in conscious humans (53). Both inspiratory duration (TI) and tidal volume (VT) were decreased, accompanied by a decrease in mean inspiratory flow rate (VT/TI) (39, 53, 112). VT was also decreased when breathing against an elastic load (59, 112). However, the TI response between elastic and resistive loads is different, with the former tending to result in a shorter TI (59, 112). These ventilatory responses are well preserved with sustained load application (148). The breathing pattern is adjusted to minimize the work of breathing (106). Reducing breathing frequency could lessen work of breathing when airway resistance is increased, while a shallower tidal volume will minimize work of breathing when the elastic forces opposing ventilation become greater.
It is also possible that those load compensatory responses are intended to minimize dyspnea during loaded breathing (33).

The changes in the breathing pattern described above are probably mediated through activation of the afferent systems. In unanesthesized subjects, the entire afferent mechanisms, including lung vagal afferents, respiratory muscle afferents, upper airway afferents, chemoreceptor afferents, etc., could be the potential sites to induce load compensation responses (148). A variety of diseases interfere with afferent mechanisms directly or through medical interventions, which provide an opportunity for investigating those mechanisms. Patients with transection of the lower cervical cord are devoided of afferent feedback from diaphragm, rib cage and/or abdominal wall, depending on the level of transection. Despite preserved ventilation (76), prolongation of TI was found depressed when breathing against resistive loads and elastic load in those quadriplegic subjects (8, 11, 81). However, the abnormal response may be related to their motor deficit rather than to deafferentation (10). Tracheostomy excludes afferent systems in the upper airway, including the mouth, nose, pharynx, larynx and upper extrathoracic trachea. O’Donnell et al. (105) studied the response to inspiratory and expiratory resistive loads and to elastic loads in patients with tracheostomy. They found that ventilatory responses in those patients were qualitatively similar and quantitatively not significantly different from those of normal healthy controls. The results suggested that loading responses to conventional mechanical loads are preserved in the absence of afferent information from the upper airways.

Patients with lung transplantation offer an opportunity to study the role of vagal afferents in resting breathing pattern and load response. Shea and co-workers (129)
compared resting breathing pattern in heart-lung transplant patients and heart transplant patients, as well as in normal subjects. They found no difference in ventilation, VT, frequency, TI and expiratory time among all three groups during wakefulness and sleep. But several other studies have reported elevated frequency and reduced TI in lung transplant recipients (88, 122). Since the lung transplant subjects in those studies had a restrictive spirometric pattern, the reduced TI and greater frequency of breathing is probably related to the presence of underlying pulmonary restriction instead of the result of lung deafferentation. The load compensation response was studied by Peiffer et al. (108). They reported that despite high intersubject variability, the ventilatory responses differed significantly between lung transplant recipients and controls when breathing against resistive loads, with the former group producing higher peak mouth pressure and inspiratory flow rate. However, in their protocol, the load was applied after a short vocal cue. Therefore, the subjects’ breathing patterns might be changed due to the effect of cue.

2.1.3 Summary

There are four kinds of external mechanical loads: resistive load, elastic load, threshold load and airway occlusion. The load response varies depending on the type of the load, with TI prolongation observed when breathing against resistive load and VT decreased for elastic load. The breathing pattern is adjusted to reduce the work of breathing and dyspnea during loaded breathing, as well. The afferent mechanisms involved in the load compensation response are still unclear. Although differences in resting breathing pattern and load response have been reported in lung transplant recipients, such a difference might not be entirely due to lung vagal deafferentation, because of the abnormal lung function in the patients. In the present proposal, the author plans to study the resting breathing pattern and the breathing pattern associated with
inspiratory resistive loads in double lung transplant patients and matched normal subjects. Lung function will also be measured in all subjects. Two hypotheses were tested in this portion of research:

1. The resting breathing pattern is similar between double lung transplant patients and matched normal subjects.
2. The loaded breathing pattern is also similar between double lung transplant patients and matched normal subjects.

### 2.2 Perception of Mechanical Loads

Respiration is unique because it is regulated not only by automatic centers located in the brainstem but also by voluntary signals initiated in the cortex (5). For the most part, the regulation of breathing is an automatic process controlled by the brainstem respiratory center, which acts as an intrinsic oscillator that sets the basic pattern and rate of activity of the respiratory muscles. The activity of the respiratory controller is modified by two principal afferent feedback systems: chemoreceptors in the blood and brain, and mechanoreceptors in the airway, lung and respiratory muscles. Those peripheral inputs adjust the brainstem respiratory center activity to meet metabolic and mechanical demands. Furthermore, this automatic regulation process can be modified or overridden by willful actions controlled by the forbrain. Associated with this forebrain motor control is conscious perception of respiratory sensations.

Psychophysics is the scientific study of the relationship between stimulus and sensation. Psychophysical studies demonstrate that subjects can sense position, pressure, volume, and a broad general appreciation of the appropriateness of the act of breathing. This section will first review some basic psychophysical methods that have been used to quantify respiratory sensations associated with mechanical loads. Then the two processes involved in load perception, load detection and magnitude estimation, will be reviewed.
Finally, the potential afferents mechanism mediating load perception will be discussed. For more details see the general reviews by Lansing and Banzett (92) and Zechman and Willey (156).

2.2.1 Psychophysical Methods

2.2.1.1 Load detection: threshold measurements

Load detection is usually studied by threshold measurements, which is concerned primarily with determining the limits of sensory performance. There are two kinds of thresholds: absolute threshold and difference threshold. Absolute threshold is the smallest stimulus that the subject can sense. Difference threshold is the smallest change in a stimulus that a subject can detect. The difference lies in whether to include the zero-stimulus condition in the final result.

For most sensory modalities, including respiratory sensation, the change in stimulus intensity that can just be perceived ($\Delta \phi$) is a constant fraction of the initial or basal intensity of the stimulus ($\phi$) over some limited range. This relationship ($\Delta \phi/\phi = K$) is known as Weber’s law. $K$ (Weber fraction) is a constant, which differs for different sensory modalities. Since the method to remove or negate the effect of the intrinsic elastance and resistance of the pulmonary system has not been developed, absolute thresholds cannot be determined. Therefore, studies of threshold measurement have focused on determining difference thresholds of load detection (156). The difference threshold is a statistical average based on repeated presentations of a range of stimuli intensities, which is usually defined as the stimulus level that evokes a positive response 50% of the time (92). Because the background load is a variable that could affect the
difference threshold (144), the Weber fraction provides a better index than a single difference threshold to assess subject performance in detecting added loads to breathing.

There are numerous non-stimuli related factors that can affect the detection threshold. Those factors include the variations in the testing procedure (such as instructions, practice, stimulus order, the opportunity to preview stimuli, feedback on success of discrimination, etc.) and in subjects (including the subject’s attention, motivation, personality, etc.). These potential factors, which might induce response bias, could be held constant, if not eliminated, through the use of highly experienced observers and standard testing procedures (92).

2.2.1.2 Magnitude estimation: scaling methods

Scaling methods assess the ability of subjects to judge the magnitude of sensations produced by suprathreshold stimuli. Numerous scales are used in respiratory sensation studies. Some scales consist of familiar words arranged in a fixed order of increasing intensity; some scales use numbers to represent perceived intensity; some use a combination of verbal and numerical categories (e.g. Borg scale); while some other scales use another sensory modality to match the sensation of loaded breathing. The main scaling methods that have been employed to study respiratory sensation will be briefly described here.

Numerical scale. This method requires the subject to make numerical estimations of the sensory magnitudes generated by a series of suprathreshold stimuli. The numerical estimates can be based on a closed scale (82, 89) or an open scale (24, 84). For the closed scale method, the subject is presented with a reference stimulus and told that the sensation it produces has a certain numerical value. Stimuli are presented during the trials, and the subject assigns numbers to the resulting sensations relative to the modulus.
By comparison, an open scale is defined by the subject, and there is no limitation on the numbers that can be used. In either scale, the average of the numbers assigned to a particular stimulus is the psychological scale value for that stimulus.

The relationship between perceived magnitude and stimulus intensity is commonly described by a power function: $\psi = K\phi^n$, where $\psi$ is the sensation magnitude, $\phi$ is the stimulus intensity, $K$ is a constant, and $n$ is the exponent that depends on the stimulus conditions and sensory modality. This power function is termed Steven’s Power law. In practice, the exponent $n$ is often determined by plotting $\log{\psi}$ versus $\log{\phi}$ and determining the slope of the resultant regression line. This index is a measure of the sensitivity of the subject to the stimuli and is thought to reflect underlying properties of neural processing (134). The value of $\psi$ can vary a lot between subjects with such factors as the subject’s choice of how to use the scale, experimental conditions, background noise, etc.. However, it is quite stable in an individual subject under the same conditions (92).

**Category scale.** Category scales consist of familiar words arranged in a fixed order of increasing intensity (e.g., “none”, “slight”, “moderate”, “strong”, etc.). The subject is required to choose the category which most closely corresponds to his/her estimation of the sensory magnitude. The subject’s selections are limited by the words that anchor the extremes and define the steps between them (92). Adding numbers to each verbal category increases the consistency of the subject response. The combined verbal-numerical category scale, such as the Borg scale, is now widely used to study respiratory sensations (23).
Cross-modality matching. For this method, the subject matches the sensations produced in two different sensory modalities. One of the most frequently used modalities for expressing the magnitude sensation when breathing against an external load is handgrip tension (26, 99). Subjects are asked to squeeze a handgrip dynamometer to produce sensations of tension that match the magnitude of respiratory sensation in response to particular stimuli. A graph called an equal-sensation function is constructed by plotting the stimulus values of one modality against the stimulus values of the other modality that result in judgments of equal sensory magnitude (156). The equal sensation function is consistent with the power law model.

2.2.1.3 Magnitude production

In contrast to magnitude estimation methods, magnitude production requires the subject to manipulate the intensity of a stimulus (e.g. volume, pressure, ventilation) to match the subjective magnitude of a numerical value provided by the experimenter (3, 146).

In summary, all the above psychophysical methods used for respiratory sensation aim to quantify the relationship between sensory experience and the physical stimuli that evoke them (i.e., mechanical loads). Some special features of the respiratory system, such as double control (automatic and behavioral), large number of simultaneous afferent sources, language variability, etc., should be taken into account in psychophysical studies (92). Despite these difficulties, psychophysical methods provide good measures of quantification of perceptual response, and contribute to our understanding of respiratory sensory processing.
2.2.2 Perception of Added Mechanical Loads

Many types of respiratory sensations have been studied, such as chest tightness, sense of effort, air hunger, tracheobronchial irritation and excessive ventilation, etc. Studies have shown that subjects can report changes of tidal volume as small as 50-100 mL (63, 79, 146). An increase of 3-7 torr in end-tidal PCO2 can also be detected and reported as “urge to breathe” (15, 16). Gandevia et al. (60) found that the threshold for detection of a negative pressure applied at the mouth during active inspiration is about 0.5cmH2O but is elevated to about 1.0-1.5cmH2O after closing the glottis. The magnitude estimation of these respiratory sensations fit Stevens’ psychophysical power law (12).

Among all the respiratory sensations that have been studied, the most extensively studied are the sensations associated with external mechanical loading to breathing. This section will focus on research about the perception of added mechanical loads. The perception of respiratory mechanical events is dependent on two processes (48). The first is the detection of the change in breathing pattern, which has been studied primarily by using difference threshold methods. The second perceptual process includes differentiation of the load type and estimation of the load magnitude by using scaling methods.

2.2.2.1 Load detection

Campbell and his colleagues (27) were the first to study the ability of humans to detect added elastic loads to breathing. A series of graded elastic loads (1.4 – 4.9 cmH2O/L) were applied to inspiration. The load magnitude detected 50% of the time was defined as the detection threshold and was about 2.47 cmH2O/L. Subsequently, Zechman and Davenport (150) reported a detection threshold of 1.03 cmH2O/L for elastic loads ranged from 0.48 to 5.27 cmH2O/L. It has been suggested that the difference in the
threshold values between these two studies might be due to the difference in the breathing circuit used to deliver elastic loads. The value reported by Campbell et al. (27) might be overestimated because some of the loaded inspirations were initiated with circuit pressures above atmospheric pressure, resulting in the actual load less than the calibrated value (156). The detection of resistive loads has been studied much more extensively. Bennett et al. (19) found that the detection threshold was about 0.59 cmH2O/l/s when a series of 5 resistive loads with a range of 0.40 – 0.98 cmH2O/L/s were added to breathing. Wiley and Zechman (144) studied the relationship between the background resistance and the detection threshold. They found that the mean detection threshold was about 0.6 cmH2O/L/s under a variety of situations with different background resistance.

Difference thresholds have been shown to be most reliable when they are expressed as a fraction of the background resistance (Weber fraction). Wiley and Zechman (144) designed a series of studies to examine the effects of background resistance on the detection threshold. The background resistance included the subject’s intrinsic resistance and the resistance of the breathing apparatus. For various experimental conditions with different background resistance, the Weber fraction remained constant (0.25-0.3) despite a large variability in the reported difference threshold values. The influence of increased background elastic loads on load detection ($\Delta E_{50}$) has not been systematically studied. Based on the values of reported threshold and the normal range of total elastance for the respiratory system, a Weber fraction of 0.1-0.2 is expected (156). The interactions between resistive loads and elastic loads have been studied by Shahid et al. (127, 128). They found that added background resistive loads did not significantly alter the thresholds for detection of added elastic loads. However, added
background elastic loads impaired the detection of added resistive loads. The value of detection threshold for resistive loads ($\Delta R_{50}$) increased with increased background elastance. These phenomena could be explained by the temporal difference in the pattern of resistive- and elastic-load information (25, 155).

The latency of detection ($T_{\text{det}}$), the time required for a subject to signal the perception of a load, is dependent on the magnitude and the type of the load. For both resistive and elastic loads, $T_{\text{det}}$ decreases with increasing load magnitude. $T_{\text{det}}$ for the resistive load occurred in mid-inspiration near peak inspiratory flow, whereas $T_{\text{det}}$ for elastic loads occurred significantly later near the end of breathing when the inspired volume reached the peak (150). These findings suggest that resistive loads and elastic loads generate different patterns of load information: resistive load information depends on load magnitude and flow, whereas elastic load information depends on load magnitude and volume. Killian et al. (86) compared $\Delta R_{50}$ under control conditions with that obtained when loads were applied at different times in inspiration with different inspiratory flows, at different lung volumes, and with different background loads. Their results suggested that external resistive load detection was subserved by the relationship between pressure and flow over the early part of inspiration, rather than changes in pressure itself. All these studies imply that the load specific breathing pattern is very important for load detection (48).

### 2.2.2.2 Ability to discriminate between added resistive and elastic loads

The temporal difference in the breathing pattern for resistive and elastic loads might enable subjects to discriminate between these two loads. Zechman et al. (154) found that when the load is very close to threshold, it was very difficult for a subject to
discriminate correctly between the two types of loads. However, the group mean scores for correct identification increased significantly to 77% and 82% for the moderately high suprathreshold resistive and elastic loads, respectively. The patterns of perceived loads expressed by handgrip tension were different between these two types of loads: for elastic loads, there was a progressive rise in grip response that reached maximum at the end of inspiration, whereas the maximal handgrip response appeared at mid-inspiration for resistive loads. These grip responses were consistent with the previously reported difference in Tdet responses to resistive load and elastic load, and were more marked when the load magnitude was higher.

2.2.2.3 Magnitude estimation

Many studies have examined the relationship between the magnitude of perceived load and the intensity of the load (26, 66, 84, 137, 138, 146). Subjects are usually exposed to a series of suprathreshold loads and asked to provide an estimate about the magnitude of the load by using numerical scales, or cross-modality matching. The collective results of these studies have shown that increasing load intensity is associated with increased perceptual estimate about the load magnitude. There is a linear relationship between the load magnitude and the perceptual estimate of the load when a log-log transformation is made. The slope of the line, which is also the exponent of the psychophysical power function, is a measure of the sensitivity of the subject to the stimulus. The exponent for elastic loads is higher than that for resistive loads. In normal subjects, the mean exponent for added resistive loads was 0.80 +/- 0.35, and 0.99 +/- 0.45 for elastic loads using a numerical scale (84). Despite a wide intersubject variation, the intrasubject variation was much less, and individual exponents for elastance and resistance were highly correlated (r = 0.92) (84).
Breathing against an external mechanical load will change the breathing pattern in a load-specific manner. Inspiration against a suprathreshold load results in a reduction of airflow for a resistive load and volume for an elastic load (150). Such changes in breathing pattern will affect the magnitude estimation. Killian et al. found that when the subjects were required to maintain flow when breathing against resistive loads and fix tidal volume during addition of elastic loads, there was an increase in the exponents (84). They later found that when resistive, elastic and mixed resistive and elastic loads were presented while inspiratory duration was kept constant, the perceived magnitude was directly related to airway pressure \((r=0.99)\) irrespectively of the type of the load (83). When inspiration is interrupted with a resistive load for only a portion of inspiration, there is a reduction in the perceived magnitude compared with when the load was applied at the onset of inspiration, with no change in slope (90). These studies imply that both spatial and temporal processes are involved in the perception of suprathreshold loads (48).

Magnitude estimation might also be affected by background loads. Revelette et al. (119) found that increased background loading was associated with a significant increase in the exponent for magnitude estimation for resistive loads. Adjustment of the stimulus scale by subtracting the difference in peak mouth pressures generated during resting breathing between control and background-loaded conditions results in a similar exponent obtained during background-loaded condition to control. It was thus suggested that an increase in detection threshold, produced by background load, is responsible for the increase in exponent for magnitude estimation. However, Burdon et al. (24) found that increased background resistance or elastance did not change either the exponent or
the intercepts of the power function between peak inspiratory pressure and the perceived magnitude. The difference between these two studies might be due to different methods employed.

In summary, the perception of respiratory mechanical loads involves load detection, load differentiation and magnitude estimation. Both resistive and elastic loads cause mechanical changes in a load-related pattern. Changes in breathing pattern play an important role in load perception. The background load is also important in load detection. The Weber fraction is constant (0.25-0.3) despite a large variability in detection thresholds reported in previous studies. However, the effect of the background load on magnitude estimation remains uncertain.

2.2.3 Potential Sites and Neural Mechanisms Subserving Load Perception

Increases in inspiratory extrinsic load changes the pattern of airflow, volume, and pressure in the respiratory system. These mechanical changes may be transduced by afferents from the mouth, airways, and the respiratory muscles. There are a variety of potential sensory mechanisms that contribute to respiratory sensation when breathing against a mechanical load. These include sensory information from peripheral afferent pathways, such as receptors in upper airway, lungs, respiratory muscle, etc.. Afferent information about outgoing motor command from within the central nervous system, which is termed corollary discharge, is also a potential mechanism. Finally, the dissociation between the motor command and the mechanical response of the respiratory system may be involved in the perceptual process, as well. It is difficult to determine the specific afferent mechanism mediating the perception of mechanical loads because load-dependent mechanical changes are transmitted throughout most of the respiratory tract and pump. Up to now, the neural systems and mechanisms subserving this respiratory
awareness are largely unknown. The following section will provide a brief summary about these potential mechanisms that mediate respiratory perception of mechanical events.

2.2.3.1 Peripheral afferent mechanisms

**Upper airway.** A variety of mechanoreceptors reside in the upper airway, including the mouth, nasal passage, pharynx, larynx and upper extrathoracic trachea. Application of external loads to the airway increases the transluminal pressure gradient across the airway. This changes wall configuration and consequently modulates the sensory information arising from those upper ways. However, the role of receptors in the upper airway remains somewhat equivocal.

Aerosolized local anesthetic is sometimes used to eliminate upper airway receptor activation. Chaudhary and Burki (29, 30) have shown that airway anesthesia in normal subjects does not alter the detection thresholds for either resistive or elastic loads. Later, Burki et al. (26) used a series of suprathreshold elastic and resistive loads to study magnitude estimation under airway anesthesia. They found that the exponent of the power function between load and handgrip response was not altered significantly during the airway anesthetized state for either load. These results suggest that upper airway receptors are not essential for detection and magnitude estimation of extrinsic mechanical loads. However, in their methods, lidocaine inhalation anesthesized both the upper and lower airways. It is difficult to differentiate the role of the upper airway from lung receptors in those studies. Furthermore, it is unknown whether the anaesthetics penetrate deeply enough to affect non-epithelial mechanoreceptors (130). Similarly, Fitzpatrick et al. (57) found that there was no significant difference in detection thresholds after blocking superior laryngeal nerve, suggesting that the larynx was not an important site for
resistive load detection. On the contrary, Davis et al. (51) found that when intra-oral sensation was considerably reduced by local anesthesia, there was a further impairment of detection of resistive loads in patients with upper spinal cord lesions, indicating that oral mechanoreceptors at least partially contributed to respiratory sensation.

Tracheostomy bypasses the afferent systems in upper airway, thus providing an opportunity to examine the role of upper airway mechanoreceptors in load perception. Noble et al. (100, 103) studied resistive load detection in patients breathing through traechostomies and found that these patients were able to detect loads as well as normals. When the upper airway receptors were exposed to the load by deflating the tracheostomy tube cuff there was little improvement in detection threshold. The investigators therefore conclude that the upper airway is not a sensitive detector of added load. However, these patients had various degrees of obstructive disease, and no measurements of base-line respiratory mechanics were made, which make it difficult to interpret these findings (156).

Younes and collegues (149) have shown that loads confined to the chest wall were not detected as well as loads applied to the extrathoracic airways, suggesting that upper airway mechanoreceptors are more sensitive than chest wall receptors. In contrast, Gandevia et al. (60) showed that pressure swings were not detected as well when confined to the oropharynx as when the glottis was open, suggesting that upper airway mechanoreceptors are less sensitive than lower airway receptors.

An animal model of resistive load detection was developed by Davenport et al. (44). They found that dogs with a tracheal stoma could reliably respond to inspiratory resistive loads and occlusion. Their detection thresholds and Weber fractions were found
to be similar to humans. So it appears that afferents in the mouth, nose, pharynx, larynx and upper extrathoracic tracheal are not mediating load detection in these dogs.

In summary, the contradictory findings from these studies and a general lack of baseline measurements make it difficult to determine the role of upper airway receptors to respiratory load perception.

**Lung and lower airway.** There are a variety of mechanoreceptors located in lung and lower airways, which discharge in response to volume and pressure changes. These receptors are innervated by the vagus nerves. They respond rapidly to mechanical changes, allowing for reflex adjustment of respiratory timing and muscle activity. At the same time, afferent information from those receptors may also contribute to respiratory perception of external mechanical loads.

Two strategies have been adopted to determine the role of pulmonary receptors in respiratory load perception: either the principal afferent nerve (vagus nerve) is selectively blocked; or alternatively, all other possible sources are eliminated leaving only the vagi intact. Guz et al. (67) studied the effect of bilateral block of the vagus and glossopharyngeal nerves in two healthy subjects. The difference threshold for elastic load detection was not affected by the nerve block. Furthermore, there was no change in the sensation associated with a high resistive load in one subject. These results suggest that pulmonary vagal afferents do no contribute to load detection. Similarly, Chaudhary and Burki (26, 29, 30) also showed that upper and lower airway anesthesia in normal subjects did not alter the detection thresholds and exponents for magnitude estimation of either resistive or elastic loads. However, it is possible that some pulmonary stretch receptors
may escape anesthesia because the anaesthetic could not penetrate to the smooth muscle or because the drug was carried away rapidly by the rich blood flow (14).

High-level quadriplegic subjects with tracheostomy provide indirect evidence about the role of pulmonary afferents in respiratory sensation, because both respiratory muscle afferents and upper airway receptors are ruled out, leaving only the pulmonary receptors intact. Studies reported that these patients could reliably detect changes in tidal volume as little as 100 mL, which was comparable to that of normal subjects (15). In contrast, a subsequent study by Lansing et al. demonstrated that volume perception in a tracheostomized C1-C2 tetraplegic patient was abolished by airway anesthesia (93). These data suggest that pulmonary stretch receptors can provide conscious perception of volume, at least in the absence of all other signals. However, their role in extrinsic load perception remains unknown.

Lung transplantation recipients provide a good model to clarify the role of lung and lower airway receptors in respiratory sensation because all the afferent traffic from receptors located distal to the surgical anastomosis are interrupted. Both vagal and sympathetic nerves are denervated in such a model. Tapper et al. (139) compared the detection threshold of inspiratory resistive load in heart-lung transplant recipients and normal subjects. There was no significant difference in the Weber fraction associated with a 50% probability of load detection (0.32±0.05 and 0.34±0.05 in the transplant patients and normal subjects, respectively). It was also shown that there was no difference between the normal control and lung transplant groups in their ability to reproduce either VT or more than 50% inspiratory capacity, while there was a difference between the two groups in their ability to spontaneously double VT (109). However, the interpretation of
such differences is confounded by a large variation in lung volume doubling observed among normal subjects (123). Peiffer et al. (108) studied magnitude estimation of inspiratory resistive loads in lung transplant patients and matched control subjects. They found that the slope of the linear relationship between the Borg scores and peak inspiratory mouth pressure were significantly lower in lung transplant recipients. Their results suggest that pulmonary afferent nerves may contribute to the sensory responses to external loads. However, the difference in the slope may be solely due to the difference in peak mouth pressure and the range of mouth pressure between the two groups, instead of the absolute Borg scales.

Sakurai et al. (121) used cats to study the role of pulmonary vagal afferents in relief of respiratory distress developed during airway occlusion. After vagotomy, the effect of lung expansion on the duration from the start of airway occlusion to the onset of the positive response (DOCCL) was totally abolished, supporting the contribution of vagal afferents in respiratory perception.

In summary, there is still no uniform support for an exclusive role of pulmonary afferent receptor input in the development of respiratory perception of mechanical loads.

**Chest wall and respiratory muscle.** Muscle spindles, tendon organs, and joint receptors are abundant in the rib cage and respiratory muscles, including chest wall respiratory muscles and diaphragm. Afferent information from these somatic receptors is expected to play an important role in respiratory sensation.

About forty years ago, Campbell et al. (27) discussed possible mechanisms by which elastic loads are detected. They concluded that the most likely was one that involved correlation of volume (length) changes with pressure (tension) changes, which
they described as “length-tension appropriateness”. They favored the possibility that all
the sensory information came from muscle and joint receptors. Up to now, there has been
a growing body of evidence that support a dominant role for muscle afferents. In 1967,
Davis (51) studied patients with upper spinal cord lesions who were lack of afferent
information from chest wall somatic receptors. Their mean detection threshold for
resistive loads was 3.39cmH₂O/L/s, which was significantly higher than the detection
threshold found in control subjects (1.19cmH₂O/L/s). The authors concluded that the
normal threshold for resistive load detection was dependent on somatic receptors in chest
wall. Similar to their findings, Killian et al. (85) showed that detection of added resistive
loads grossly deteriorated when ventilation was mechanically assisted. They argued that
active respiratory muscle contraction played an essential role in load detection. Gandevia
et al. (60) showed that pressure swings were not detected as well when confined to the
oropharynx as when the glottis was open, suggesting that chest wall receptors were more
sensitive than upper airway receptors. Respiratory muscle afferents have also been
reported to be involved in magnitude estimation. Leech et al. (95) assessed magnitude
estimation performance for both resistive and elastic loads in quadriplegic patients. They
found that the mean exponent for either resistive or elastic loads was reduced in the
quadripletic group. However, caution must be taken in the interpretation of these results
in patients who have substantial sensory and motor deficits (156). Such deficits may
significantly alter the behavior of the ventilatory pump when impeded by the mechanical
loads.

Further evidence supporting the role of respiratory muscle afferents comes from
studies about the pressure generated by inspiratory muscles. Killian et al. (150, 154)
demonstrated that the perceived magnitude of both added resistive loads and elastic loads were directly related to inspiratory pressure and its duration ($\psi = KP^{1.3}t^{0.56}$) and only indirectly related to the load. The inspiratory pressure patterns developed by subjects during resistive loading and elastic loading have temporal characteristics similar to the patterns of perception. Zechman et al. (151) found that augmentation of transdiahragmatic pressure (Pdi) occurred progressively earlier with increasing load magnitude. Transdiahragmatic pressure augmentation preceded Tdet and correlated well with Tdet. Their results suggested that changes in diaphragmatic tension might be involved in load detection. This hypothesis is further supported by the similarity in the Weber fractions for pressure predicted at times associated with detection of threshold resistive and elastic loads (about 0.1 for both loads) (156).

However, Zechman et al. (152) reported that resistive-load detection in two quadriplegic patients was normal when their elevated background resistance was taken into account. Similar results were also found for elastic load detection by using spinal anesthesia to denervate the entire rib cage (55). When the motion of the rib cage was restricted, Zechman et al. (153) found that the detection was unaffected in normal subjects. They proposed that the diaphragm might provide a primary source subserving load detection. But Nobel et al. (100) found there was no change in resistive load detection in one patient who had a complete paralysis of the chest wall and diaphragm. They also found that the ability of the subjects to detect resistive loads was unaltered by bilateral block of the phrenic nerves, which was the primary nerve innervating diaphragm (101). However, the investigators also indicated that the block was incomplete as judged by fluoroscopy.
In summary, although it is generally believed that respiratory muscle afferent mechanisms may play a primary role in load perception, it would be mistaken to think that they are the only mechanisms contributing to load perception. In addition, no single group of respiratory muscles has been found to be essential for load perception.

2.2.3.2 Respiratory motor command corollary discharge

There is a conscious awareness of the outgoing respiratory motor command to the ventilatory muscles, which is attributed to a corollary discharge from brainstem respiratory neurons to the sensory cortex during automatic reflex breathing or from cortical motor centers to the sensory cortex during voluntary respiratory efforts (5). Specific receptors and pathways mediating corollary discharge have not been identified. However, rostral projections from brainstem respiratory center to the midbrain and thalamus in the cat provide a possible neural substrate for the conscious appreciation of respiratory corollary discharge (31, 32).

Evidence concerning contributions of outgoing motor command to respiratory perception come from experiments in which subjects make voluntary inspiratory efforts after interventions that increase the motor command needed to develop a given force in the muscles: partial paralysis, fatigue, etc.. By using partial curarization to weaken the respiratory muscles, Campbell et al. (28) found that the difference threshold for detection of resistive loads was unaffected. However, the exponent for the power function relating perceived magnitude of the load to the intensity of the load was reduced. At each level of resistive load, perceived magnitude was overestimated during neuromuscular block. The authors argued that overestimation of load size indicated that subjects placed more reliance on sensing increased motor signals when making perceptual judgments than relying on peripheral afferent signals related to the load. Gandevia et al. (61) used
respiratory muscle fatigue to study the possible involvement of motor command in load perception. Their results showed that during fatiguing contractions all subjects overestimated the inspiratory load. Such findings are consistent with the hypothesis that awareness of motor command contributes to the estimation of respiratory loads.

The quality of sensation arising from corollary discharge may depend on the source of command. Voluntary commands may result in a sense of effort, whereas automatic commands may give rise to sensations of an urge to breathe (130). However, there has been no study designed to separate these two commands. Patients with the locked-in syndrome provide a unique opportunity for studying the role of the motor cortex due to their loss of influence of motor cortex on breathing. Although it has been shown that load compensatory responses were altered in those patients (148), there has been no experiment investigating load perceptual response in those patients.

2.2.4 Summary

In summary, numerous studies using psychophysical methods have shown that humans can detect the presence and type of a load, and assign a perceptual scale to estimate the magnitude of the load, as well. Application of extrinsic mechanical loads causes load-dependent mechanical changes in the respiratory system. Breathing pattern associated with mechanical events play an important role in the load perceptual response. These mechanical changes can be sensed simultaneously via various afferent systems. Although it is generally believed that respiratory muscle afferent mechanisms may play a primary role in load perception, the neural mechanisms subserving the perception of mechanical loads remains largely unknown. All the above conflicting results about afferent mechanism in load perception studies suggest that the afferent system involved in the perceptual activity during loaded breathing is a redundant system. Each individual
afferent system may not be essential, but all contribute partly to load perception. In this proposal, the author plans to compare load detection and magnitude estimation of inspiratory resistive loads in double lung transplant patients and matched normal subjects. The objective of this research is to investigate the role of lung vagal afferents in load perception. Two hypotheses are tested in this portion of the research:

1. Detection threshold, as well as Weber fraction, is higher in double lung transplant patients as compared with normal subjects.
2. The sensitivity of the subject to the stimulus, which is measured by the slope of the linear relationship between load magnitude and perceptual estimate of the load after a log-log transformation, is lower in double lung transplant patients as compared with normal subjects.

### 2.3 Respiratory-Related Evoked Potential (RREP)

Humans are able to consciously control their breathing and have conscious awareness of some aspects of respiration. Breathing against mechanical loads will induce changes in respiratory mechanics. These changes in lung volume, pressure and flow can be transduced into a sensory neural code by afferent nerves supplying the airway, lung, and respiratory muscles. The fact that respiratory sensations are appreciated at a conscious level during application of external mechanical loads means that there must be pathways for the afferent information from those respiratory system receptors to reach cortex and be centrally processed. The standard method for studying cortical activation by afferent stimulation is via recording cortical evoked potentials (CEPs), also known as event-related potentials (ERPs). The ERPs evoked by visual, auditory and somatosensory stimuli have been well studied (115). Only recently have ERPs evoked by respiratory stimuli been recorded (45). This section will present an overview of respiratory-related evoked potentials (RREPs).
2.3.1 RREP Methodology

In a pioneer study, Davenport et al. (45) recorded electroencephalographic activity on scalp in six healthy subjects when occlusion was applied at the onset of inspiration. Electroencephalographic activity was recorded from scalp electrodes placed over the frontal (Fz) and somatosensory regions (Cz, C3) of the cerebral cortex. Bipolar recordings were obtained with a cephalic reference, Cz. The inspiratory occlusion pressure was used to synchronize the occlusions and trigger the signal averager. The inspiration preceding each occlusion was averaged as the control. Results demonstrated a specific occlusion-related evoked potential, which was characterized as a consistent pattern of four voltage peaks, identified as P1, N1, P2 and N2. The waveform of this RREP was found similar to those previously reported somatosensory evoked potentials mechanically elicited by the arm and leg (52, 94, 113, 114). Subsequently, Revelette and Davenport (118) reported that RREPs could be recorded bilaterally from the somatosensory region of the cortex with a cephalic reference, Cz - C3 and Cz - C4. The peak amplitude of the RREP recorded over the left cerebral cortex was greater than that recorded over the right. In the same study, they also found that mid-inspiration occlusion produced a larger amplitude RREP with a shorter latency than the occlusions presented at the onset of inspiration. The authors argued that the difference in amplitude might be due to a more simultaneous activation of the afferents with the interrupted occlusion, and the difference in latency was probably due to the greater rate of pressure change produced by the interrupted occlusion bringing the afferents to threshold earlier.

Use of only one or two electrodes to record the RREP in these studies has its limitations. EEG activity recorded from one electrode only suggests the presence of a particular pattern of activation, but not the location of the generator of the source of the
activity. Simultaneously recording of multiple channels of EEG data is better in terms of source localization. In an attempt to specify the topographic distribution of the various RREP components, Davenport et al. (42) recorded RREPs induced by inspiratory occlusion from Cz - C3, Cz - C4, and 17 sites referenced to the linked earlobes. They found that there was a distinct difference in the RREP waveform recorded from pre-central and post-central electrodes when the earlobe was used as reference. A negative peak with a latency of 47-79ms (Nf) was found maximal at frontal electrode sites and the P1 was maximal at parietal sites. These findings provide evidence for radial dipole sources both pre (motor cortex) and post centrally (somatosensory cortex). This hypothesis was further supported by Webster and Colrain (141), who recorded RREPs in response to brief inspiratory occlusion by using 29 scalp sites, referenced to linked ears.

The respiratory-related evoked potentials can be elicited by occlusions presented both on the inspiratory phase (42, 45, 118, 141) and on the expiratory phase (70, 71). Respiratory-related evoked potentials are also reported in response to a series of graded resistive loads (20, 21, 89, 90), elastic loads (20) and negative mouth pressure (136). The results from the above studies demonstrated a similar RREP pattern. However, comparison between RREP studies is difficult due to the difference in methodology of recording, such as the choice of reference, bipolar recording versus monopolar recording, signal averaging method, number and sites of electrodes applied, etc.. There has been no study designed to investigate the difference in RREPs in response to different respiratory stimuli mode.

Some studies showed that there was a close relationship between the RREP and psychophysical measures. Knafelc and Davenport (90) reported that when a series of
resistive loads were applied, there was a log-log relationship between magnitude estimation of the load and P1 amplitude ($R^2 = 0.996$). However, a close correspondence between a RREP component and a perceptual response does not necessarily mean that the two have identical neural generators. They may simply have a common sensory input, causing the two to vary together as the stimulus intensity changes (92). Whether the scalp recording of ERPs can reflect the complex patterns of neural activity that lead to perceptual judgments is still unknown. It is argued that the ERPs recorded on the scalp represent net electrical activity from a neuronal population. Only those neurons which are activated in synchrony and have a certain geometric configuration can be measured at the scalp (2, 115). A further complication is that the spatial and temporal overlapping of the field potentials from different sources makes it difficult to determine whether an ERP waveform has a single neural origin or is a composite of several cerebral generators (92). Finally, it is also possible that the RREP might be contaminated by some non-respiratory artifacts. The inspiratory occlusion method produces auditory signals that may produce an auditory evoked potential artifact. But actually, the sound was produced when the occlusion valve was reopened rather than when it is closed. Davenport et al. (48) reported that RREP could also be recorded in a deaf subject and was similar to those with no hearing deficit. In addition, the fact that P1 peak was unaltered when the control, no-load average was subtracted for each load’s averaged RREP demonstrated that the P1 observed with resistive load application was not contaminated by sound or apparatus related artifacts (90). In the same study, Knafelc and Davenport (90) investigated the electrocardiographic (ECG) activity from scalp electrodes. They found that RREP was unaffected by ECG activity when $C_3$ and $C_4$ were referenced to $C_Z$. Furthermore, RREP
can also be recorded when the load stimulus occurred during the t-p interval of the ECG, where ECG peaks were absent. Their results demonstrated that the RREP recorded from \( C_z - C_3 \) and \( C_z - C_4 \) was not contaminated by ECG artifact. Head motion was recorded as a change in the pressure of an air-filled pillow (90). The absence of load-related changes in head motion, which could cause movement of the scalp electrodes, meant that such a motion artifact did not contaminate the RREP. A further problem is contamination of RREP by electromyographic (EMG) artifacts due to the activation of upper airway, neck, jaw muscles, etc. Masseter EMG activity was found to be concurrent with a bipolar scalp recording during voluntary jaw clenching (1). It has been suggested that the global field power (GFP) method, which provided a reference–free measure of somatosensory activation elicited by large negative pressures, may provide partial insulation from EMG contamination (40).

In summary, human studies have demonstrated that a consistent RREP pattern can be recorded in response to a variety of respiratory stimuli, including inspiratory occlusion, inspiratory resistive loads, etc. RREP measures are closely related to psychophysical measures. The artifact analysis also demonstrates that the RREP is an important and valid measure of neural activity related to the sensation of mechanical loads.

2.3.2 RREP Components

Event-related potentials typically consist of a series of negative and positive peaks, or components, which are hypothesized to reflect the activity of different generator structures within the brain. These components are generally described in terms of their scalp distribution and labeled according to their amplitude and latency. There are two main categories of components: exogenous component (appeared < 100 ms after
stimulus) and endogenous components (appeared > 100 ms after stimulus). The exogenous component (e.g. P1, P1a, Nf) reflects the arrival of impulses in the primary sensory area and are determined mainly by the physical characteristics of the stimuli. In comparison, endogenous components (e.g. N2, P3) are associated with cognitive processes of the sensory information and are highly sensitive to psychological variables. There may be some overlap between endogenous and exogenous components that are elicited about 100 ms after the stimuli, such as N1. This section will briefly describe the main characters of these components elicited by respiratory stimuli.

2.3.2.1 Early-latency components

In a pioneer study done by Davenport et al. (45), a characteristic RREP pattern was induced in response to inspiratory occlusion. The initial positive peak, with an average latency of about 60 ms, was identified as P1. This early exogenous component appeared to be unaffected by attention (143). It was suggested that P1 reflected the arrival of the occlusion related afferent information at the somatosensory cortex.

There is evidence supporting the idea that this early component is sensory in nature. First, source localization has suggested that the likely generator of P1 was primarily within the somatosensory cortex (96). P1 was consistently observed at post-central electrodes with the largest amplitudes found at the C3, C4, P3 and P4 electrode positions (42). About 13ms after P1, a negative peak, Nf, was found primary in the precentral electrodes (F3, F4, F7, F8, and to a lesser extent, C3 and C4). The source of the Nf peak may be associated with the motor cortex. The result of this study suggested that there were potentially two load-related generator sites, the somatosensory cortex and the motor cortex. Second, the latency of P1 was shorter in children than adults (42). The shorter height of the children, and the associated shorter nerve path length, would
possibly account for the age-related difference. Third, P1 and N1 remained relatively unchanged from wakefulness to at least Stage 2 sleep (141). Finally, P1 amplitude and latency are largely dependent on the intensity of the eliciting stimulus. Changes in the inspiratory drive will alter the rate at which the load-dependent mechanical changes occur and thus affect the afferents that transduce mechanical information. Davenport et al. (45) found that the P1 latency was related to the P0.1, i.e., decreased P1 latency with increased P0.1. This suggested that with a larger driving pressure, the stimulus threshold would be exceeded sooner with a shorter latency for the onset of the sensory signal. The relationship between inspiratory drive and P1 latency was further studied by comparing the RREP with steady-state hypercapnia and normocapnia (46). Similarly, increased P0.1 was found associated with a decrease in P1 latency. However, P1 amplitude was not significantly different between normocapnic and hypercapnic conditions. Revelette and Davenport (118) reported that mid-inspiration occlusion produced a larger P1 amplitude and a shorter P1 latency than the occlusions presented at the onset of inspiration. The authors argued that the difference in amplitude might be due to a more simultaneous activation of the afferents with the interrupted occlusion, and the difference in latency was probably due to the greater rate of pressure change produced by the interrupted occlusion bringing the afferents to threshold earlier. Recently, the correlation of RREP with the perception of resistive loads and the associated changes in mechanics in adults were studied (89, 90). The log of the P1 peak amplitude linearly correlated to the log of the magnitude estimation of the loads and log of the resistive load magnitude. Similar results were also found by Webster and Colrain (142). These findings support the idea that P1 is a response to the arrival of sensory input in the cortex.
The N1 component is a scalp-recorded negative peak with a latency of approximately 100ms. This component can also be produced by respiratory stimuli (42, 45, 71, 141). N1 has properties similar to exogenous components. Bloch-Salisbury et al. (21) assessed the effects of stimulus magnitude on the late RREP components using resistive loads. Their results showed that N1 amplitude decreased as the load size decreased. Harver et al. (71) recorded RREPs to both inspiratory occlusions and expiratory occlusions presented during both attend and ignored conditions. They found N1 was unaffected by attention in the young subjects. In contrast, Webster et al. found that the latency of N1 was significantly shorter and its amplitude was significantly larger in the attend condition as compared with the ignore condition (143).

2.3.2.2 Late-latency components

The P3 component, also known as P300, is a positive deflection with a latency in the range of 300-600 ms. A respiratory P3 was recently reported by Webster and Colrain by using brief inspiratory occlusions (141). Electroencephalograph was recorded from 29 scalp locations during wakefulness, stage 1, stage 2, and slow-wave sleep. A positive peak was observed with a latency of approximately 290ms. This component was interpreted as a P3-like response, which was similar to the P3 previously identified from recording an auditory oddball task (37). This late component was also reported by Gora et al. using similar methods (64). The P3 component was also seen in response to resistive load stimuli (20, 21, 142) and elastic load stimuli (20).

More than ten years ago, Johnson proposed a model to explain variations in P3 amplitude (78). He hypothesized that the variables that influenced P3 amplitude could be categorized along three dimensions: subjective probability (the subject’s perception of the likelihood that a certain stimulus will be delivered), stimulus meaning and information
transmission. These three variables in this triarchic model represent independent neural processes, and in an additive or multiplicative fashion contributing to P3 amplitude (77). Johnson’s idea seems appropriate for respiratory P3. Strobel and Daubenspeck (136) sought to assess the effect of stimulus predictability by presenting trials in two conditions. In condition one the negative mouth pressure was presented on every breath, while in condition two it was randomly presented every second to seventh breath. The negative component (with a latency of about 238msec) were either severely diminished or absent during condition one when load presentation was made completely predictable. The early-latency activity was resistant to manipulations of stimulus predictability. Similarly, Colrain et al. (36) assessed the effects of target stimulus probability for the late positive component of the RREP. A single respiratory stimulus paradigm was employed where normal breaths served as the standard stimuli and the occluded breaths presented randomly at various probability levels served as the target stimuli. P3 amplitude was found to increase with decrease in target probability in a linear fashion, whereas early components were unaffected by target probability.

It is generally believed that P3 component does not appear to reflect the physical parameters of the eliciting stimuli as the earlier components do, but rather appears to reflect the active cognitive processing of stimulus information. The effect of attention on the P3 component was examined by Harver et al. (71). They recorded P3 by both inspiratory and expiratory occlusions. P3 was found markedly affected by attention. Specifically, P3 amplitude was larger and its latency was shorter when occlusions were attended to, compared with when they were ignored. Amplitudes for this component were largest at the Cz central and P3, P4 parietal sites. By using more electrode sites, Webster
and Colrain (143) recently recorded the RREP from twenty-nine EEG channels. The evoked potentials were elicited by a pseudorandom sequence of 100-, 200-, 400-, and 800-ms inspiratory occlusions, under attend and ignore conditions. Attention resulted in augmentation of the N1, P2, and P3 components, but did not affect the early latency Nf and P1 components. P3 occurred with shorter latencies in the attend condition. This result highlights the relationship between P3 and perception of respiratory somatosensory information.

The respiratory P3 can also be affected by the type and magnitude of the external mechanical loads. Bolch-Sailsbury and Harver (20) recorded P3 in response to small and large resistive and elastic loads. Similar to the N2 component, the P3 was larger and occurred earlier for the larger loads than smaller loads, and for resistive loads than elastic loads. Subsequently, they presented inspiratory resistive loads in a detection paradigm (21). P3 was absent when a sub-threshold load was applied, but was observed with the other three larger loads, which were near or above detection thresholds. Similar to their previous finding, P3 amplitudes increased and latencies decreased as load size increased. This phenomenon was again demonstrated by Webster and Colrain (142). These data show that this late component of the RREP is sensitive to the physiological effects of the stimuli. It has been demonstrated that P1 amplitude and latency could be affected by the magnitude of load (90, 142). Respiratory P3 and P1 might reflect different stages of the cortical response. Therefore, the effects of the stimuli on P3 might be due to its effects on P1. However, no data has shown the correlation between these two components.

In summary, RREP components can be divided into two main categories according to their latencies: early components (e.g. P1) and late components (e.g. P3). P1
is generally believed to be sensory in nature, whereas P3 appears to reflect the active cognitive process. However, the exact neural process associated with those RREP components remains largely unknown.

2.3.3 Afferent Mechanism of RREP

As described in the above sections, inspiratory occlusions and graded suprathreshold mechanical loads can elicit RREP. Animal and human studies have demonstrated that afferents from various regions of respiratory system project specifically to somatosensory region of cerebral cortex. However, the afferents mediating the RREP remain largely unknown. This section will first present an overview of respiratory afferent activation of the cerebral cortex, and then discuss the possible afferent mechanisms that might mediate the RREP.

2.3.3.1 Afferent activation of cerebral cortex and anatomical pathways

**Facial nerve, vagal and superior laryngeal afferents.** A positive peak of the evoked potential with the latency of about 21-22msec was elicited by natural cutaneous stimulation directed to the skin of the face (125). Similarly, two subsequent studies also showed that trigeminal and facial nerve afferents could elicit neural activity in the somatosensory cortex in humans (72, 126).

A series of cat studies demonstrated that stimulation of vagal and superior laryngeal nerve (SLN) elicited neural activity in the somatosensory region of the cortex. Korn and Massion (91) used an electrical stimulus applied to the cat cervical vagus nerve. They recorded CEPs in the contralateral cortex in four frontal regions: the motor precruciate region, a region caudal to the ansate sulcus, and two fronto-orbital regions. Later, Aubert and Legros (7) found a wider distribution of contralateral cortical activity elicited by vagus stimulation in cats. Superior laryngeal nerve stimulation in cats elicited
CEPs which were recorded in the regions of the cruciate sulcus, anterior ectosylvian sulcus, anterior sylvian sulcus, suprasylvian sulcus and orbital cortex (6). Similar studies of vagal afferent activation have also been performed in monkeys (104). The specific cortical areas were identified histologically. Results demonstrated that single pulse electrical vagal stimulation elicited activity in the sensory and motor cortices. The projection was bilateral and the pattern of responses was similar for both ipsilateral and contralateral cortices. Neurons responsive to vagal afferent stimulation have been identified in the monkey thalamus (69). It is hypothesized that there are at least two vagal and SLN pathways: one projects to the somatosensory and motor cortex, another projects to the mesocortex (47). However, there is still very limited information about the vagal and SLN afferent pathways to cortex. Furthermore, somatosensory cortical activation by vagal afferents in humans is still unknown yet. Given that afferents mediating respiratory-related information from the larynx, pharynx, airways, and lungs are found in these nerves, it is reasonable to hypothesize that vagal and SLN afferents might be responsible for a portion of cortical activation in response to respiratory stimuli.

**Respiratory muscle afferents.** Electrical stimulation of myelinated phrenic afferents has been shown to elicit evoked potentials in the somatosensory region of the cat cerebral cortex (50). In this study, the phrenic afferents activated by the electrical stimulation were primarily group I and II, and to a lesser extent group III afferents. The majority of the phrenic afferent evoked activities were in the areas 3a and 3b. Bolser et al. (22) recorded spinothalamic tract neurons activated by group II or III phrenic afferents. The group III afferent elicited CEPs were found more laterally in the forelimb region of area 4γ in the cortex (47).
Intercostal muscle afferent projections to the cerebral cortex have also been identified by both electrical and mechanical stimulation (49). Both methods resulted in localization of cortical foci in area 3a medial (2-3mm) to the phrenic foci. Cortical event-related potentials were found with only 50um stretch of the intercostals space. The amplitude increased with increasing stretch magnitude and reached a plateau with a stretch of 300um, which indicated that intercostals muscle spindles played a major role in the CEPs response.

Both phrenic nerve stimulation and mechanical probing of the diaphragm could elicit thalamic neuron activity (47, 48). Neurons in the ventroposterior lateral (VPL) nucleus of the thalamus were activated following diaphragmatic afferent stimulation, whereas mechanical and electrical stimulation of intercostals afferents activated a separate populations of neurons in the VPL nucleus of the thalamus. Yates et al. (147) recorded CEPs in the right somatosensory cortex following stimulation of the left C5 root. The majority of primary CEP sites were seen in the area of postcruciate dimple, in area 3a near the 3a/3b border. Retrograde fluorescent tracers injected at the sites of primary activation produced labeled cells in the oralis nucleus of the ventroposterior complex within the thalamus. These findings suggest that phrenic nerve afferents project to the trunk region of somatosensory motor cortex via specific thalamocortical projections, originating in the VPO.

This thalamocortical pathway seen in cats, is also present in humans (62). The investigators elicited CEPs following needle electrical stimulation of intramuscular afferents of the second parasternal and fifth lateral intercostal muscles. This study provides direct evidence for a short- latency projection from intercostal muscle afferents
(group I and/or II) to the human cerebral cortex. Phrenic nerve stimulation induced CEPs in human subjects were also recorded by Straus et al. (135).

Therefore, both animal and human studies have provided evidence of the existence of neural substrates for mechanoreceptors in the pump muscles to project to the somatosensory cortex. Pump mechanical activity would be transduced by the afferents and projected to the somatosensory region of the cerebral cortex via a dorsal column-lemniscal pathway that is relayed at the VPL region of the thalamus. This neural pathway may be part of the sensory limb for perception of mechanical loads (48).

In summary, it is hypothesized that there are two cortical systems involved in respiratory central neural processing (47). One is the pathway for respiratory muscle afferents. Neural information arising from mechanoreceptors enters the spinal cord, ascends in the dorsal column, relays in the brainstem, projects to the VPO in thalamus via the medial lemniscal tract, and is projected through a thalamocortical pathway to the sensorimotor cortex. The function of this pathway could relate to the proprioceptive control of respiratory muscles. The second pathway involves ascending afferent information from the vagus nerve and its branches, and possibly phrenic afferents. They relay from the brain stem to the amygdala before projecting to the mesocortex. This circuit may deal with some of the behavioral aspects of respiration.

2.3.3.2 Afferent mechanisms of RREP

Respiration against mechanical loads can alter the afferent activity of several populations of mechanoreceptors: mouth, pharynx, larynx, lung, and respiratory muscles. The afferent information from these receptors is mediated via facial nerve, vagus nerve, SLN, phrenic nerve and other respiratory muscle afferent nerves. Although many studies
have demonstrated activation of cortical neurons by these afferents, the specific afferents mediating the RREP elicited by respiratory mechanical loads are still largely unknown.

Davenport et al. (46) reported that increasing respiratory drive with steady-state hypercapnia resulted in a decreased latency for the P1 peak by ~5ms but no change in peak amplitude. The mouth pressure (Pm) associated with the interrupted occlusions was tripled during hypercapnia. The significant increase in Pm should be associated with an increased recruitment of mouth mechanoreceptors. The lack of a change in amplitude suggested that mouth and pharyngeal afferents were not the primary source of the afferents mediating the RREP with inspiratory interruptions. Daubenspeck et al. (41) compared RREP responses to negative oral pressure (-10cmH2O) before and after insertion of a laryngeal mask airway (LMA) that prevented supraglottal airway receptors from sensing the applied stimulus. They found that evoked potentials with LMA were smaller, with delayed or missing features. It was estimated that supraglottic receptors contributed about one-half of the response. The authors concluded that upper airway receptors (above larynx) were responsible for a major portion of the mid-latency (not the P1) afferent information arriving at the somatosensory cortex in response to applied negative oral pressure in normal humans. However, applying a large negative pressure is not comparable to breathing against a mechanical load.

Changes in the inspiratory drive will alter the rate at which the load dependent mechanical changes occur and will similarly affect the afferents that transduce those mechanical parameters. Davenport et al. (45) found that P1 latency was related to P0.1, that is, decreased P1 latency with increased P0.1. Similar results were also found in a later study (46). P0.1 is an index of the overall motor output from the respiratory center, which
parallels the changes in phrenic nerve activity (56). Thus it appears that respiratory muscle afferents might contribute a portion of RREP response. However, the mechanical changes due to increased inspiratory drive will act on the whole respiratory afferent system. Therefore it can not be concluded which afferent population plays the primary role in RREP. The role of respiratory muscle afferent mechanism is also indirectly supported by the finding that CEPs recorded following phrenic nerve stimulation in cats can be modified by diaphragm fatigue (13). The authors reported progressive lengthening in onset and peak latencies of the CEPs associated with diaphragmatic failure produced by direct muscle stimulation. These findings suggest that a reduction in phrenic sensory activity is associated with delayed cortical responses. However, there have been no study assessed the effects of respiratory muscle fatigue on RREP. Recently, Knafelc and Davenport (89) investigated the relationship between P1 peak and inspiratory pressures during application of graded resistive loads. They found a close log-log relationship between P1 amplitude and Pm, Pes, Pdi, Pdi slope. Although this study could not identify the afferents mediating the RREP, it was hypothesized that the P1 peak of the RREP is elicited by changes in mechanical forces related to the inspiratory pump.

2.3.4 Summary

Cortical activation has been recorded by using a variety of respiratory stimuli in both human and animal studies. Animal studies have demonstrated that afferents in the respiratory system, including respiratory muscle, lung etc., have projection pathways to the cerebral cortex. Respiratory mechanical changes associated with extrinsic loads are transmitted to the central nervous system via those afferent pathways. The respiratory-related evoked potentials provide a convenient and non-invasive method of studying the neural activity in high brain center in response to the respiratory stimuli. However, the
afferent mechanisms involved in RREP response remain largely unknown. Double lung transplant recipients provide a good model to investigate the role of lung vagal afferent system in RREP response. Therefore, in this proposal, the author plans to compare both early and late latency RREP components elicited by inspiratory occlusion in double lung transplant patients and matched normal subjects. For each subject, the RREP response will be recorded in three separate trials: attend trial, ignore trial, and control trial. To the author’s knowledge, this will be the first study to investigate the lung vagal afferent mechanism in RREP response. Two hypothesis were tested in this part of research:

1. RREP response will still be elicited in double lung transplant patients.
2. Both early and late-latency components will have longer latency and smaller amplitude in double lung transplant recipients due to the loss of lung vagal afferent system.

2.4 Chapter Summary

Ventilation of the lung is a mechanical process. The respiratory muscles act as a pump to generate the driving force for air to flow and increase the lung volume. Application of extrinsic mechanical loads will alter this mechanical process and lead to a conscious awareness of the loads. The perception of respiratory mechanical loads is dependent on two processes: load detection and magnitude estimation. These have been studied by using psychophysical methods in both humans and animals. In addition, the RREP method provides a unique way to investigate the neural activity involved in the perceptual response to external loads.

The neural mechanism and the afferent pathway mediating the perception of respiratory mechanical loads are still largely unknown. It is difficult to determine the specific afferent mechanism involved in load perception because load-dependent changes are transmitted through all the respiratory afferent systems, including upper airway, lung,
respiratory muscle, etc.. Double lung transplant recipients provide a good model to clarify the role of lung and lower airway receptors in respiratory sensation because all the afferent traffic from receptors distal to the surgical anastomosis are interrupted. Therefore in this proposal, we plan to compare the breathing pattern, inspiratory resistive load detection and magnitude estimation, as well as RREP components elicited by inspiratory occlusion in double lung transplant patients with matched normal subjects to determine the role of lung vagal afferents. Based on previous conflicting results, it appears that respiratory sensation related to loaded breathing may be due to multiple and simultaneous sensory input. No single afferent mechanism is essential, but all contribute to load perception and the RREP response. Therefore it is hypothesized that double lung transplant patients may have impaired load perceptual activity and attenuated RREP response. The result of this study will provide important information about the relationship between the selected afferent respiratory mechanoreceptor populations and the mechanical load perception.
CHAPTER 3
METHODS

3.1 Subjects

In this project, 11 double lung transplant patients (DLT), and 12 normal subjects (NOR) were recruited. All subjects were recruited from the age group of 20-65 years, and they were matched for gender, ethnicity, and age. Subjects were classified as normal on the basis of habitual good health, no history of cardiorespiratory disease, no history of smoking and no evidence of current major or minor illness. Patients with double lung transplant surgery were recruited from the University of Florida Medical Center. All these patients had no evidence of current respiratory or neurological disease. Forced vital capacity (FVC) and forced expiratory volume within 1 second (FEV1) were tested for each subject. Subjects with a FVC value less than 70% of predicted values were excluded from this study. Only one double lung transplant subject was excluded from this study due to abnormal lung function (FVC: 60.8% of predicted value; FEV1: 41.9% of predicted value). All participants were provided an informed consent on an approved form prior to participating in this study. The IRB has reviewed and approved this study.

3.2 Procedures

All subjects initially performed pulmonary function testing. Then all three experiments: load detection, magnitude estimation and RREP recording were performed in each subject. The order of the three experiments was randomized for each subject.
3.2.1 Pulmonary Function Testing

Subjects were asked to refrain from strenuous physical activity, large meals and caffeine for at least four hours prior to the test. Subjects were seated upright in a chair when they performed the test. Spirometry testing conformed to American Thoracic Society Standards. Standard instructions were given to each subject. Spirometry Data were collected by a computerized spirometer. All subjects performed pulmonary function testing of FVC and FEV1. Each test was repeated 2-4 times with at least one minute’s rest between each repetition. The highest number obtained was accepted as the final result. Values were also contrasted with age and sex-predicted values.

Background respiratory resistance was measured using the forced oscillation method. The subject was seated in front of the apparatus and breathed “normally” through the mouthpiece, with his or her cheeks supported by both hands. Approximately 10 tidal breaths were collected continuously to analyze the resistance by computer (Jaeger Toennies, Medizintechnikmit System, V. 4.5). The test was repeated for at least three times for each subject with a one-minute rest between every two repetitions. The average of three acceptable measures was used as the subject’s respiratory system resistance.

Maximal inspiratory pressure (MIP) was defined as the greatest negative pressure obtained at the mouth and sustained for at least one second while performing a maximal inspiratory effort from residual volume. Maximal inspiratory pressure was commonly used to reflect inspiratory muscle strength indirectly. Subjects were in a standing position when they performed the test. The test was repeated until 3 measurements within 10% variation were obtained. There was at least one-minute rest between every two repetitions. The maximal value obtained was recorded as the subject’s MIP.
3.2.2 Detection of Graded Resistive Loads

The subject was seated in a lounge chair in a sound isolated chamber, separated from the experimenter and the experimental apparatus. During the detection experiment, the subject was instructed to breathe through a mouthpiece connected to a non-rebreathing valve (Hans Rudolph, 2600 series) with their nose clamped. The inspiratory port of the valve was connected to the resistive loading manifold (Hans Rudolph, Model 4813). The apparatus is shown in Figure 3-1. Mouth pressure (Pm) was measured at the center of the non-rebreathing valve and recorded on a polygraph. Inspiratory airflow was measured by a differential pressure transducer (Validyne, Model MP45) and signal conditioner (Validyne, Model CD316) connected to a pneumotachograph. Inspired volume was obtained by electrical integration of the airflow signal. Mouth pressure, inspiratory airflow and volume were recorded on a polygraph (Grass Instruments, Model 7), and stored and analyzed on a computer (Chart, Powerlab AD Instrument). The resistive loads were sintered bronze disks placed in series in the loading manifold and separated by stopped ports. The load was applied for the entire inspiration and then removed. The subject was asked to press the signal button held in their dominant hand as soon as they sensed the presence of a load. A series of test loads were presented in a practice session to familiarize the subject with the load sensation and the range of loads. During the subsequent experimental session, the subject listened to music of their choice to mask experiment sounds. A series of resistive loads (0.2, 0.8, 1.24, 1.64, 2.48, 3.26, 6.95, 11.46 cmH2O/l/s) were presented in a randomized block design, with each loaded breath separated by 2-6 control breaths. A total of 10 presentations of each load magnitude were presented in two experimental trials with a 5-minute break between trials. The subject was monitored by video camera throughout the experiment.
3.2.3 Magnitude Estimation of Graded Resistive Loads

The apparatus used for magnitude estimation is similar to that used for load detection, as shown in Figure 3-2. Initially, the subject was asked to breathe normally with eyes closed. A line was placed on the oscilloscope screen that coincided with the peak inspiratory flow rate with quiet breathing. This is determined as the target flow rate. The subject then opened their eyes and breathed while watching the oscilloscope screen. They were instructed to have each breath “hit” the target line during the whole experiment. The light was illuminated during expiration, cueing the subject that the next inspiration would be loaded. The subject estimated the test breath each time when the light above the oscilloscope was illuminated. If they sensed the presence of the load, they squeezed the handgrip using their dominant hand, according to how hard it was to breathe in.

Before the experimental sessions, each subject squeezed the handgrip as hard as possible for three times and his or her handgrip response was recorded on the polygraph.
All handgrip magnitude estimations were expressed as a percentage of their maximum handgrip responses. A practice session was then presented while informing the subject of the size of the load. After practice, a series of resistive loads (1.64, 2.48, 3.26, 6.95, 11.46, 20.48, 42.62 cmH2O/l/s) were presented in a randomized block design during the experimental session. Each loaded breath was separated by 2-6 unloaded breaths. The load was applied for the entire inspiration. A total of 10 presentations of each load were presented in two trials with a 5-minute rest period between trials.

3.2.4 RREP Elicited by Inspiratory Occlusion

The subjects were seated in a sound insulated room (Figure 3-3), with the back, neck and head comfortably supported. The experimenter was in the adjacent room and monitored the subject with a video camera. An electrode cap with integral electrodes (Electro-cap International) was used to record scalp EEG activity. The electrodes were placed at the scalp positions Cz, C3, C4, Cz', C3', C4', Fz, F3, F4, Pz, P3, P4, based on the
International 10-20 System (Figure 3-4, (34)). The cap was placed on the subject’s head, positioned and secured with a strap. Scalp and electrode contact was made by the application of electro-conducting paste administered through the center opening in the electrode. Two tin electrodes were placed on both earlobes, which were used as the reference. Two Ag/AgCl electrodes were placed over the lateral edge of the eye for recording vertical electrooculogram (EOG). The impedance levels for each electrode was checked and maintained below 5 KΩ. The electrode cap was connected to a 20-channel electroencephalograph (EEG) system (model 12, Neurodata Acquisition System, Grass Instruments, Quincy, MA). Electroencephalograph activity was monitored with an oscilloscope monitor. The EEG signals was band-pass filtered (0.3Hz-1kHz), amplified and led into an on-line signal averaging computer system (Cambridge Electronic Design).

The subjects were instructed to relax all postural and facial muscles and breathe as normally as possible through a non-rebreathing valve with the inspiratory port.
connected to a pneumotachograph and an occlusion valve. The inspiratory load was presented by silently inflating the occlusion valve at approximately the mid-inspiration point of the breath. Each occlusion was separated by 2-6 unoccluded breaths. A transistor-transistor logic (TTL) pulse generated by the inspiratory occlusion valve controller triggered the collection of 50 ms of pretrigger and 950 ms of posttrigger EEG and Pm data. The duration of the occlusion was about 350ms.

There were three RREP experimental trials: ignore trial, attend trial and control trial. All three trials were separated by a 5-minute rest period off the breathing apparatus. During the ignore trial, the subject watched a video tape with the sound masking experimental noises. One hundred occluded breaths were presented. The control trial was designed to provide the same sounds and vibrations without occlusion. One hundred activations of the occlusion valve were presented similar to the ignore trial. However, the breathing circuit was connected to room air by opening a separate stopped port, so the
airflow was not interrupted. During the attend trial, the subject was asked to attend to their breathing and count the number of occluded breaths. Experimental noises were masked by music. Similar to the other two trials, a total of 100 occluded breaths were presented during attend trial.

3.3 Data Collection and Statistical Analysis

The descriptive statistics of all the variables were calculated and expressed as mean ± standard error of mean (SEM). Significance level was set at 0.05, unless multiple contrast analysis was used.

3.3.1 Detection of Resistive Loads

The number of detections for each load was summed and divided by the number of total presentations to obtain the detection percent (det%) for each load. The det% was plotted against the magnitude of added load. The detection threshold (ΔR_{50}) was determined as the magnitude of load corresponding to a det% of 50%. The Weber Fraction (ΔR_{50}/R_0) for each load was computed by dividing ΔR_{50} by the sum of the subject’s respiratory background resistance and the resistance of the apparatus. The resistance of the apparatus is about 1.6cmH_2O/L/s. Detection latency (Tdet) for each load was also computed by measuring the time from the start of air flow to the onset of the detection signal.

A two-tailed T-test was used to compare ΔR_{50} and Weber fraction between the DLT and the NOR group. A two-way repeated measure ANOVA was performed to study the effects of group and load on det% and Tdet. Contrast analysis was performed to compare the effect of different loads. P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.
Peak mouth pressure (Pm), peak volume (Vmax), peak inspiratory airflow, inspiratory duration (TI), expiratory duration (TE), Time to peak (TP), breathing frequency (f), and minute ventilation (VE) were recorded for each loaded breathing and the previous unloaded breathing, which was used as a control breath.

The breathing pattern was compared by using two-way repeated measure ANOVA to study the effects of group and load. Contrast analysis was performed to compare the effects of different loads. P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.

3.3.2 Magnitude Estimation of Resistive Loads

The handgrip response during loaded breathing was divided by the maximal handgrip response for each subject to obtain the percentage of handgrip response (HG%). Only the handgrip responses that corresponded to the load level greater than each subject’s $ΔR_{50}$ were used for further analysis. The HG% was then plotted against actual load magnitude and peak Pm using a log-log transformation, respectively. The regression line was obtained by using the method of least squares and the slopes from each subject were determined (including slope of logHG%-logR and slope of logHG%-logPm).

A two-tailed T-test was used to compare the slope in the DLT group with the NOR group. A two-way repeated measure ANOVA was performed to estimate the effects of group and different level of loads on the value of %HG, Pm, PI, Vmax, peak airflow, TI, TE, f, VE. Contrast analysis was performed to compare the effects of different loads. P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.
3.3.3 RREP Data

The EEG activity in each subject was averaged for each RREP trial. An individual EEG sample was included in the signal average if the onset of the occlusion-related changes in mouth pressure was present and coincident with the majority of the samples. A second criterion for inclusion was the absence of artifact (e.g. movement or EOG). A minimum of 64 presentations were included in each average. The baseline trace was DC corrected if necessary. The presence, latency and amplitude of RREP components: P1, P1a, Nf, N1 and P3 were determined for each scalp location from the averaged EEG traces. Peak latencies were measured as the time from the onset of the occlusion valve closure, indicated by the change in mouth pressure, to the EEG peak. The zero-to-peak amplitude was recorded at the peak of each component. The nomenclature for the peaks is based on previous reports (42, 45, 118). The RREP components were identified in the following manner: P1 and Nf were defined as the first positive and negative deflections occurring between 25-40ms and 40-60ms following the onset of the change in Pm, respectively. Following Nf, a positive deflection occurring between 50-60 ms after the onset of the change in Pm was defined as P1a. N1 was defined as the negative deflection, which occurred between 90 and 130ms. P3 was identified as a positive deflection in the range of 250-450ms.

Latencies of the RREP components were compared using two-way repeated measure ANOVA to determine the effects of group and trial. Peak amplitudes of the RREP components were compared using three-way repeated measure ANOVA to determine the effects of group, trial and scalp location. Contrast analysis was performed to compare the amplitude of various RREP components among different scalp locations (frontal region: F3, Fz, F4; central region: C3, Cz, C4; centroparietal region: C3’, Cz’).
C4; and parietal region: P3, Pz, P4). P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.
CHAPTER 4
RESULTS

4.1 Demographic Characters of Subjects

The results from ten double lung transplant (DLT) subjects and twelve normal subjects (NOR) aged between 20 and 64 years were analyzed. The time since the DLT patients received transplant surgery varied from 1.5 to 5.5 years. None of these patients had evidence of rejection when they participated in this study. All DLT subjects were still on immunosuppressives and steroid medications. The major medications used for each DLT subject was listed in Table 4-1. None of the normal subjects were taking medications.

Table 4-1. Medications Listed by Double Lung Transplant Patients

<table>
<thead>
<tr>
<th>DLT</th>
<th>Time post-surgery (yrs)</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>Imuran, Prednisone, Prorocal, Neoral, Mycelex.</td>
</tr>
<tr>
<td>2</td>
<td>2.9</td>
<td>Imuran, Prednisone, Dapsone, Cyclospoiiin.</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>Imuran, Prednisone, Ultrase, Prograf, Verapamil, Monopril.</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>Imuran, Prednisone, Prograf, Dapsone, Prilosec, Mycelex, Promethazine, Glipizide.</td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>Imuran, Prednisone, Prograf, Lipitron, Dapsone.</td>
</tr>
<tr>
<td>6</td>
<td>4.0</td>
<td>Imuran, Prednisone, Neoral.</td>
</tr>
<tr>
<td>7</td>
<td>5.5</td>
<td>Imuran, Prednisone, Prograf, Septra, Nortryline, Tagamet.</td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>Imuran, Prednisone, Prograf, Provera.</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>Imuran, Prednisone, Prograf.</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>Imuran, Prednisone, Prograf.</td>
</tr>
</tbody>
</table>

The group mean demographic characteristics and pulmonary functions of all the subjects that participated in this project are shown in Table 4-2. Both groups are matched
with gender, age, height, and weight. All subjects are Caucasian. T-test showed no significant difference in MIP and background respiratory resistance between the DLT group and the NOR group. Both FVC and FEV1 were significantly lower in the DLT group than the NOR group (97.0 ± 4.9% of predictive value vs 119.5 ± 5.0%, 83.8 ± 6.1% vs 108.3 ± 3.7% of predictive value, respectively). However, both FVC and FEV1 were greater than 80% of predictive values for the DLT group, indicating normal lung functions in the DLT subjects who participated in this study. FEV1/FVC ratio was not significantly different between the two groups (88.1 ± 6.0% of predicted value for DLT vs 93.9 ± 2.8% of predicted value for NOR, p = 0.371).

Table 4-2. Demographic Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>DLT</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>5 females, 5 males</td>
<td>7 females, 5 males</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>46.5 ± 4.4</td>
<td>46.6 ± 4.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.4 ± 2.9</td>
<td>173.8 ± 2.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.0 ± 4.4</td>
<td>81.1 ± 6.2</td>
</tr>
<tr>
<td>MIP (cmH2O)</td>
<td>77.0 ± 5.1</td>
<td>86.7 ± 7.6</td>
</tr>
<tr>
<td>R (cmH2O/L/s)</td>
<td>4.7 ± 0.8</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>FVC (% of predicted value)</td>
<td>97.0 ± 4.9</td>
<td>119.5 ± 5.0 *</td>
</tr>
<tr>
<td>FEV1 (% of predicted value)</td>
<td>83.8 ± 6.1</td>
<td>108.3 ± 3.7 *</td>
</tr>
<tr>
<td>FEV1/FVC (% of predicted value)</td>
<td>88.1 ± 6.0</td>
<td>93.9 ± 2.8</td>
</tr>
</tbody>
</table>


Data are presented as means ± SEM. SEM, standard error of the mean.

* indicates a significant difference between DLT and NOR, p < 0.05.

4.2 Load Detection

All subjects completed the load detection experiment. The total number of false positive responses were compared between DLT and the NOR groups, and no significant difference was found (4.8 ± 2.7 for DLT vs 4.2 ± 1.1 for NOR, p = 0.621). The detection
Threshold (ΔR₅₀) was found significantly higher for the DLT subject (2.91 ± 0.5 cmH₂O/L/s for DLT vs 1.55 ± 0.3 cmH₂O/L/s for NOR, p < 0.05). The Weber fraction was also significantly elevated in the DLT group (0.502 ± 0.1 in DLT vs 0.295 ± 0.05 in NOR, p < 0.05). The results of detection threshold and Weber fraction are shown in Figure 4-1 and Figure 4-2.

Detection latency (Tdet) and detection percent (det%) in response to seven resistive loads (R1 (0.2cmH₂O/L/s), R2 (0.8cmH₂O/L/s), R3 (1.24cmH₂O/L/s), R4 (1.64cmH₂O/L/s), R5 (2.48cmH₂O/L/s), R6 (3.26cmH₂O/L/s), R7 (6.95cmH₂O/L/s), R8 (11.46cmH₂O/L/s)) were shown in Figure 4-3 and Figure 4-4. Detection latency tended to decrease and det% to increase as the magnitude of the resistive load increased. Two-way repeated measure ANOVA found significant group (p < 0.05), load (p < 0.001) and interaction effects (p < 0.05) on det%. Specifically, the DLT group had significantly higher det% than the NOR group. Further contrast analysis among different levels of resistive loads found that det% for R1 and R2 were significantly lower than the other loads. The det% for R3, R4 and R5 were significantly lower than the det% for R6, R7 and R8. The det% for R6 is also significantly lower than the det% for R7 and R8 (p < 0.0018, since a total of 28 comparisons were made, p-value was corrected by dividing 0.05 by 28). Surprisingly, Tdet did not display significant effects of group (p = 0.67) or load (p = 0.084), nor was there significant interaction (p = 0.422) between the two factors.
Figure 4-1 Detection Threshold

Figure 4-2 Weber Fraction
Figure 4-3 Detection Latency during Resistive Loading

Figure 4-4 Detection Percent during Resistive Loading
4.3 Breathing Pattern and Load Response

During the load detection experiment, all the subjects were asked to breathe normally. There was no cue and airflow targeting involved. Therefore, the breathing pattern during unloaded and loaded breathing can be used to reflect the subjects’ resting breathing pattern and load compensation responses. The breathing pattern during control breathing and loaded breathing are shown from Figure 4-5 to 4-12.

Two-way repeated measure ANOVA found no significant group, load and interaction effects on all the above parameters during control breathing. During loaded breathing, group and interaction effects were also not significant, except for the interaction effect of VE. The main effects of load were significant during loaded breathing, as the magnitude of resistive load increased, Pm, TI, TP increased, while Vmax, airflow, TE, f, and VE decreased. Further pair wise contrast analysis results for each parameter were displayed in Table 4-3.

![Figure 4-5 Peak Mouth Pressure during Load Detection](image)
Figure 4-6 Peak Volume during Load Detection

Figure 4-7 Peak Air Flow during Load Detection
Figure 4-8  Inspiratory Duration during Load Detection

Figure 4-9  Expiratory Duration during Load Detection
Figure 4-10 Time to Peak Air Flow during Load Detection

Figure 4-11 Breathing Frequency during Load Detection
Figure 4-12 Minute Ventilation during Load Detection
### Table 4-3. Contrast Analysis of Load Effects on Breathing Pattern

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air flow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>TI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Pm, peak mouth pressure. Vmax, peak volume. TI, inspiratory duration. TE, expiratory duration. TP, time to peak airflow. f, breathing frequency. VE, minute ventilation.

* indicates a significant difference in comparison between two loads, p < 0.00018 (a total of 28 comparisons for each parameter, so p-value is corrected as 0.05 divided by 28, which equals to 0.00018).

### 4.4 Magnitude Estimation

The relationships between ME of breathing difficulty (represented by HG%) and stimulus intensity (R and Pm) of 7 resistive loads (R4 (1.64cmH2O/L/s), R5 (2.48cmH2O/L/s), R6 (3.26cmH2O/L/s), R7 (6.95cmH2O/L/s), R8 (11.46cmH2O/L/s), R9 (20.48cmH2O/L/s), R10 (42.62cmH2O/L/s)) are displayed in Figure 4-13 and
Figure 4-14. As the magnitude of resistive load increased, HG% increased. Similarly, as resistance increased, Pm and the HG% increased, as well. This suggested that at higher loads, the subjects generated higher Pm and squeezed the handgrip harder. The main effect of load on HG% is significant (p < 0.001). Further contrast analysis showed that HG% was significantly different between R4 and R8, R9, R10, between R5 and R8, R9, R10, between R6 and R9, R10, between R7 and R9, R10, between R8 and R10 (p < 0.0024, since a total of 21 comparisons were made, p-value was corrected as 0.05 divided by 21, which equals to 0.0024). However, two-way repeated measure ANOVA found no significant difference between the DLT group and the NOR group in their handgrip response.

The exponents of the power function relationships between load and estimated magnitude of load, i.e., the slope of logHG%-logR and the slope of logHG%-logPm, reflects the sensitivity of the subject to the load. The mean slope of logHG%-logR for the DLT group and the NOR group was 0.39 ± 0.08 and 0.42 ± 0.06, respectively. The mean slope of logHG%-logPm for the DLT group and the NOR group was 0.66 ± 0.15 and 0.57 ± 0.07, respectively. There was no significant difference between the two groups for either slope.

Two-way repeated measure ANOVA showed no main effects of group for Pm, Vmax, peak airflow, TI, TE, TP, f and VE. This indicated that the loaded breathing pattern during magnitude estimation was not significantly different between the DLT group and the NOR group. All the above breathing pattern parameters displayed a significant load effect (p < 0.05), except TE (p = 0.068). There was significant load and group interaction effects for airflow, TI and f (p < 0.05).
Figure 4-13 Handgrip Response to Resistive Load

Figure 4-14 Handgrip Response to Mouth Pressure during Resistive Loading
4.5 Respiratory Related Evoked Potential (RREP)

There were no identifiable RREP components during control trial for both DLT and the NOR group (Figure 4-15 and Figure 4-16, respectively).

The mean numbers of trials used in group averaging during attend trial and ignore trial for DLT patients was 81.5 and 82.1, respectively; and 79.5 and 84.1, for NOR subjects, respectively. No significant differences in the number of averaged trials were observed either between trials (attend vs ignore, p > 0.05) or between DLT and the NOR group (p > 0.05).

A constant pattern of RREP components (P1, Nf, P1a, N1, P3) were identified during attend and ignore trial in most subjects for both the DLT group and the NOR group. Two DLT subject and five NOR subjects did not have recognizable P1 during attend trial. Four DLT subjects and three NOR subjects did not have recognizable P1 during ignore trial. P1a, Nf, N1 were identified in all subjects for both trials. All subjects had identifiable P3 during attend trial. However, three DLT subjects and one NOR subject did not have recognizable P3 during ignore trial.

<table>
<thead>
<tr>
<th>RREP latency</th>
<th>DLT attend</th>
<th>DLT ignore</th>
<th>NOR attend</th>
<th>NOR ignore</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>25.6 ± 1.9</td>
<td>28.1 ± 2.4</td>
<td>25.3 ± 1.1</td>
<td>26.0 ± 1.9</td>
</tr>
<tr>
<td>P1a</td>
<td>58.0 ± 2.7</td>
<td>58.7 ± 1.7</td>
<td>65.5 ± 3.7</td>
<td>66.0 ± 4.2</td>
</tr>
<tr>
<td>Nf</td>
<td>50.8 ± 3.9</td>
<td>48.2 ± 2.0</td>
<td>46.3 ± 1.4</td>
<td>48.7 ± 3.1</td>
</tr>
<tr>
<td>N1</td>
<td>107.0 ± 3.3</td>
<td>106.0 ± 3.2</td>
<td>99.0 ± 2.4</td>
<td>102.0 ± 6.9</td>
</tr>
<tr>
<td>P3</td>
<td>340.0 ± 9.9*</td>
<td>380.0 ± 2.4</td>
<td>292.0 ± 9.9*#</td>
<td>314.0 ± 1.4#</td>
</tr>
</tbody>
</table>

DLT, double lung transplant group. NOR, normal group.
Data are shown as mean ± SEM.
* indicates a significant trial effect (p < 0.05). # indicates a significant group effect (p < 0.05).
Figure 4-15 Signals of RREP during Control Trial in a Double Lung Transplant Patient

Figure 4-16 Signals of RREP during Control Trial in a Normal Subject
The mean latency of each component is shown in Table 4-4. Two-way repeated measure ANOVA was conducted to investigate the effect of group (DLT vs NOR), trial (attend vs ignore), and the interaction effect between group and trial for each component. For all the early-latency components (P1, P1a, Nf, N1), no significant main effects of group, trial and interaction effects were found (p > 0.05). Both group and trial main effects were significant for P3 component, but not for the interaction effect between group and trial. Specifically, P3 occurred significantly later in the DLT group than the NOR group (p = 0.012), and significantly earlier during attend trial than ignore trial (p = 0.004). Central process time (CPT) was calculated by subtracting P1a latency from P3 latency. For DLT subjects, mean CPT was 282.0 ± 9.8ms during attend trial, and 323.0 ± 23.4ms. For NOR subjects, mean CPT was 226.0 ± 11.9ms during attend trial, and 246.0 ± 17.0ms during ignore trial. The main effect of group was significant (p = 0.007), so was the main effect of trial (p = 0.014). Specifically, CPT was significantly longer for the DLT group than the NOR group, and significantly shorter during attend trial than during ignore trial. No significant interaction effect between group and trial was found for CPT. The group result of P3 and CPT were shown in Figure 4-17 and Figure 4-18, respectively.

The mean peak amplitudes of each RREP component (P1, Nf, P1a, N1, P3) were measured in all 12 scalp positions: F3, Fz, F4, C3, Cz, C4, C3’, Cz’, C4’, P3, Pz, P4. When the P1 was absent, amplitudes were treated as missing data in further analysis. When the P3 was absent, amplitudes were treated in two ways in further analysis: missing data and zero amplitude. Three-way repeated measure ANOVA was used to investigate the main effects of group (DLT vs NOR), trial (attend vs ignore), scalp location, the interaction effects between group and trial, group and scalp location, trial and scalp
location, and the interaction effect among the three factors: group, trial and scalp location. All RREP components showed a significant main effect of scalp location (p < 0.05). The mean amplitudes of each component in major scalp locations where they were found maximally were shown in Table 4-5.

Further contrast analysis was conducted to compare the amplitude of each RREP component among frontal region (F3, Fz, F4), central region (C3, Cz, C4), centroparietal region (C3’, Cz’, C4’) and parietal region (P3, Pz, P4). A total of 6 comparisons were made for each component, therefore p-value was corrected by dividing 0.05 by 6, which equals to 0.00833. The results of contrast analysis were shown in Table 4-6. Similar to previous reports (42, 141), P1 was found maximally in central and centroparietal regions. Nf was found maximally in frontal regions. N1 was found maximally in central regions. P1a and P3 were found maximally in centroparietal and parietal regions.

A three-way repeated measure ANOVA found no significant group main effects on amplitudes for all the components, except for P3, when unidentifiable P3 was treated as zero amplitude in analysis (p = 0.0235). A significant interaction effect between group and scalp location were found for P1 and N1. A significant interaction effect between trial and scalp location were found for Nf and N1. A significant group, trial and scalp location interaction effect was found only for Nf. In an effort to increase statistical power, an additional two-way repeated measure ANOVA was conducted only for attend trial, during which P3 were identifiable in all subjects. The two factors were group (DLT vs NOR) and scalp location. The main effects for both group and scalp locations were significant. the DLT group had significantly lower P3 amplitude than the NOR group (p
< 0.05). And no significant interaction effect was found between group and scalp location.

Figure 4-17 Latency of P3 (* indicates significant trial effect (attend vs ignore, p < 0.05). ** indicates significant group effect (DLT vs NOR, p < 0.05))

Figure 4-18 Central Process Time (* indicates significant trial effect (attend vs ignore, p < 0.05). ** indicates significant group effect (DLT vs NOR, p < 0.05))
Table 4-5. Amplitudes of RREP

<table>
<thead>
<tr>
<th>RREP amplitude (uV)</th>
<th>Electrode position</th>
<th>DLT</th>
<th>NOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>attend</td>
<td>ignore</td>
<td>attend</td>
</tr>
<tr>
<td>P1♣</td>
<td>C3</td>
<td>-2.93±0.6</td>
<td>-2.10±0.4</td>
</tr>
<tr>
<td></td>
<td>Cz</td>
<td>-2.49±0.5</td>
<td>-1.62±0.5</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>-2.63±0.4</td>
<td>-1.38±0.4</td>
</tr>
<tr>
<td></td>
<td>C3'</td>
<td>-2.47±0.8</td>
<td>-1.83±0.4</td>
</tr>
<tr>
<td></td>
<td>Cz'</td>
<td>-2.36±0.5</td>
<td>-1.33±0.3</td>
</tr>
<tr>
<td></td>
<td>C4'</td>
<td>-2.28±0.5</td>
<td>-0.83±0.3</td>
</tr>
<tr>
<td>P1a</td>
<td>C3'</td>
<td>-1.12±0.6</td>
<td>-1.62±0.7</td>
</tr>
<tr>
<td></td>
<td>Cz'</td>
<td>-0.84±0.5</td>
<td>-1.38±0.4</td>
</tr>
<tr>
<td></td>
<td>C4'</td>
<td>-1.12±0.5</td>
<td>-1.06±0.3</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>-1.15±0.5</td>
<td>-2.12±0.6</td>
</tr>
<tr>
<td></td>
<td>Pz</td>
<td>-1.03±0.4</td>
<td>-1.67±0.5</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>-1.24±0.5</td>
<td>-1.57±0.4</td>
</tr>
<tr>
<td>Nf</td>
<td>F3</td>
<td>3.76±0.9</td>
<td>4.25±0.5</td>
</tr>
<tr>
<td></td>
<td>Fz</td>
<td>3.83±0.9</td>
<td>4.93±0.7</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>3.36±0.8</td>
<td>4.63±0.6</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>2.03±0.6</td>
<td>1.32±0.4</td>
</tr>
<tr>
<td></td>
<td>Cz</td>
<td>2.38±0.5</td>
<td>2.20±0.4</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>1.93±0.5</td>
<td>2.03±0.2</td>
</tr>
<tr>
<td>N1</td>
<td>C3</td>
<td>6.36±0.9</td>
<td>4.57±0.5</td>
</tr>
<tr>
<td></td>
<td>Cz</td>
<td>4.27±0.8</td>
<td>2.83±0.6</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>4.43±0.7</td>
<td>3.74±0.6</td>
</tr>
<tr>
<td></td>
<td>C3'</td>
<td>4.59±0.6</td>
<td>3.49±0.6</td>
</tr>
<tr>
<td></td>
<td>Cz'</td>
<td>3.05±0.7</td>
<td>2.62±0.6</td>
</tr>
<tr>
<td></td>
<td>C4'</td>
<td>3.15±0.8</td>
<td>2.84±0.7</td>
</tr>
<tr>
<td>P3♣</td>
<td>C3'</td>
<td>-5.33±1.2</td>
<td>-2.85±1.1</td>
</tr>
<tr>
<td></td>
<td>Cz'</td>
<td>-5.37±1.2</td>
<td>-3.36±1.0</td>
</tr>
<tr>
<td></td>
<td>C4'</td>
<td>-5.54±1.1</td>
<td>-2.53±0.7</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>-5.57±1.3</td>
<td>-3.80±0.7</td>
</tr>
<tr>
<td></td>
<td>Pz</td>
<td>-5.49±1.3</td>
<td>-3.00±1.1</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>-5.44±1.1</td>
<td>-2.61±0.7</td>
</tr>
<tr>
<td>P3♦</td>
<td>C3'</td>
<td>-5.33±1.2</td>
<td>-1.90±0.8</td>
</tr>
<tr>
<td></td>
<td>Cz'</td>
<td>-5.37±1.2</td>
<td>-2.35±0.9</td>
</tr>
<tr>
<td></td>
<td>C4'</td>
<td>-5.54±1.1</td>
<td>-1.78±0.6</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>-5.57±1.3</td>
<td>-2.66±0.8</td>
</tr>
<tr>
<td></td>
<td>Pz</td>
<td>-5.49±1.3</td>
<td>-2.10±0.9</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>-5.44±1.1</td>
<td>-1.83±0.6</td>
</tr>
</tbody>
</table>

Data were shown as mean ± SEM. DLT, double lung transplant group. NOR, normal group.
♣ Absent peak were treated as missing data in analysis
♦♦♦♦ Absent peak were treated as zero amplitude in analysis.
Table 4-6. Contrast Analysis for RREP Amplitudes in Different Scalp Regions

<table>
<thead>
<tr>
<th></th>
<th>F3,</th>
<th>C3,</th>
<th>C3',</th>
<th>P3,</th>
<th>F3,</th>
<th>C3,</th>
<th>C3',</th>
<th>P3,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fz,</td>
<td>Cz,</td>
<td>Cz',</td>
<td>Pz,</td>
<td>F4,</td>
<td>C4,</td>
<td>C4',</td>
<td>P4,</td>
</tr>
<tr>
<td>P1a</td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nf</td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3a</td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F3,Fz,F4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3,Cz,C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C3',Cz',C4'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P3, Pz, P4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Absent peak were treated as missing data in analysis
♦♦♦♦ Absent peak were treated as zero amplitude in analysis.
* indicates a significant difference in comparison between two regions, p < 0.00833 (a total of 6 comparisons for each parameter, so p-value is corrected as 0.05 divided by 6, which equals to 0.00833).

The grand average signals for mouth occlusion pressure were shown in Figure 4-19 and Figure 4-20. At the onset of occlusion, mouth pressure changes were very similar in the DLT group and the NOR group during both attend and ignore trials.

Figure 4-19 Mouth Occlusion Pressure (Grand Average) Trace During Attend Trial
Table 4-7. Mouth Occlusion Pressure

<table>
<thead>
<tr>
<th>Pressure (cmH2O)</th>
<th>DLT attend</th>
<th>DLT ignore</th>
<th>NOR attend</th>
<th>NOR ignore</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.42 ± 0.1</td>
<td>1.57 ± 0.1</td>
<td>1.62 ± 0.2</td>
<td>1.61 ± 0.2</td>
</tr>
<tr>
<td>Nf</td>
<td>1.97 ± 0.1</td>
<td>2.08 ± 0.1</td>
<td>2.12 ± 0.2</td>
<td>1.95 ± 0.2</td>
</tr>
<tr>
<td>P1a</td>
<td>2.11 ± 0.1</td>
<td>2.34 ± 0.1</td>
<td>2.47 ± 0.3</td>
<td>2.20 ± 0.2</td>
</tr>
<tr>
<td>N1</td>
<td>2.78 ± 0.1</td>
<td>3.06 ± 0.2</td>
<td>2.90 ± 0.3</td>
<td>2.70 ± 0.3</td>
</tr>
<tr>
<td>P3</td>
<td>2.56 ± 0.5*</td>
<td>4.54 ± 0.4</td>
<td>3.22 ± 0.6*</td>
<td>3.56 ± 0.3</td>
</tr>
</tbody>
</table>

DLT, double lung transplant group. NOR, normal group.
Data were shown as mean ± SEM.
* indicates a significant difference between attend trial and ignore trial (p < 0.05).

Table 4-7 displays mouth occlusion pressure recorded corresponding to each RREP component: P1, Nf, P1a, N1, P3. These pressure points can be used to reflect the mouth pressure trace elicited by inspiratory occlusion. Two-way repeated measure ANOVA was used to investigate the effect of group (DLT vs NOR), trial (attend vs ignore condition), and interaction effect between group and trial for each pressure point.
There is no significant difference between DLT and NOR on all the mouth pressure values (p > 0.05). The main effect of trial is not significant for all the pressure values, except that mouth pressure recorded corresponding to P3 was significantly lower in the attend trial, compared with the ignore trial (p = 0.003). No interaction effects between group and trial were found for all the pressure points.
CHAPTER 5  
DISCUSSIONS

5.1 Summary of Results

The results of this project are summarized as the followings:

1. Breathing pattern and load compensation: no significant differences in the unloaded breathing pattern (Pm, Vmax, flow, TI, TE, TP, breathing frequency, VE) were found in the DLT group, as compared with the NOR group. Load compensation responses in the DLT group were similar to the NOR group: as the magnitude of the resistive load increased, Pm, TI and TP increased, while Vmax, peak inspiratory airflow, TE, breathing frequency and VE decreased.

2. Load detection: detection threshold and Weber fraction were significantly elevated in the DLT group, as compared with the NOR group. Consistently, detection percent was also significantly lower for the same magnitude of resistive loads in the DLT group. These results suggest that lung vagal afferent input contributes to load detection.

3. Magnitude estimation of resistive loads: handgrip responses were similar in the DLT and the NOR group. No significant differences were found in the slopes of logPm-logHG% and logR-logHG% between the two groups. These results suggest that the sensitivity of the subject to the load magnitude was not changed after lung denervation.

4. RREP: a constant pattern of RREP components (P1, Nf, P1a, N1, P3) were identified during attend trial and ignore trial in most subjects for both the DLT group and the NOR group. The peak latencies and peak amplitudes of early-latency components (P1, P1a, Nf, N1) were similar in the DLT group and the NOR group. However, the peak latency of the late-latency component (P3) was significantly longer in the DLT group. The peak amplitude of P3 during attend trial was also significantly lower in the DLT group. These results suggest that lung denervation did not significantly affect the early-latency RREP components, but attenuated the P3 response.

The following discussion will be divided into four parts, based on the experiment results summarized as above.
5.2 Breathing Pattern and Load Compensation Response

5.2.1 Resting Breathing Pattern in Double Lung Transplant Patients

The absence of pulmonary vagal afferents in many mammals has been found to be associated with an increased tidal volume (VT) and reduced breathing frequency (38, 110). This is due to abolition of pulmonary stretch receptor input to the Hering-Breuer inflation reflex. However, the inflation reflex is relatively weak in humans in comparison with animals, and is demonstrable only with large inflations. It would thus be expected that the vagal influence on breathing pattern might be substantially less in humans.

The effects of vagal afferents upon the pattern of breathing in man at rest are still controversial. Vagal blockade (68) and airway anesthesia (145) experiments have failed to show any significant effects of pulmonary afferents on resting breathing patterns in humans. However, the results from those studies may be confounded by the technical limitation on the completeness of lung deafferentation.

Lung transplantation interrupts afferent traffic from receptors located distal to the surgical anastomosis, thus providing an ideal model to investigate the role of lung vagal afferents in regulation of breathing in human. In this study, the breathing pattern was recorded for both loaded breathing and the breathing cycle prior to each load (control breath) during the detection experiment in both DLT subjects and matched normal control subjects. The control breathing pattern recorded during the detection experiment reflects the subjects’ spontaneous resting breathing pattern, because all subjects were instructed to breathe normally throughout the experiment, and there is no visual or auditory cue provided to indicate the loaded inspiration. We found that there were no significant differences in peak Pm, Vmax, peak inspiratory airflow, TI, TE, TP, breathing frequency and VE between DLT subjects and normal subjects. Our results suggest that
lung vagal afferents are not essential to the regulation of resting breathing pattern in humans.

Our findings were consistent with other lung transplant studies (87, 129). Shea and co-workers (129) compared resting breathing pattern in heart-lung transplant patients (HLT), heart transplant patients, as well as in normal subjects. They found no difference in ventilation, VT, frequency, TI and expiratory time among all three groups during wakefulness and sleep. Kimoff et al. (87) also failed to find any major differences in ventilatory level or pattern between HLT patients and normal subjects.

In contrast, other studies have reported elevated frequency and reduced TI in lung transplant recipients (88, 122). However, the lung transplant subjects in those studies had a restrictive spirometric pattern. A relationship between increased lung elastance and increased breathing frequency has been reported by Renzi and colleagues (116, 117). Furthermore, Sanders et al. (124) observed that the HLT recipients with a restricted spirometric pattern had a higher breathing frequency during wakefulness and sleep, compared with those recipients without a restrictive pattern. Therefore, the reduced TI and greater frequency of breathing are probably related to the presence of underlying pulmonary restriction rather than lung-lower airway deafferentation. Our DLT subjects did not display a restrictive spirometric pattern. The group mean value of FVC for DLT was 97 ± 4.9% of predictive values, and the FEV1/FVC ratio was 88.1 ± 6.0% of predicted value. All the measurements of pulmonary function in the DLT group are within normal limits, and FEV1/FVC did not differ significantly from the NOR group. The difference in our result and those of Kinnear and Sanders (88, 122) is probably due
to the difference in the pulmonary function of lung transplant patients recruited. Thus, the role of lung vagal afferents is not evident in spontaneous ventilation.

5.2.2 Loaded Breathing Pattern in Double Lung Transplant Patients

The ventilatory response to added mechanical loads can be regarded as the sum of two components: one representing the effect of the passive respiratory system and one representing the effect of neural load-compensating mechanisms (9). The load-compensating component represents the action of neural mechanisms that modify the pressure developed by loaded respiratory muscles. Receptors in lung and lower airway could potentially contribute to these neural adjustments. However, our results showed no significant group difference. For both the DLT and NOR subjects, as the magnitude of the resistive load increased, Pm, TI and TP increased, while Vmax, flow, TE, breathing frequency and VE decreased. These results indicate that lung vagal afferent input doesn’t play a significant role in load compensation response.

Load compensation response in DLT patients was studied by Peiffer et al. (108). In contrast to our findings, they reported that despite higher intersubject variability, the ventilatory responses differed significantly between lung transplant recipients and controls when breathing against resistive loads. The lung transplant recipients produced higher peak mouth pressure and inspiratory flow rate. However, in their protocol, the load was applied after a short vocal cue. Subjects’ breathing patterns might change in response to the cue. It is not known whether there is a difference in their reaction to the cue between DLT and the NOR group, but it is likely that the cue allowed the subject to prepare for the load, thus adding a voluntary component to the load compensation response. Moreover, the inspiratory resistive loads were presented for the duration of two consecutive inspiratory breaths. It is not known whether their data came from the first or
the second loaded breath. Load compensation response will be different for a first-breath response as compared to a second-breath response. Finally, muscle strength was not compared between their lung transplant recipients and controls. Lung transplant recipients usually have weakened respiratory muscles because of the use of steroid medications and deconditioning after surgery. Most studies demonstrated a close relationship between weak muscle strength and increased respiratory drive (4, 65). The increase in peak mouth pressure and inspiratory flow rate during loaded breathing might be due to increased respiratory drive in those lung transplant patients (17, 140). In our study, MIP, a commonly used measurement representing inspiratory muscle strength, was not significantly different in the DLT group from the NOR group. This is, in fact, due to the enrollment of our DLT patients in an inspiratory muscle strength training program as part of their pre and post transplant rehabilitation. Although we did not measure inspiratory drive directly, it would be reasonable to believe the drive would be similar due to similar inspiratory muscle strength in these two groups.

Forster et al. (58) studied resistive load compensation response in awake intact (I), diaphragm deafferentation (DD), pulmonary vagal (hilar nerve) denervation (HND), and DD+HND ponies. They found that first-breath load compensation remained after DD, HND, and DD+HND, but after DD+HND, tidal volume and inspiratory minute ventilation were compensated 5-10% less than in I ponies. They concluded that diaphragm and pulmonary afferents contributed to but were not essential for inspiratory load compensation in awake ponies. Our results were in line with Forster’s (58). It is thus reasonable to conclude that the afferent pathway from the lung is not providing a major afferent effect on the regulation of the ventilatory response to inspiratory loads.
An important assumption of this study is that double lung transplants are, and remain, entirely denervated after surgery. The results of several investigations performed in animals found reappearance of a weak Hering-Breuer inflation reflex as early as 5 months after pulmonary autotransplantation (54, 97). However, reinervation would be less likely in the context of human allotransplantation than with simple reimplantation of an excised lung as in the canine model since no attempt is made to approximate nerves in DLT patients, which may vary widely in position in donor and recipient structures (87). In a study investigating the integrity of the cough reflex, which is mediated mostly by pulmonary receptors, following lung transplant, Higenbottam and co-workers (73) observed a significantly diminished cough response to ultrasonically nebulized distilled water for up to 3 years after lung transplant. More compelling evidence for persistent lung denervation following human lung transplant has been provided by Iber et al. (75). They recently reported persistently absent expiratory prolongation following passive lung inflation during sleep in bilateral lung transplant recipients for a period of 49 months after surgery. In this study, the time since the patients received double lung transplant surgery varied from 1.5 to 5.5 years, with an average of 3.45 years. Although we did not test the possibility of reinnervation in our patients, it seems highly unlikely that reinnervation had occurred based on previous findings (73, 75).

In summary, the present results failed to find any significant difference in breathing pattern during both unloaded and loaded inspiration between the DLT group and the NOR group. These results suggest that lung vagal afferent inputs are not active in the regulation of resting breathing pattern and load compensation responses in humans.
5.3 Detection of Resistive Loads

5.3.1 Load Detection in Normal Subjects

In the present study, the detection threshold to resistive loads and Weber Fraction in the NOR group are $1.55 \pm 0.31 \text{ cmH}_2\text{O/L/s and } 0.30 \pm 0.05$, respectively. Compared with the early work done by Bennett et al (19) and Wiley and Zechman (144), the threshold value found in this study was much higher. Kellerman et al. (80) recently reported the mean detection threshold for resistive loads in normal subjects was even higher ($3.43 \text{ cmH}_2\text{O/L/s}$). The variability in the detection threshold value among those studies is due to the variation in the background resistance, which includes both the subject’s intrinsic respiratory resistance and the resistance of the apparatus. Wiley and Zechman (144) studied the relationship between the background resistance and the detection threshold. They found that for various experimental conditions with different background resistance, the Weber Fraction remained constant (0.25 - 0.3) despite a large variability in the reported detection threshold values. In the present study, the value of the Weber Fraction ($0.3 \pm 0.05$) in normal subjects was similar to previous reports.

Load specific breathing pattern plays an important role in load detection (48). Application of resistive loads impedes inspiratory airflow during breathing. During a loaded inspiration, mouth pressure continues to increase and reaches its peak at approximately mid-inspiration, when airflow peaks. Figure 5-1 shows the breathing pattern and detection signal for one normal subject in this study. As shown in this figure, when the magnitude of the resistive load increased, airflow tended to decrease due to the impedance imposed by the resistive load, and reached maximum at mid-inspiration. Inspiratory volume decreased as the load increased and reached peak at the end of
inspiration. As the load increased, more negative pressure is needed to overcome the resistance. The mouth pressure pattern was consistent with the airflow pattern, that is, the most negative pressure occurred at about mid-inspiration when airflow was highest. In Figure 5–1, the subject could not detect the lowest load, but detected all the remaining 7 larger loads. And the detection signal started at approximately mid-inspiration, when airflow and mouth pressure reached peak. ANOVA analysis found a significant load magnitude effect on det%. Specifically, the larger the magnitude of a load, the higher the detection percentage of the load.

Our results were consistent with previous studies. It has been found that detection for the resistive load occurred in mid-inspiration near peak inspiratory flow, whereas Tdet for elastic loads occurred significantly later near the end of breathing when the inspired volume reached the peak (150). Killian et al. (86) compared ΔR50 under control
conditions with that obtained when loads were applied at different times in inspiration, with different inspiratory flows, at different lung volumes, and with different background loads. Their results suggest that external resistive load detection is subserved by the relationship between pressure and flow over the early part of inspiration, rather than changes in pressure itself.

5.3.2 Load Detection in Double Lung Transplant Patients

Despite a similar loaded breathing pattern, as discussed in section 5.2, the DLT group had a significantly higher detection threshold (2.91 ± 0.5 cmH₂O/L/s, p < 0.05) and Weber Fraction (0.50 ± 0.1, p < 0.05) than the NOR group. The group effect on det% was significant, with a lower det% found in the DLT group. Our results suggest that pulmonary vagal afferents may contribute to load detection. The breathing pattern and detection signals in one DLT subject were shown in Figure 5-2.

Figure 5-2 Breathing Pattern during Resistive Load Detection in a Double Lung Transplant Subject
Although the detection of inspiratory loads has been studied extensively, the site at which such detection occurs is still not known. There are a variety of mechanoreceptors located in lung and lower airway, which are innervated by the vagus nerves. Afferent information from those receptors related to respiratory mechanical changes during loaded breathing may contribute to the detection of external loads.

Two strategies have been adopted to determine the role of pulmonary receptors in respiratory load perception: either their principle afferent nerve (vagus nerve) is selectively blocked, or alternatively, all other possible sources are eliminated, leaving only the vagal input intact. High-level quadriplegic subjects with tracheostomies provide indirect evidence about the role of pulmonary afferents in respiratory sensation, because both respiratory muscle afferents and upper airway receptors are bypassed, leaving only the pulmonary receptors intact. It has been reported that these patients could reliably detect changes in tidal volume as little as 100 mL, which was comparable to that of normal subjects (15). Similarly, other studies (102, 152) also found that detection of external loads did not appear to be impaired in quadriplegic patients in whom afferent pathways from the chest wall are disrupted. These finding suggest the possibility that pulmonary receptors may contribute to load detection.

Contradictory results about the role of lung vagal afferents in load detection were reported by Guz et al. (67). They studied the effect of bilateral block of the vagus and glossopharyngeal nerves in two healthy subjects. The difference threshold for elastic load detection was not affected by the nerve block. Furthermore, there was also no change in the sensation associated with a high resistive load in one subject. These results suggest that pulmonary vagal afferents do no contribute to load detection. Chaudhary and Burki
(25, 29, 30) showed that upper and lower airway anesthesia in normal subjects did not alter the detection thresholds of either resistive or elastic loads. Nonetheless, it is possible that some pulmonary stretch receptors may escape topical anesthesia because the anaesthetic could not penetrate to the smooth muscle or because the drug was carried away rapidly by the rich blood flow (14). Moreover, since both upper and lower airway receptors were blunted in their methods, it is not possible to make a conclusion about the specific role of lung and lower airway afferents in load detection.

Lung transplantation, through a total interruption of afferent nerve fibers from the lung and lower airways, provides an opportunity to study the contribution of neural feedback from lung and lower airways to respiratory sensation. Besides the present study, there is only one another investigation of the resistive load detection in lung transplant recipients by Tapper and his co-workers (139). They compared the detection threshold of inspiratory resistive loads in heart-lung transplant recipients (HL), heart transplant recipients (H) and normal subjects (C), and found no significant difference in the Weber fraction associated with a 50% probability of load detection between HL and C group (0.32±0.05 and 0.34±0.05 in the HL patients and normal subjects, respectively). Therefore, they concluded that lower respiratory tract afferents did no play a significant role in the perception of respiratory resistive loads. In contrast to their results, we found a significantly higher detection threshold and Weber Fraction in double lung transplant recipients than normal subjects (2.91 ± 0.5 cmH$_2$O/L/s vs 1.55 ± 0.3 cmH$_2$O/L/s, and 0.50 ± 0.1 vs 0.30 ± 0.05, respectively). The difference between our study and Tapper’s study might be due to the difference in lung transplant patients and the method applied to determine detection threshold. Their HL patients are younger than our DLT patients (33.7 ± 1.5 years old vs
Although neither of our studies found a significant correlation between age and Weber Fraction, the effect of age on resistive load perception is still unclear. In the Tapper et al. (139) study, a tracking procedure was performed to determine the detection threshold. The tracking procedure causes more false positive responses. Moreover, the imposition of resistance or shams was signaled by an audible during the preceding exhalation. It is reasonable to believe that a cue would improve a person’s detection performance, which might result in their lower Weber Fraction in HL patients. Finally, trials were excluded when more than two sham presentations were inappropriately signaled as added resistance. The relationship between false positive response rate and detection threshold has never been investigated. It is also unknown whether there is a difference in the number of false positive responses during resistive load detection between HL patients and normal subjects. It is possible that the lower detection threshold found in HL patients in Tapper et al. (139)’s study were due to differences in the methodology.

In the present study, the DLT patients’ FEV1 and FVC values were significantly lower than the NOR subjects (83.8 ± 6.1% vs 108.3 ± 3.7% of predictive values, and 97.0 ± 4.9% vs 119.5 ± 5.0% of predictive values, respectively). However, both FEV1 and FVC are well within normal limits. It is unlikely that an increased detection threshold found in the DLT group was due to their lung function. The Weber Fraction, which controlled for the effect of background resistance, was significantly higher in the DLT group than the NOR group. Furthermore, no significant correlation has been found between either FVC or FEV1 and Weber Fraction in both DLT and NOR subjects (p > 0.05).
It is possible that the medications taken by DLT recipients would have caused their higher detection threshold. In the present study, all DLT subjects were still on immunosuppressive agents and steroid medications. None of those medications have been studied for their effect on resistive load detection. Further studies are necessary to investigate the effect of these drugs on resistive load detection.

In summary, we found that DLT recipients had a significant higher detection threshold and Weber Fraction than the NOR group. The impaired detection capability is likely due to the loss of lung vagal afferent inputs in those lung denervated patients. Resistive load detection did occur in DLT patients. This means that non-vagal afferents are activated during breathing against resistive loads and do elicit load detection response. The results of this study suggest that vagal afferents play a role in resistive load detection. The detection threshold is increased with the loss of vagal afferents. However, the effect of DLT medications on load detection cannot be ruled out.

5.3 Magnitude Estimation

5.3.1 Magnitude Estimation in Normal Subjects

Many studies have examined the relationship between the perceived magnitude of loads and the intensity of the load (26, 66, 84, 137, 138, 146). In the present study, the subjects were asked to provide an estimate about the magnitude of the resistive load by using their handgrip response (HG%). Similar to previous studies, as the load increased, larger handgrip responses were given by all subjects. The sensitivity of the person to the stimulus, i.e., the mean slope in the NOR group was 0.57 ± 0.07 and 0.42 ± 0.06, when HG% was plotted against peak Pm and resistive load magnitude (R) using a log-log transformation, respectively.
Revelette et al. (119) found that increased background loading was associated with a significant increase in the exponent for magnitude estimation for resistive loads. In contrast, Burdon et al. (24) reported that increased background resistance or elastance did not change either the exponent or the intercepts of the power function between peak inspiratory pressure and the perceived magnitude. Although we did not specifically study the effect of background resistance on the perception of load magnitude, we found that the correlation between background resistance and the slope were not significantly for either slope of \( \log Hg\%-\log Pm \) or slope of \( \log HG\%-\log R \) in our subjects.

Breathing against an external mechanical load will change the breathing pattern in a load-specific manner. Such changes in breathing pattern might be involved in load magnitude estimation (83, 84). The breathing pattern and handgrip responses in a normal subject during the magnitude estimation experiment were shown in Figure 5-3. Although the subjects were instructed to target their resting airflow throughout the experiment, in reality, group mean peak inspiratory airflow at higher loads was still significantly less than those at lower loads. When the magnitude of resistive load increased, \( Pm \) also increased significantly to overcome the load. The mouth pressure pattern is consistent with the airflow pattern, that is, the most negative pressure occurred at about mid-inspiration when airflow is highest. Peak volume tended to increase as load increased, which might be due to prolonged inspiration when breathing against higher loads. As the load increased, handgrip response increased, as well. And the peak handgrip response occurred at approximately mid-inspiration, when airflow and mouth pressure reached peak. The handgrip response pattern is consistent with earlier studies (154).
The slopes obtained in normal subjects in the present study (0.57 ± 0.07 for logHg%-logPm, and 0.42 ± 0.06 for logHg%-logR, respectively) were lower compared with previous reports, in which the exponents varied from 0.57 to 0.96 (26, 66, 84, 95, 119). The difference might be due to the difference in the subjects’ age, the load range, the scale used to estimate magnitude, airflow targeting, etc.. The mean age of normal subjects in this study was 46.6 ± 4.4 years old, which was older than those previous reports. Tack et al. studied the effect of age on magnitude estimation (137). He found that the exponent for both inspiratory and expiratory loads is reduced in the older group. Because peak airway pressure and the inspiratory and expiratory duration during loaded breathing were the same in both age groups, they hypothesized that the difference in exponents in the two groups was probably due to age-related changes in sensory perception. In the present study, the resistive loads ranged from 1.64 cmH$_2$O/L/s to 42.62
A "range effect" is another possibility to explain the differences in exponent obtained from other studies. This refers to a general law in psychophysics stating that the higher the range of a given stimulus, the lower the rate of increase in the intensity of the induced sensation (107). Therefore, the lower slope found in this study might be due to a relatively large load range used in the magnitude estimation task.

Moreover, some studies used numerical scales (e.g. Borg scale) (66, 84, 95), while others (including the present study) used cross-modality matching (e.g. handgrip response) (26, 119) to estimate load magnitude. Unlike the Borg scale, for which subjects only needed to select a number from the Borg scale to match their breathing difficulty, there are two steps involved in the process when using handgrip to express their estimation of the load magnitude. The first step is the subject’s detection and quantification of the load. The second step, the subject has to translate the respiratory sensation into a handgrip response quantitatively. At the same time, the subject’s sensation arising from their handgrip squeezing has to be transferred back to the higher brain center to match the previous sensations about the external load they breathe against. It is possible that handgrip and Borg scale are results from two different types of neural processing. Muza et al. (99) compared different scales in magnitude estimation. The mean exponent and correlation coefficient obtained from numerical estimates were 1.11 +/- 0.16 and 0.94 +/- 0.04, respectively, while the exponent and correlation coefficient simultaneously obtained from handgrip matching was 0.73 +/- 0.10 and 0.91 +/- 0.05, respectively. In a recent work done in our lab, it was found that the Borg scale had slightly higher correlation with load stimulus than handgrip response (unpublished data). And it was also found that the Borg scale was used over a wider range and had smaller
inter-subjects variation. Therefore, the choice of different scaling method will affect the value of the exponent. A final explanation about the difference in exponent among studies might be due to how well airflow was targeted in those studies. In the present study, even though the subjects are instructed to maintain the airflow level during loaded breathing, airflow still decreased as load increased, especially during the three highest loads (R8, R9 and R10). Therefore, it was possible that the actual impedance of those high loads were underestimated, which might flatten the regression line when logHG% was plotted against logR.

5.3.2 Magnitude Estimation in Double Lung Transplant Patients

The loaded breathing pattern and handgrip response during magnitude estimation experiment were similar between the DLT group and the NOR group (see Figure 5-4). Both groups showed a higher handgrip response as load magnitude increased. There were no significant differences in the slope of logHG%-logPm (0.66 ± 0.15 in DLT vs 0.57 ± 0.07 in NOR, p = 0.59) and the slope of logHG%-logR (0.39 ± 0.08 in DLT vs 0.42 ± 0.06 in NOR, p = 0.76). Our results suggest that lung vagal afferents are not essential to magnitude estimation of suprathreshold loads.

Similar to our findings, Burki et al. (26) found that anesthesia of the upper and lower airways did not significantly alter the slope between log added resistive load and log handgrip response. However, it is possible that some pulmonary stretch receptors may escape topical anesthesia because the anaesthetic could not penetrate to the smooth muscle or because the drug was carried away rapidly by rich blood flow (14). Moreover, since both upper and lower airway receptors were blunted in their methods, it is not
possible to make a conclusion about the specific role of lung and lower airway afferents in load magnitude estimation.

Figure 5-4 Breathing Pattern and Handgrip Response during Magnitude Estimation in a Double Lung Transplant Patient

Lung transplantation, through a total interruption of afferent nerve fibers from the lung and lower airways, provides an opportunity to study the contribution of neural feedback from lung and lower airways to respiratory sensation. Recently, Peiffer et al. compared sensations related to inspiratory resistive loaded breathing in lung transplant recipients and healthy control subjects (108). In contrast to our results, they found that the slope of Borg scale as a function of peak Pm was significantly lower in lung transplant recipients than controls (0.63 vs 1.26, p < 0.01). Moreover, the loaded breathing pattern was also different between the two groups, with a significantly higher peak Pm and peak inspiratory airflow rate in the lung transplant recipients. Although they did not report the analysis in absolute Borg scale scores between the two groups, it appeared that the difference in the slope of Borg scale as a function of Pm was only due to the difference in
Pm between the two groups. Indeed, their lung transplant recipients had a significantly higher peak Pm and higher individual range of Pm than normal subjects (10.4 ± 5.6 cmH2O vs 4.8 ± 0.96 cmH2O, respectively) during loaded breathing. Therefore, the lowered slope found in lung transplant patients might result from the higher range of stimulus, i.e., a “range effect” (107), instead of from lung denervation. In the present study, Pm range was not significantly different between DLT and the NOR group (12.22 ± 1.2 vs 15.12 ± 1.5, respectively, p = 0.159), nor as peak Pm. The difference in lung transplant subjects’ loaded responses between the current study and Peiffer et al.’s (108) might be due to the methodology difference. In their protocol, airflow was not targeted. Moreover, inspiratory resistive loads were presented for the duration of two consecutive inspiratory breaths. It is not known whether their data come from the first or the second loaded breathing. Load responses will be different for first-breath as compared to the second-breath. Furthermore, inspiratory muscle strength was not measured. Lung transplant recipients usually have weakened respiratory muscles because of the use of steroid medications and/or deconditioning after surgery (122). Most studies demonstrated a close relationship between weak muscle strength and increased respiratory drive (4, 65). However, weakened inspiratory muscles and increased drive should increase the intensity of respiratory sensations, which was opposite to their results. Double lung transplant subjects in our study had similar inspiratory muscle strength with the NOR group. Although we did not measure inspiratory drive directly, it would be reasonable to believe the drive would be similar due to similar inspiratory muscle strength in these two groups. Therefore, the effect of inspiratory muscle strength and drive on load magnitude estimation are not present in this study.
As discussed in section 5.3, we found in the same study that load detection was impaired in DLT recipients. However, studies of load-detection ability do not allow the prediction of absolute intensity and perceptual sensitivity to suprathreshold loads. There are two major differences in these two tasks. First, the intensity of the load applied is different. In contrast to a detection task, the loads used in magnitude estimation tasks are suprathreshold loads, which are associated with the development of larger mechanical changes in the respiratory system. Therefore, with the loss of one potential afferent input, the remaining afferents mechanisms may be able to compensate during magnitude estimation. Second, temporal information was involved in magnitude estimation task (83), but not in the detection task. Kellerman et al. (80) found that despite a significant reduction of magnitude estimation of external resistive loads after inspiratory muscle training, load detection remained unchanged. In the present study, the relationship between Weber fraction and the slopes of logHG%-logPm and logHG%-logR were not significant when we combine data from both DLT and the NOR groups (p > 0.05, see Figure 5-5). All these results suggest that load detection and magnitude estimation of suprathreshold loads are two different perceptual processes, and may involve different neural mechanisms.

In summary, we found that the handgrip response and loaded breathing pattern were similar in the DLT group and the NOR group. Furthermore, the slope of logHG%-logPm and logHG%-logR were also comparable in the two groups. These finding suggest that neural feedback from the lung and lower airways does not play a significant role in inspiratory resistive load magnitude estimation tasks. It is possible that the relative importance of other potential afferent mechanisms (upper airway, respiratory muscle and
chest wall, etc.) may be altered as one site is blocked, e.g. lung denervation. Similarly, a nonexclusive role in respiratory sensation has been previously demonstrated for other potential paths, such as chest wall (55), phrenic nerve (100), or upper airway (105), suggesting that respiratory sensation related to loaded breathing may be due to multiple and simultaneous sensory inputs.

![Graph showing the relationship between Weber Fraction and Slopes of Magnitude Estimation](image)

Figure 5-5 Relationship between Weber Fraction and Slopes of Magnitude Estimation

### 5.4 Respiratory-Related Evoked Potential (RREP)

**5.4.1 Early-Latency RREP Components**

**5.4.1.1 Scalp distribution**

Early-latency RREP components (P1, Nf, P1a, N1) have been identified in response to inspiratory occlusion in previous studies (42, 43, 45, 141). In the present study, all the early-latency components were recognized in both DLT and NOR subjects,
except P1 was unidentifiable in a few subjects. All these components showed a significant main effect of scalp location. Similar to previous reports (42, 141), P1 was found maximally in central and centroparietal regions. Nf was found maximally in frontal regions. N1 was found maximally in central regions. P1a was found maximally in centroparietal and parietal regions. The scalp distribution was similar in DLT patients and NOR subjects.

5.4.1.2 Attention effect

Early-latency components, also known as exogenous components, are believed to reflect the arrival of impulses in the primary sensory area and are determined mainly by the physical characteristics of the stimuli. Recently, both Knafelc et al. and Webster et al. found that the log of P1 amplitude linearly correlated with the log of the magnitude of resistive loads (90, 142), supporting the hypothesis that this early component is sensory in nature. N1 is regarded as having both exogenous and endogenous features. Bloch-Salisbury et al. assessed the effects of stimulus magnitude on RREP components using resistive loads (21). Their results showed that N1 amplitude decreased as the load size decreased, similar to the other early-latency components. Early-latency RREP components P1 and Nf have been found to be unaffected by attention (143). However, the effect of attention on N1 is still controversial. Harver et al. (71) recorded RREPs in response to inspiratory occlusions and expiratory occlusions presented during both attend and ignore conditions, they found N1 was unaffected by attention in young subjects. In contrast, Webster et al. found that the latency of N1 was significantly shorter and its amplitude was significantly larger in the attend as compared with the ignore condition (143).
In our study, there were no significant differences in either latency or amplitude of all the four early-latency RREP components (P1, Nf, P1a, N1) between attend trial and ignore trial for both groups. In addition, we also found similar occlusion pressure changes between attend and ignore trial, which indicating similar stimulus characters between trials. Although we did not test directly the effects of external stimulus characters on those early-latency RREP components, our results were consistent with previous studies. It is thus suggested that the early-latency RREP components (P1, Nf, P1a and N1) were determined by the physical characteristics of the stimulus, and were not affected by attention. They are pre-cognitive indicators of occlusion information processing.

5.4.1.3 The role of lung vagal afferents in early-latency RREP response

Respiratory mechanical changes associated with extrinsic loads could be sensed by a variety of pulmonary mechanoreceptors which are innervated by the vagus nerve. Animal studies have demonstrated that vagus nerve stimulation could elicit neural activity in the cortex (7, 91, 104). However, no study has been conducted before to investigate the role of lung vagal afferents in the RREP response in humans. Double lung transplant recipients lose all the afferent traffic from receptors distal to the surgical anastomosis after surgery. Therefore, they provide an ideal human model to study the role of lung and lower airway receptors in perceptual and RREP responses.

In the present study, we found that the early-latency RREP components (P1, Nf, P1a, N1) can still be identified in DLT recipients. And the peak latencies and peak amplitudes of these RREP components were similar in the DLT group and the NOR group. The occlusion mouth pressure, which is commonly used to reflect the intensity of respiratory stimuli, was also found not significantly different between DLT and the NOR group. Early-latency components are generally believed to reflect the arrival of impulses
in the primary sensory area and are determined mainly by the physical characteristics of the stimuli. Inspiratory occlusion puts an infinitive load on the whole respiratory system. The neural feedback from the remaining afferent mechanisms (e.g. upper airway, respiratory muscles) will still be able to reach cortex. Loss of lung vagal input during inspiratory occlusion did not affect these precognitive peaks. It is also possible that scalp recordings are not sensitive enough to detect the loss of vagal input because of significant attenuation of the response by the skull and scalp, and because of electrical noise from extracerebral sources. The presence of these early-latency RREP components in DLT patients suggests that lung vagal afferents are certainly not essential to early-latency RREP responses. The similarity in latency and amplitudes further suggests that lung vagal afferents may not play a significant role in the early-latency RREP responses.

5.4.2 Late-Latency RREP Component: P3

5.4.2.1 Scalp distribution

In the present study, a significant main effect of site was found for P3 in all subjects. The finding that P3 was maximal at centroparietal and parietal sites is consistent with previous reports (20, 71, 141, 143).

5.4.2.2 Attention effect

Late-latency RREP components (e.g. P3), also known as endogenous components, are associated with cognitive processing of the sensory information and are highly sensitive to psychological variables. Increased amplitude and decreased latency of P3 in the attend as compared with the ignore condition has been observed in response to other sensory stimulus (18, 74, 98, 111). Recently, similar effects of attention on respiratory P3 have also been reported (71, 143).
The present results are consistent with previous studies. We found that P3 was identified in all subjects during the attend trial. While during the ignore trial, 7 out of 10 DLT patients and 10 out of 11 NOR subjects had an identifiable P3. P3 latency was significantly shorter during attend trial as compared with ignore trial. The main effect of trial condition on P3 amplitude was also significant, whether we used missing data or zero amplitude to replace those unidentifiable P3 during ignore trial in some subjects. Specifically, P3 amplitude was significantly larger during attend condition.

5.4.2.3 The role of lung vagal afferents in P3 response

In the present study, we found that P3 were still identifiable in all DLT patients during attend trial and 7 out of 10 during ignore trial. However, the percentage of identifiable P3 during the ignore trial was lower in DLT patients as compared with the NOR group (7 out of 10 vs 10 out of 11, respectively). In order to increase the statistical power, two-way repeated measure ANOVA was conducted only for the attend trial, during which P3 were identifiable in all NOR subjects and DLT subjects. A significant group effect was found, with the DLT group having significantly smaller P3 amplitudes. Moreover, DLT patients also had significantly delayed P3 latencies than the NOR group. The P3 response during the attend trial in a DLT patient and a matched normal subject was shown in Figure 5-6 and Figure 5-7. These results suggest that DLT recipients had impaired cognitive processing of the inspiratory occlusion stimulus, which might be due to the loss of lung vagal afferent inputs.

To the author’s knowledge, this is the first study designed to investigate the role of lung vagal afferent mechanisms on the RREP response. P3 is generally believed to be associated with complex cognitive functioning such as selective attention, memory, and stimulus evaluation, rather than earlier sensory processing of stimuli. Previous studies
have demonstrated that stimulus degradation could delay P3 in response to visual stimuli, which might be due to a prolonged stimulus evaluation process when less information about the stimulus was available (132, 133). The effect of deafferentation on P300 was studied by Cohen et al. (35). They compared P300 in three groups: healthy control, paraplegic and tetraplegic subjects using a transcutaneous electrical stimulation oddball task. Their results indicated P300 amplitude was significantly reduced in the two spinal cord injury groups compared to the control group.

Our results were consistent with those previous studies. It has been suggested that there are two cortical systems involved in respiratory central neural processing (47). In the first one, neural information arising from mechanoreceptors in the respiratory muscles enters the spinal cord, ascends in the dorsal column, relays in the brainstem, projects to the thalamus and is projected through a thalamocortical pathway to the sensorimotor cortex. The second pathway involves ascending afferent information from the vagus nerve and its branches, and possibly phrenic afferents. They relay from the brain stem to the amygdala before projecting to the mesocortex. This circuit may deal with some of the behavioral aspects of respiration. Lung denervation in DLT patient results in loss of afferent information ascending from lung mechanoreceptors. Early neural processing of the sensory stimuli may not be affected significantly due to intact function of other afferent mechanisms. However, the less information available, the more uncertainty will arise about the stimulus, therefore, a longer cognitive evaluation process about the stimulus will occur. P3 is a converged cognitive response, which is sensitive to the duration of the stimulus evaluation process. Loss of vagal afferent input thus might affect P3, as shown by our results. Indeed, central processing time, which was obtained by
subtracting the latency of early component P1a from P3, was also significantly longer in the DLT group than the NOR group (p < 0.05).

Figure 5-6 Response of P3 in a Double Lung Transplant Recipient during Attend Trial

Figure 5-7 Response of P3 in a Normal Subject during Attend Trial
Cognitive processing is also involved in load detection. In the present study, we found that the detection threshold, as well as the Weber fraction were significantly elevated in the DLT group, which is probably due to impaired cognitive process after lung denervation. The delayed P3 response should, result in a longer detection latency in DLT recipients. However, the main effect of group on detection latency was not significant. During the detection experiment, the detection latency was recorded as the duration from the start of inspiratory airflow to the start of detection signal. The detection signal was obtained by pressing a signal button by the subject. Detection latency can also be affected by how quick the subject will press the signal button once they have made the decision about the presence of a load. This detection latency task is not sensitive enough to identify a 60msec delay in central neural processing.

All the DLT subjects were on immunosuppressive and steroid drugs. The delayed latencies and reduced amplitudes of P3 in the DLT group might result from the medications. To our knowledge, none of the medications have been reported to have an adverse impact on human’s cognition and event-related evoked potential response. Further studies are necessary to investigate the effect of the drugs on the RREP response.

In summary, our results confirmed previous findings about the effect of attention on early- and late-latency RREP components. Early-latency RREP components were not affected by attention, while the late-latency component, P3, was observed to have a significantly shorter latency and larger amplitude during the attend trial compared to the ignore trial. We also found that despite a similar early-latency RREP response, DLT recipients had a significantly prolonged and attenuated P3 response, suggesting that lung denervation impaired cognitive processing about respiratory stimulus information.
CHAPTER 6
CONCLUSIONS

First, the breathing pattern during both unloaded and loaded inspiration were not significantly different in the DLT group and the NOR group. These results suggest that lung vagal afferent inputs are not essential to the regulation of resting breathing pattern and load compensation responses in humans.

Second, DLT recipients had a significant higher detection threshold and Weber Fraction than the NOR group. The impaired detection capability may be due to the loss of lung vagal afferent input in those lung denervated patients. These results indicate that lung vagal afferents may contribute to detection of resistive loads in humans.

Third, handgrip response and loaded breathing pattern were similar in the DLT group and the NOR group. Furthermore, the slope of logHG%-logPm and logHG%-logR were also comparable in the two groups. These finding suggest that neural feedback from the lung and lower airways does not play a significant role in the inspiratory resistive load magnitude estimation task.

Fourth, our results confirmed previous findings about the effect of attention on early and late-latency RREP components. Early-latency RREP components were not affected by attention, while late-latency component P3 was observed to have significantly shorter latency and larger amplitudes during attend trial compared to ignore trial.

Finally, the presence of both early and late-latency RREP components in DLT patients suggests that lung vagal afferents are not essential to RREP response to
inspiratory occlusions. The similarity in latency and amplitudes suggests that lung vagal afferents may not play a significant role in the early-latency RREP responses. However, DLT recipients had a significantly delayed and attenuated P3 response, suggesting that lung denervation impaired cognitive processing about respiratory stimulus information.

In summary, our results suggest that lung vagal afferents are involved in cognitive processing of respiratory stimuli during breathing against mechanical loads, but are probably not essential to load perception and the RREP response. Respiratory sensation related to loaded breathing may be due to multiple and simultaneous sensory inputs.
LIST OF REFERENCES


34. Chudler, E. Explore the brain and spinal cord. [Online]. faculty.washington.edu/chrdler/introb.html 09/12/2001


BIOGRAPHICAL SKETCH

Weiying Zhao was born in Nanjing, P. R. China. She received her Bachelor of Medicine degree from Nanjing Medical University in 1995. Later she earned her Master of Medicine degree in sports and rehabilitation medicine from Nanjing Medical University in 1998.

She joined the Rehabilitation Science program in the Department of Physical Therapy of the University of Florida in January 1999 to pursue a Doctor of Philosophy degree, and since then she has worked as a research assistant in the Department of Physical Therapy. Her research interests include respiratory sensation, regulation of breathing, and cardiopulmonary exercise physiology.