INFLUENCE OF HYPOXIA, HYPERCAPNIA AND INSPIRATORY EFFORTS ON SYMPATHOEXCITATION DURING APNEA

by

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ABSTRACT

INFLUENCE OF HYPOXIA, HYPERCAPNIA AND INSPIRATORY EFFORTS ON SYMPATHOEXCITATION DURING APNEIC EPISODES

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Obstructive Sleep Apnea (OSA) is characterized by frequent periods of apnea resulting in hypoxia, hypercapnia and fluctuations of intrathoracic pressure (inspiratory efforts). Hypoxia, hypercapnia and inspiratory efforts lead to stimulation of peripheral chemoreceptors, central chemoreceptors and baroreceptors, respectively, which in turn influence Muscle Sympathetic Nerve Activity (MSNA). We hypothesized that hypoxia and hypercapnia result in an elevated MSNA response, while inspiratory efforts result in a reduced MSNA response during apnea. To test the hypothesis, we determined the contributions of hypoxia (at 12% O_2), hypercapnia (at 3% CO_2) and inspiratory efforts (mueller maneuvers at 20 mmHg) to MSNA (using microneurographic techniques) during simulated apnea, respectively. Six healthy subjects and four OSA patients participated in this study. We found that, hypoxia and hypercapnia during apnea

resulted in a significant increase in MSNA response from 88.5 ± 9.8 AIU for room air conditions (simulated apnea after breathing room air) to 116.4 ± 9.0 AIU for hypoxia and 121.1 ± 7.4 AIU for hypercapnia, respectively. However, inspiratory efforts during apnea resulted in a significant decrease in MSNA to 78.8 ± 9.3 AIU. Further, it was also found that hypercapnia had a significantly greater influence on MSNA response during apnea as compared to hypoxia and inspiratory efforts. These findings indicate that central chemoreceptors are more sensitive during apneic episodes than peripheral chemoreceptors and baroreceptors.

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CHAPTER 1

INTRODUCTION

The central controlling area for breathing, called the respiratory centre controls breathing. The afferent inputs to this central system are provided by several receptors. The effector nerves (efferent nerves) consist of intercostal nerves and the phrenic nerves [1].

1.1 Chemoreceptors

Chemoreceptors detect the levels of Carbon dioxide (CO_2) and Oxygen (O_2) in the blood and transmit this information to the central controlling area for breathing, called the respiratory center (medulla oblongata). Medulla oblongata then sends nervous impulses to the external intercostals muscles and the diaphragm, via the intercostals nerve and the phrenic nerve, respectively, to increase breathing rate and the volume of the lungs during inhalation [1]. There are two major types of chemoreceptors: central and peripheral.

1.1.1 Central Chemoreceptors

There are cells in the floor of the fourth ventricle that respond to pH of the Cerebrospinal Fluid (CSF) and do not respond to a drop in O_2 (hypoxia). CO_2 in the blood can easily diffuse across into the CSF, and an increase of CO_2 in blood

(hypercapnia) leads to an increase in CO_2, hydrogen ion and bicarbonate ion concentration in CSF. This in turn, increases the acidity in CSF and hence pH increases in CSF which activates central chemoreceptors [1].

1.1.2 Peripheral Chemoreceptors

The carotid body and the aortic body contain chemoreceptors that respond to the O_2 and CO_2 concentrations in the arterial blood. The aortic body detects changes in blood oxygen and carbon dioxide, but not pH, while carotid body detects all three. Of the aortic body and carotid body, the carotid body is more important and is situated at the division of common carotid artery into the external and internal carotid arteries in the neck. The aortic body is found on the aortic arch. The information from the carotid body is carried along the glossopharyngeal nerve (9^{th} cranial nerve), and the information from the aortic body is carried along the vagus nerve (10^{th} cranial nerve), to medulla oblongata, the respiratory center. These peripheral chemoreceptors monitor the physically dissolved O_2 and CO_2 (PO_2 and PCO_2). They are simulated by increase in PCO_2 and/or decrease in PO_2. However, these chemoreceptors only detect the levels of physically dissolved O_2 and not the O_2 that is chemically attached to hemoglobin [1].

1.2 Baroreceptors

Baroreceptors in the human body detect the pressure of blood flowing through them, which in turn then increase or decrease total peripheral resistance and cardiac

output. They are stretch-sensitive mechanoreceptors. Baroreceptors can be divided into two categories: high pressure arterial baroreceptors and low pressure baroreceptors [2].

1.2.1 High Pressure Arterial Baroreceptors

Arterial baroreceptors are present in the arch of the aorta, and the carotid sinuses of the left and right internal carotid arteries. Baroreceptors act to maintain mean arterial blood pressure to allow tissues to receive the right amount of blood. If blood pressure falls, baroreceptors firing rate decreases. While signals from the carotid baroreceptors are sent via the glossopharyngeal nerve, signals from the aortic baroreceptors travel through the vagus nerve. Baroreceptors work by detecting the amount of stretch applied to the walls of the arteries where they are located. The more the baroreceptor walls are stretched, the more frequently they generate action potentials [2].

1.2.2 Low Pressure Baroreceptors

Low pressure baroreceptors are in veins and the atria of heart. The low pressure baroreceptors are involved with the regulation of blood volume. The blood volume determines the mean pressure throughout the system, in particular the venous side where most of the blood is held [2].

1.3 Lung Stretch Receptors

Stretch receptors are located in lungs which are activated by either a stretch or nonstretching of lungs. The impulses are transmitted from the receptor sites through the

vagus nerve to the medulla oblongata. The inflation reflex (Hering-Breuer inflation reflex) produced by stretching of lungs is responsible for terminating inspiration, whereas the deflation reflex (Hering-Breuer deflation reflex) produced by deflation of lungs produces the opposite stimulus, thereby increasing respiration [3].

1.4 Cardiovascular Responses of Chemoreceptors and Baroreceptors

In response to inputs from chemoreceptors and baroreceptors, a nervous impulse is sent to the cardiovascular centre in the medulla, which would then feedback to the sympathetic ganglia, increasing the sympathetic activity [2].

1.5 Relationship of Chemoreceptors and Baroreceptors with Sympathetic Nervous System

Hypoxia is known to elicit peripheral chemoreflex activation of the sympathetic nervous system [4]. Moreover, hypercapnia is known to simulate sensitive central chemoreceptors, which also serve to activate the sympathetic nervous system [5]. Changes in intrathoracic pressure, due to inspiratory efforts (mueller maneuvers), may alter the activity of baroreceptors (cardiopulmonary arterial mechanoreceptors) leading to reflex sympathoexcitation (sympathoinhibition).

1.6 Sleep Apnea

The Greek word “Apnea” literally means “without breath”. During apnea, there is no movement of the muscles of respiration and the volume of lungs initially remains unchanged. Sleep apnea in this sense is a sleep disorder caused by cessations in breath

during sleep. More than 18 million American adults suffer from sleep apnea. Apnea occurs in all age groups, both sexes and across different populations, but some factors that increase the risk include small upper airway, being overweight, smoking and alcohol use and being age 40 or older.

According to the National Sleep Foundation *Sleep in America* 2005 Poll, 26% (31% of men and 21% of women) of the 1,506 respondents being interviewed using Berlin questionnaire criteria were indicated to be at a high risk of sleep apnea. Of those at risk, 66% were experiencing daytime sleepiness at least 3 days per week and 58% of them were obese. Also, of the adults in the population who had been diagnosed with high blood pressure or depression, almost 50% of them were at a risk for sleep apnea. Untreated sleep apnea can cause high blood pressure and cardiovascular diseases, memory problems, weight gain, impotency, and headaches. Moreover, untreated sleep apnea may be responsible for job impairment and motor vehicle crashes [6].

1.6.1 Types of Sleep Apnea

There are three types of sleep apnea: central, obstructive and mixed (combination of central and obstructive). Central sleep apnea occurs when the brain's respiratory control system is impaired and the neural drive to breathe is absent resulting in decreased efforts to breathe during sleep. Obstructive Sleep Apnea (OSA) occurs when air cannot flow into or out of the person's airway despite efforts to breathe. It is mostly associated with fat buildup or loss of muscle tone with aging [7, 8].

According to a study conducted by Mayo clinic in 2006 on 243 sleep apnea patients, 0.4% were reported to suffer from Central Sleep Apnea (CSA) and 15% suffered from Mixed Sleep Apnea (MSA), whereas 84% of them suffered from OSA [8]. This clearly shows that obstructive sleep apnea is the major type of sleep apnea and its physiological effects are discussed below.

1.6.2 Physiological Effects of OSA

In patients suffering from OSA, the typical progression of apnea and cardiovascular responses is shown in Figure 1.1. First, there is an initial decrease in the drive to breathe caused by two reflex mechanisms: baroreflex and chemoreflex, which are discussed later in this chapter. As a result of this, the airway collapses, leading to an apneic event that reduces gas exchange at the lungs. Also, this leads to reduced activity of the pulmonary stretch receptors (Hering-Breuer deflation reflex) which in turn increase the drive to breathe. Moreover, the subsequent hypoxic-hypercapnic state in turn increases the drive to breathe. In addition, chemoreceptor activation also increases sympathetic activity which consecutively increases arterial blood pressure, which then activates the baroreceptors. Activation of baroreceptors in turn increases the drive to breathe accompanied by a decrease in heart rate and arterial blood pressure. These reflexes eventually terminate the apneic event and elicit arousal, which is repeated many times during the night [9]. OSA interacts with various physiological systems and has vital effects on many parameters, which are discussed below.

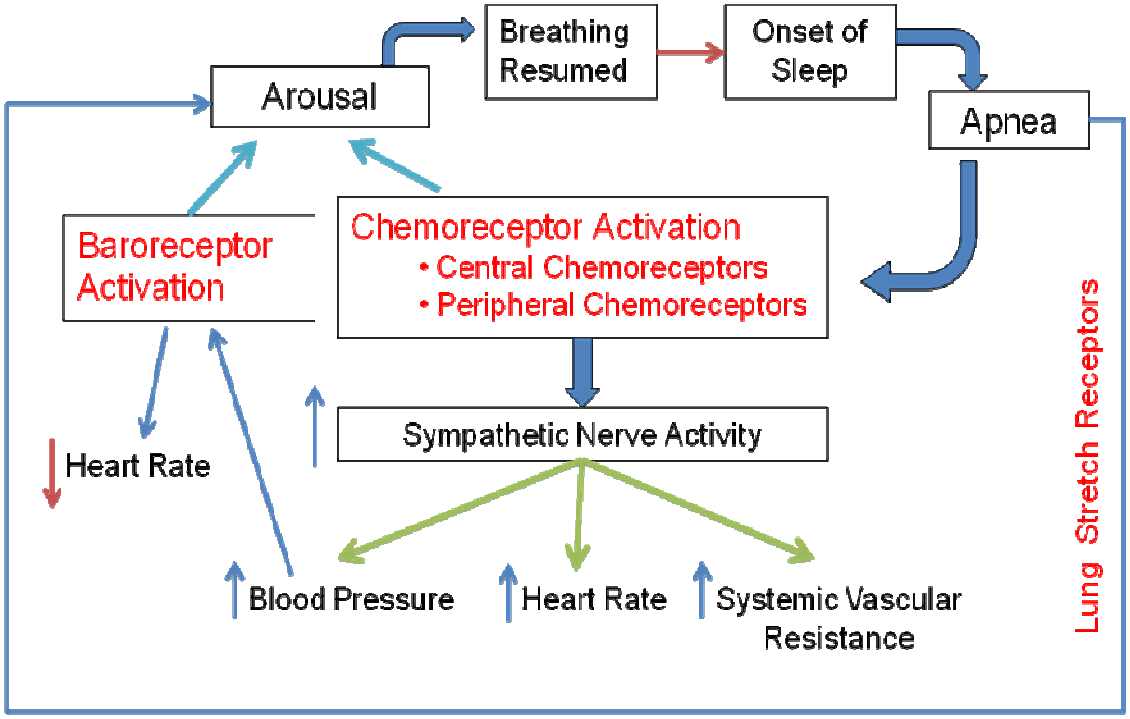

Figure 1.1 Progression of apnea [9].

1.6.2.1 MSNA and OSA

Watanabe et al. showed that MSNA is enhanced during sleep apnea in healthy elderly individuals [10]. Moreover, Narkiewicz et al. showed that, there is a significant increase in MSNA in obese subjects with OSA as compared to obese subjects without OSA [11]. Hedner et al. and Watanbe et al. have previously found that, MSNA becomes stronger and increases towards the end of apneic episodes in patients with OSA [5, 10].

1.6.2.2 Blood Pressure and OSA

T Shimizu et al. recorded MSNA from 4 OSA patients and showed that arterial systolic and diastolic blood pressure peaks increased sharply 4-10 cardiac cycles after

the end of apneic episodes, whereas heart rate gradually became slower during the first 10 cardiac cycles after the onset of apneic episodes [12]. Irrespective of the magnitude of the MSNA burst, it always occurs during spontaneous transient blood pressure reductions than during transient increases in blood pressure. When measured for individual heart beats, not only the occurrence but also the mean voltage amplitude of the sympathetic bursts tended to increase with decreasing diastolic pressure [13].

1.6.2.3 Hypertension, Cardiovascular Disorders and OSA

The prevalence of hypertension is high in OSA; whereas the incidence of OSA in patients with hypertension is 20-30% as reported by Fletcher et al. [14]. Recent findings suggest that, OSA is associated with an increased incidence of dysrhythmias, myocardial infarction, hypertension, stroke and sudden death [14-21]. Changes in intrathoracic pressure during apnea affect the cardiovascular responses and can influence venous return, ventricular filling, arterial and cardiopulmonary baroreflexes [22, 23]. Somers et al. recently found that obesity and OSA are each very strongly associated with hypertension [24]. In addition, apnea index is predictive of early death in untreated patients with OSA [25]. Hence, OSA is accompanied by significant cardiovascular morbidity which is related to the extent of the sleep disorder.

1.7 Differences between OSA Patients and Normal Subjects

The sympathoexcitatory responses to end-expiratory apneas and end-inspiratory apneas in healthy control subjects were quantitatively similar to those of patients with moderate sleep apnea. Also, Mean Arterial Pressure (MAP) responses were similar

between patients and healthy subjects [26]. Conversely, Hedner et al. observed that MAP increased significantly when hypoxia occurred in patients with OSA, while normotensive control subjects demonstrated no pressor responses to similar hypoxia [27]. Somers et al. also found that MSNA was markedly elevated in patients with OSA compared with control subjects [28]. Also, they found that the blood pressure profile during sleep in patients with OSA is dominated by responses to obstructive events that occur repetitively throughout the sleep. In addition, patients with OSA had a high sympathetic drive and have a blunted increase in MSNA, in response to baroreceptor deactivation [29].

1.8 Simulation of Apnea versus Actual Apnea

Simulation of apnea using breath hold is well established [12, 30-32]. Moreover, it is also shown by Imadojemu et al. that all responses like MSNA, vascular resistance and mean arterial pressure to voluntary apnea is same as to those of actual OSA [33]. As such, breath hold experiments elicit same physiological responses as OSA. Simulated apnea is preferred over actual sleep apnea as it would be extremely difficult to avoid movements of the leg during sleep and as such microneurographic readings will not be as accurate. Microneurographic technique is described in detail in the following section.

1.9 Microneurography

Microneurography was first performed in humans in Uppsala, Sweden in 1965-1966 within the department of clinical neurophysiology at the Academic Hospital. Hagbarth first inserted a needle into his own ulnar nerve, but the signal to noise ratio was very low. To improve this, a tungsten electrode with electrode tip of 5-10 μm provided acceptable recordings of nerve activity in humans. The efferent nature of this activity was confirmed by inserting two electrodes 100 mm apart; the conduction velocity was found to be 1 m/s and the direction was efferent. One of the main research areas of microneurography was assessing sympathetic efferent nerve activity [34]. Most bursts have an approximately triangular shape with mean burst duration of 0.62 ± 0.01 second in peroneal nerve. They also showed that there is a positive correlation between the burst amplitude and burst duration [35].

Although microneurography is a powerful technique, it has a few limitations. First, the experiments are time consuming and require long search periods to locate the exact nerve and make manual adjustments of electrode positions. Second, this technique does not allow for large movements of the subject [35].

1.10 Significance of Research

Several factors may contribute to sympathoexcitation during periods of apnea. Hypoxia and hypercapnia are known to elicit sympathoexcitation during sleep apneic episodes [32]. Inspiratory efforts against a closed airway may lead to sympathoinhibition during sleep apnea, but this has not been studied systematically and

hence this relationship is explored in this study. Moreover, we compared effects of these parameters on both healthy subjects and patients suffering from OSA. A quantitative predictive model was built, which relates independent variables like O_2%, CO_2% and inspiratory efforts with Muscle Sympathetic Nerve Activity (MSNA) level for both healthy subjects and OSA patients. We determined the relative effects of each parameter on MSNA.

1.11 Research Problem

Our prime focus was to assess the effects of hypoxia, hypercapnia and inspiratory efforts on muscle sympathetic nerve activity on both healthy subjects as well as patients suffering from OSA. We hypothesized that, hypoxia and hypercapnia elicit sympathoexcitation, while inspiratory efforts tend to cause sympathoinhibition.

CHAPTER 2

METHODS

2.1 MSNA Quantification: Historical Perspective

MSNA has been over years studied by various researchers and has been related to a variety of physiological responses. Quantification of MSNA has been a very important issue, and researchers have come up with various ways of quantifying MSNA. Tamisier et al. quantified MSNA as burst frequency (Bursts/minute) in 5 minute periods [36]. Hansen et al. used burst frequency and burst incidence (Bursts/100 Heart beats) as two different metrics for representing MSNA [37]. Shimizu et al. also used a similar approach to quantify MSNA with burst frequency and burst incidence [38].

It was later observed that, using the burst frequency alone as the metric for MSNA would fail to take account of burst amplitudes and the relative strength of each burst [39]. Amplitude of bursts is an important factor because a change in both the firing rate of single fibers and the recruitment of additional fibers is reflected in the amplitude of the integrated signal [40]. In addition, we also observed that there is a significant increase in MSNA energy as the apnea progresses. Hence, ignoring the burst amplitude to quantify MSNA would be a blunder. Mark et al. then represented MSNA as a product of burst frequency and mean burst amplitude (bursts/minute X mean burst amplitude). This quantification method was also used by many other

researchers including Somers et al., Loring et al., Morgan et al. and Katraggada et al. [32, 41-43]. Other researchers like Shoemaker et al. and Leuenrberger et al. used total burst amplitude as a metric for quantifying MSNA [44, 45]. A few researchers like Narender et al. and Tank et al. also tried area under the burst to quantify MSNA, but electrode movements, EMG and afferent nerve traffic tend to induce baseline shifts in MSNA signal, making it difficult to measure the area under the curve accurately [46, 47]. Moreover, Shimizu et al. also suggest that MSNA area being dependent on firing rate, number of firing fibers and proximity of recording electrode to firing fibers cannot be compared between individuals [12].

Wallin et al. showed in 1977, that significant differences have been observed in MSNA between individuals. They propose that emotional stress, blood pressure, age, personality and physical fitness could explain the differences in MSNA between individuals [48]. They also proposed that, since the strength of the sympathetic signal varies with the microelectrode position for measuring MSNA within the fascicles, there could be inter-individual differences [49]. This necessitates the use of some kind of normalization which would eliminate the inter-individual differences. Welch et al. and Wallin et al. used a normalization technique where in, the average burst amplitude voltage during the control period was arbitrarily assigned a value of 100 units. The voltage of all bursts during the rest of the experimental periods is normalized to this scale, for a particular subject. Total nerve activity was then estimated as the sum of all normalized burst amplitudes during a specific unit of time [50, 51]. We used a similar approach for this study.

2.2 Experimental Validation

2.2.1. Subject Group

Ten subjects volunteered to take part in this study. Six of the subjects were healthy and four of them were diagnosed with OSA (patient group). There were seven male and three female subjects. Of the healthy group subjects, three were female and three were male, whereas of the patient group subjects all four were male. Age ranged from 33 to 56 years with a height of 176.1 ± 3.8 cm, weight of 96.7 ± 7.1 Kg and BMI of 30.9 ± 1.6 Kg/m^2. Subjects had a left arm measured systolic blood pressure of 128.4 ± 4.4 mmHg and diastolic blood pressure of 80.8 ± 2.5 mmHg before conducting the experiments. The age, sex, weight, height, BMI and Blood Pressure for the subjects is as shown in Table 2.1. Physical exams were conducted to confirm subject's health, which included baseline 12 lead ECG. Moreover, medical histories were also thoroughly checked to exclude subjects with a history of diabetes or heart disease. Subjects were asked not to consume caffeine, alcohol and medications for at least 12 hours prior to their participation in the study. They also did not perform any strenuous exercise for 12 hours prior to their participation in the study, so as to maintain normotensive state. They were also advised to have a good night's sleep. Informed written consent was obtained from all the subjects. All subjects were explained the basis of study, details of procedures and the risks and pain associated with the study. All subjects underwent familiarization and practice sessions with the protocol until they were fully comfortable with all procedures and capable of performing all breath hold

maneuvers. The University of North Texas Health Science Center Institutional Review Board has approved this research [52].

Table 2.1 Subject Data

Subject No.	**Age**	**Sex**	**Height**	**Weight**	**BMI**	**Measure left arm Blood pressure**	
	Years		**cm**	**kg**	**kg/m^2**	**Systolic (mmHg)**	**Diastolic (mmHg)**
Healthy 1	43	M	184.5	128	37.6	92	140
Healthy 2	33	M	191	108.5	29.7	78	144
Healthy 3	56	F	160	87.2	34.1	80	130
Healthy 4	47	F	163	72	27.1	68	98
Healthy 5	34	M	175	82.3	26.9	80	122
Healthy 6	38	F	164	71	26.4	72	118
Patient 1	35	M	187.5	116	33	76	122
Patient 2	42	M	178	126.2	39.8	90	134
Patient 3	39	M	168	73.6	26.1	82	138
Patient 4	49	M	190	102.5	28.4	90	138
Mean	41.6	N/A	176.1	96.7	30.9	80.8	128.4
S.E.	2.3	N/A	3.8	7.1	1.6	2.5	4.4

2.2.2. Measurements

After obtaining the informed written consent from each subject, they were instrumented as shown in the schematic in Figure 2.1. The following measurements are as described below.

2.2.2.1 ECG

ECG was obtained from an electrocardiogram with electrodes attached on the chest of the subject.

2.2.2.2 Arterial Blood Pressure

Arterial blood pressure was continuously measured noninvasively with a Finapres monitoring system placed around the middle or adjacent fingers. Finapres model 2300 (Ohmeda, Inc. Englewood, CO) was used for this purpose. The Finapres finger cuff was adjusted to give readings that matched diastolic pressure measured by a conventional arm auscultation blood pressure measurement as shown in the Figure 2.1.

2.2.2.3 Arterial Oxygen Saturation

Arterial oxygen saturation (SaO_2) was measured at the forehead using pulse oximetry (Nellcor N-100, Nellcor Inc., Hayward, CA). A forehead sensor was used as, forehead is a site closer to heart and so the signal delay is significantly reduced relative to the finger. Moreover, the forehead is less prone to patient motion artifact than the finger.

2.2.2.4 Respiratory Monitoring Band

A respiratory monitoring band (Grass Instruments, West Warwick, RI) is placed around the subject's abdomen generating a voltage signal output in response to the motion of the torso during human respiration. It measures the respiration signals to ensure that all breath holds were performed at end tidal expiration.

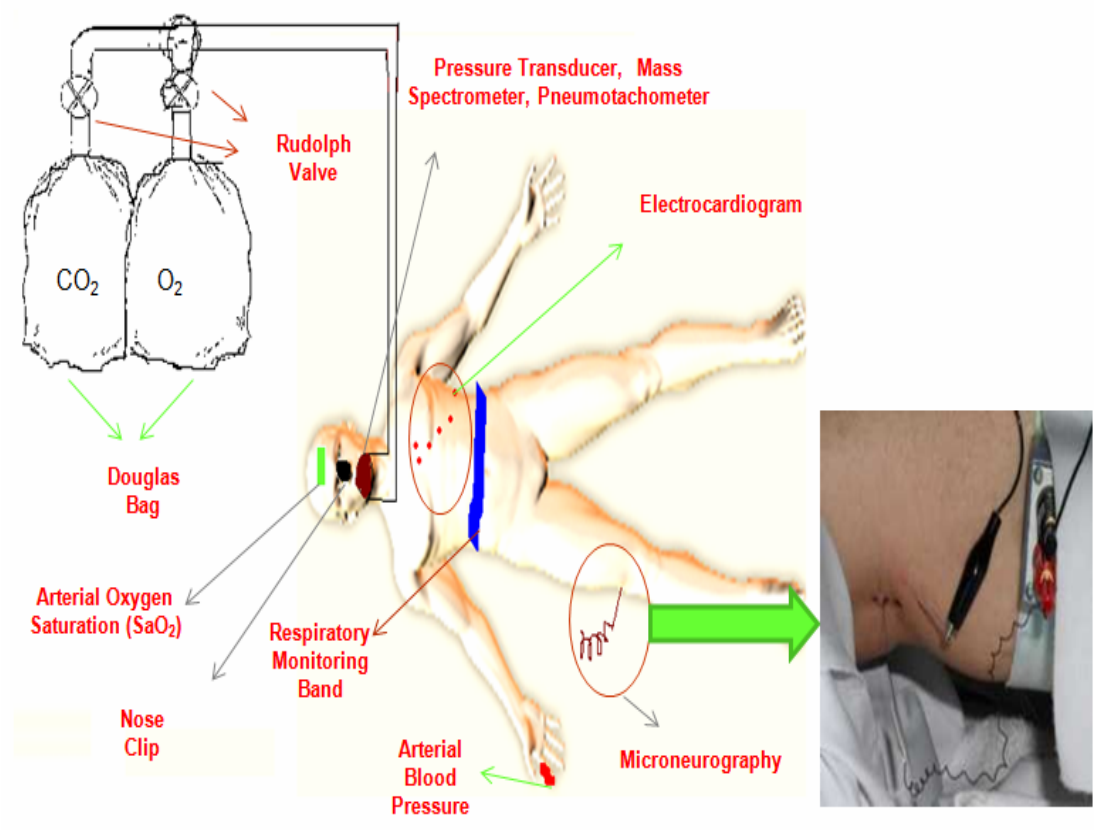

Figure 2.1 Schematic of experimental setup [53, 54].

2.2.2.5 Breathing Circuit

Subjects were fitted with an airtight mouthpiece and three-way rudolph valve (Hans Rudolph Series 2400) connected to a douglas bag containing various gas mixtures of O_2 and CO_2, which subjects breathe in after baseline to breath hold. A nose clip was used to prevent nasal breathing. End tidal O_2 and CO_2 was measured using a mass spectrometer connected to the mouthpiece. Tidal volume was measured using pneumotachometer connected to the mouthpiece. A pressure transducer was also attached to the mouth piece to measure and control inspiratory breathing efforts.

2.2.2.6 MSNA Measurements

Muscle Sympathetic Nerve Activity (MSNA) is measured at the leg using standard microneurographic techniques. MSNA is measured from the right peroneal nerve (near the fibular head) at the popliteal fossa, which is located at the posterior lateral aspect of the knee. Initially, the course of the nerve was determined by stimulating through the skin, with a pencil shaped electrode. When the nerve was stimulated, involuntary twitching of the calf or foot and/or tingling sensations in these areas occurred. Once the nerve is localized, two sterile, wire electrodes were inserted through the skin. This procedure was done without local anesthesia as the microelectrodes are too small to cause any considerable pain, and because anesthesia might interfere with local nerve function. First, a sterile reference tungsten microelectrode with a tip diameter of 5-10 μm and length of 35 mm (Frederick Haer and Co., Bowdoinham, ME, USA) was inserted into the skin 2-3 cm from the identified nerve path and directed towards, but not into the nerve. A similar recording microelectrode is then inserted into the nerve. The nerve is slowly and gently probed with the microelectrode while monitoring the signal for insertion discharges and action potentials. Skin sympathetic activity is excluded by performing several startle stimuli and observing a lack of response.

The raw MSNA signal thus obtained was first amplified 9 x 10^4 times (University of Iowa Bioengineering, Iowa city, Iowa, USA) and then band pass filtered (700-2000 Hz), rectified, and then discriminated (model 662C-3, nerve traffic analyzer, University of Iowa Bioengineering, Iowa city, Iowa, USA). These signals were

integrated by a Resistance-Capacitance circuit (RC circuit) with a time constant of 0.1 sec which gives an integrated MSNA signal. RC circuit has the effect of producing a moving averaged (smoothing) output or in other terms integration. The process is visually summarized in Figure 2.2.

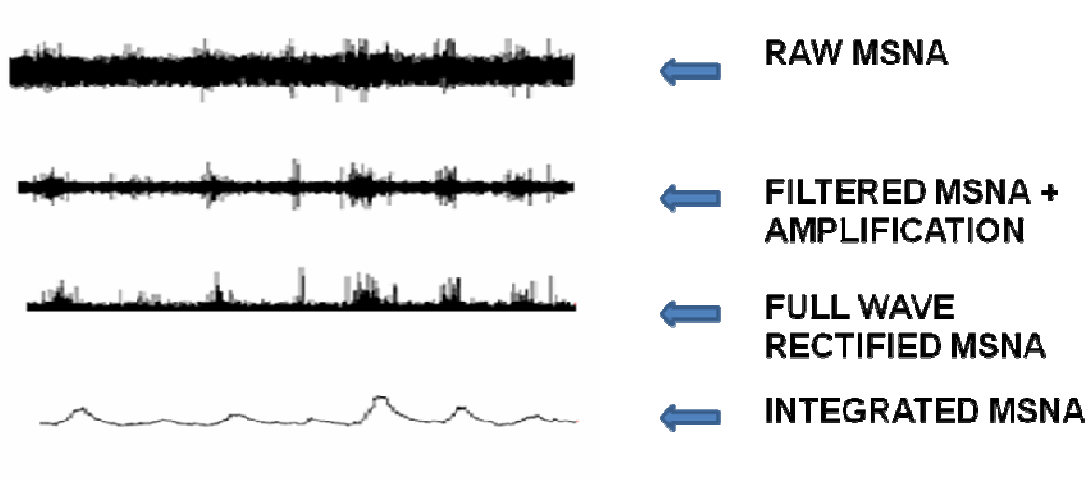

Figure 2.2 MSNA processing techniques [55].

2.2.2.7 Data Acquisition

All the above mentioned signals were digitized using Windaq data acquisition hardware (DI220) and software modules (Dataq Instruments, Akron, OH, USA) and data thus acquired were exported to MATLAB/Excel. Windaq Waveform Browser (WWB) was used to view the signal offline, and could also be used for post-processing, if required. Figure 2.3 shows a typical recording using the Windaq software. It contains 10 channels as acquired from various reading instruments. Channel 1 contains the MSNA signal followed by ECG and arterial blood pressure on channels 2 and 3, respectively. Channel 4 contains the exhaled volume, as measured by the pneumotachometer. Channels 5 and 6 display the end tidal O_2 and CO_2 measured by

mass spectrometer. The inspiratory effort measured with a pressure transducer is displayed in channel 7. Channel 8 shows the arterial oxygen saturation measured with a forehead pulse oximeter. Channel 9 contains the respiratory airflow signal and channel 10 contains the respiratory tidal volume. Table 2.2 summarizes the experimental measurements made in this study.

Table 2.2 Experimental Measurements

Channel No.	Measurements
1	Muscle sympathetic nerve activity
2	Electrocardiogram
3	Arterial blood pressure
4	Exhaled volume
5	End tidal O_2
6	End tidal CO_2
7	Inspiratory Efforts
8	Arterial oxygen saturation
9	Respiratory airflow
10	Respiratory tidal volume

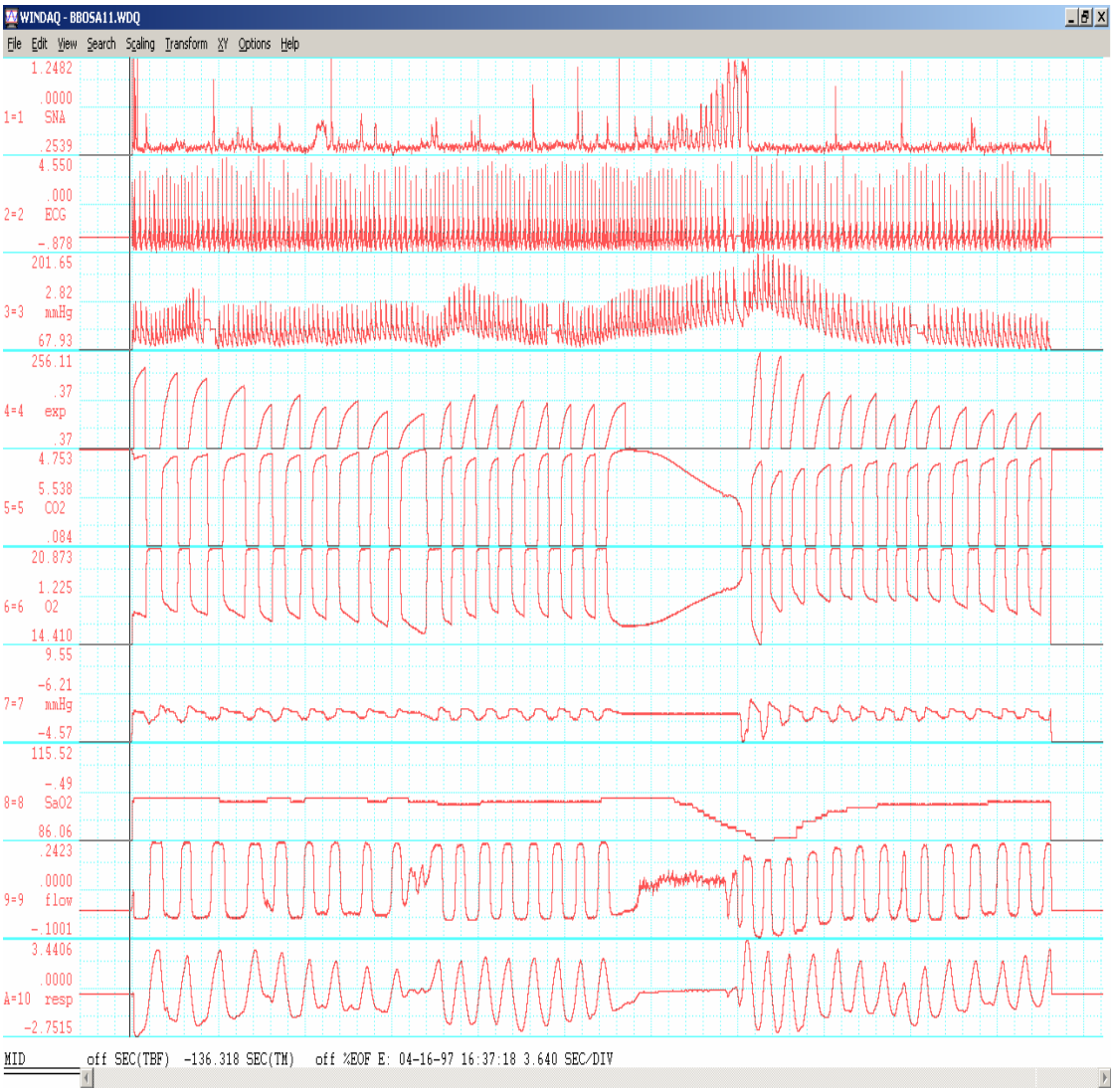

Figure 2.3 Typical recording using WINDAQ software.

2.2.3. Experimental Protocol

The experimental protocol is designed to evaluate the effects of hypoxia, hypercapnia and inspiratory efforts to the sympathoexcitation which occurs during simulated apnea events (breath hold). To test the effects of hypoxia and hypercapnia subjects breathed in two levels of O_2 and CO_2. Two levels of O_2 being used were 21%

(room air) and 12% (hypoxic), whereas two levels of CO_2 being used were 0% (room air) and 3% (hypercapnic). To assess the effects of inspiratory efforts, mueller maneuvers were performed on a closed mouthpiece connected to the pressure transducer. Mueller maneuvers of 20 mmHg were performed in order to evaluate the effect of inspiratory efforts.

There were in all 8 different experiments to evaluate the effects of hypoxia, hypercapnia and inspiratory efforts on MSNA during simulated apnea. An experiment map was established as shown in Table 2.3, with two levels of O_2% (21% and 12%), two levels of CO_2% (0% and 3%) and two levels of inspiratory efforts (0 mmHg and 20 mmHg). For example, as shown in the Table 2.3, experiment 2 corresponds to the experiment in which the subject inhaled 21% O_2 in combination with 3% CO_2 without any inspiratory efforts (0 mmHg), and experiment 7 corresponds to the experiment in which the subject inhaled 12% O_2, 0% CO_2 with 20 mmHg inspiratory efforts.

Table 2.3 Experimental Conditions Map

Inspiratory Efforts (mmHg)	O_2%	CO_2% : 0%	CO_2%: 3%
0	21	Experiment 1	Experiment 2
20	21	Experiment 3	Experiment 4
0	12	Experiment 5	Experiment 6
20	12	Experiment 7	Experiment 8

The baseline conditions are obtained after a 30 minute period of rest in the laboratory. The room is isolated and quarantined during the experiment. Moreover, adequate care is taken to make the subject comfortable. The timeline of the protocol is

as shown in Figure 2.4. The subject first breathed in room air (21% O_2 and 0% CO_2) for 60 seconds (Baseline Phase), followed by 60 seconds inhaling (breathing gas mixture phase) of each combinations of O_2 and CO_2 gases from the douglas bag connected to rudolph valve. To initiate apnea, an investigator closed the Rudolph valve to the subject at the end of a normal expiration. Subjects held their breath at end-expiration for approximately 30 seconds (apnea phase/breath hold phase) from then. If necessary, the subject signaled the investigator to open the valve to end the apnea before the 30-second target -apnea duration. After the simulated apnea episode, the Rudolph valve was again opened to room air and the data was recorded for 60 seconds (recovery phase). Breathing gas mixture serves to more closely approximate sleep apnea, and to maximize apnea-induced arterial oxygen desaturation. The instructor also put in event markers marking the start and end of each phase. These event markers guided subdivision of data for later offline analysis.

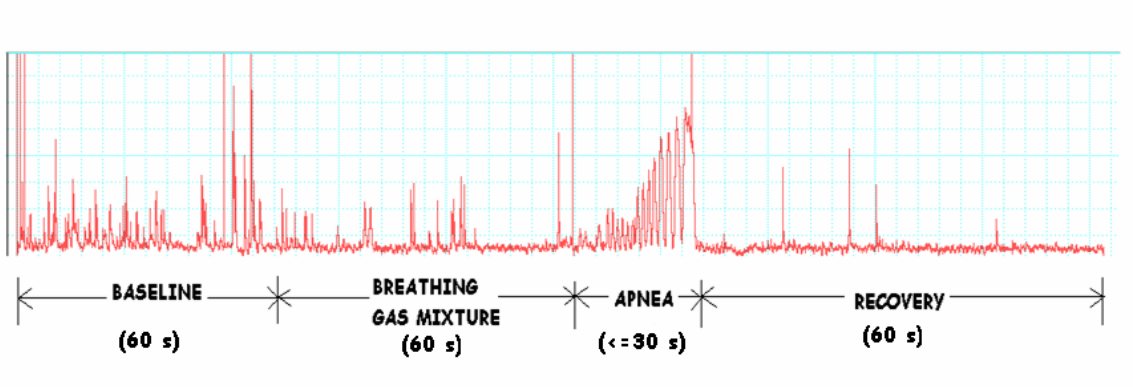

Figure 2.4 Time line of experimental protocol.

2.3 Computer Algorithm for MSNA Peak Detection

Detecting MSNA peaks accurately has been a very critical issue over years, as accurate quantification of sympathetic nerve activity provides for very important information about physiological systems and their interaction. It has been noticed that a relation exists between R peaks of electrocardiographic QRS complexes and the pulse synchronous MSNA signal. The MSNA bursts correspond to an R peak 1.0 to 1.5 seconds before the burst. This delay is known as baroreflex latency and is well established by Fagius and Wallin [56]. With this interesting observation, R peak would serve as an important indicator of MSNA peaks. This relation between R Peak and corresponding MSNA peak is the basis of the MSNA peak detection algorithm. The algorithm is described in the form of a simple flowchart in Figure 2.5.

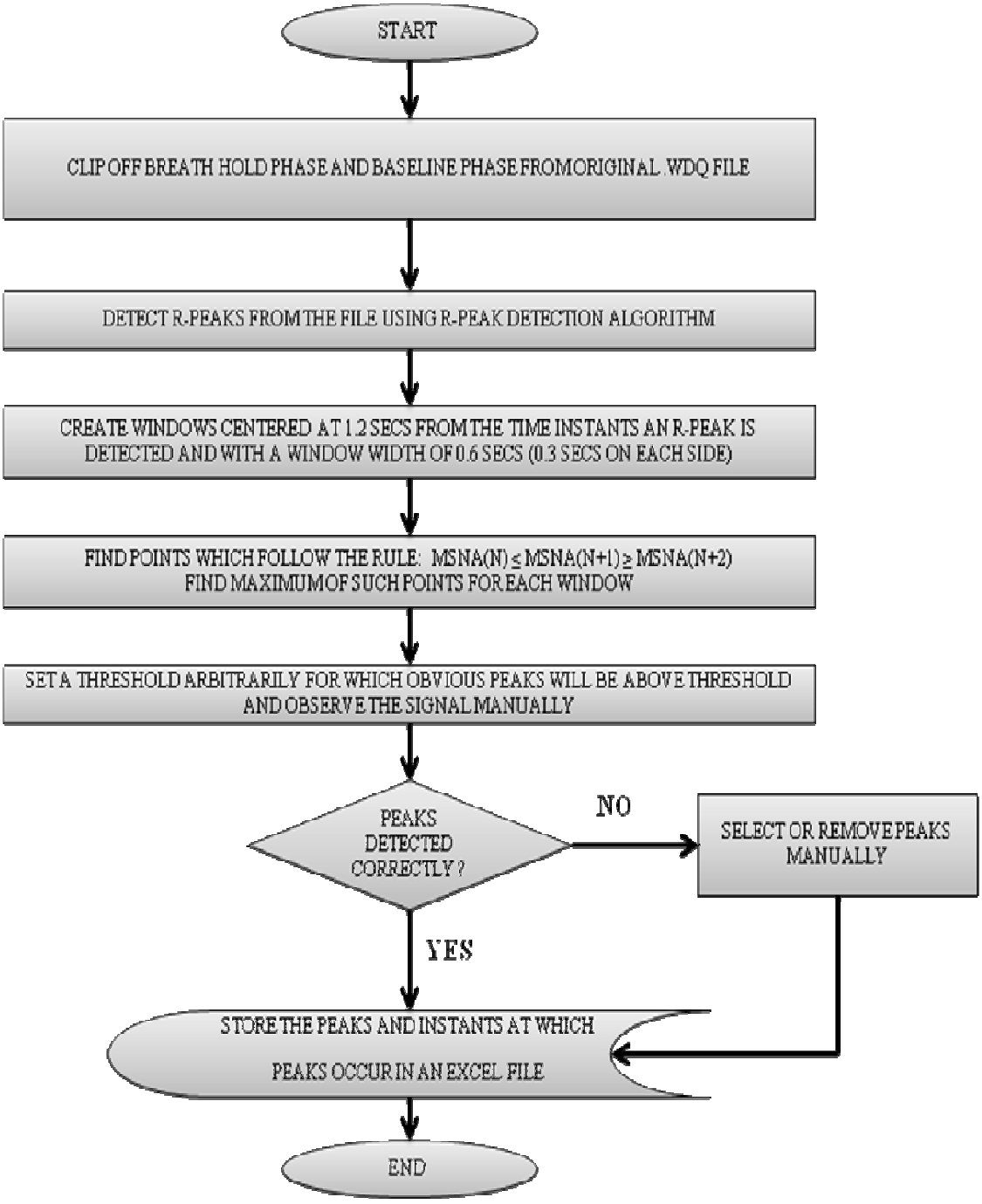

Figure 2.5 Flowchart of the MSNA peak detection algorithm.

Each steps of the algorithm are as described below:

2.3.1. Reading Windaq files into MATLAB

First, the breath hold phase and baseline phase are clipped off from the original raw Windaq file, based on the event markers placed by the investigator at the time of experiment. This clipping is done using the Advanced CODAS module (Dataq Instruments, Akron, OH, USA) and the clipped files are stored as .calc files. These .calc files are then read into MATLAB 7.1 (The Mathworks Inc., Natick, MA, USA) using Activex control commands. These commands acquire all the data points from all channels, as well as the sampling rate from the .calc files.

2.3.1. R Peak Detection

Once all the channels are acquired in MATLAB domain, an R peak detection algorithm developed by Vijendra et al. was used to detect R peaks. This R peak detection has been tested by Vijendra et al. on MIT-BIH arrhythmia database which produces a very low mean detection error of 1.14% [57]. This algorithm was developed by using an approach as proposed by Benitez et al., which uses first differential of the ECG signal and its hilbert transformed data to locate the R wave peaks from the original ECG signal [58].

2.3.2. MSNA Peak Detection

Once the R Peaks and the corresponding time instants at which these peaks occur are detected, a window is created centered at 1.2 seconds following each R peak

time instants. The width of the window is chosen arbitrarily as 0.6 seconds, with 0.3 seconds on each side of the centre of the window. Now, for each window of MSNA signal, those points are found which follow the rule: MSNA (n) $\leq$ MSNA (n+1) $\geq$ MSNA (n+2), where n varies from the (first data point) to (last data point-2) for that particular window and stored in a temporary variable 'tpeak'. Of all the points in the temporary variable 'tpeak', the maximum of these points is chosen to be as the peak for that window. An arbitrary threshold is determined for each case by visual inspection of the signal, which would screen for true MSNA peaks. It should be noted here that, for each MSNA peak there has to be a corresponding R peak occurring 1.0 to 1.5 seconds before it occurs, but it is not necessary that for each R Peak, a MSNA peak occurred. As such, the number of MSNA peaks may be less than the number of R peaks.

This algorithm works almost perfectly fine for most of the cases, but in cases where the signal is very noisy, undetected peaks or peaks detected falsely are removed manually. After the automated MSNA peak detection, each peak detected waveform is examined manually for any corrections using the following criteria [59]:

1. The MSNA peaks should have a triangular shape with a clear rising slope which rises to peak followed by a falling slope which falls again to the baseline.
2. Only one MSNA peak is marked per R-R interval. So, number of MSNA peaks cannot be greater than the number of R peaks.
3. The signal to noise ratio should be considerably high in the peak detected region.
4. There is a preference for false negatives over false positives.

2.4 MSNA Quantification

MSNA can be quantified in various ways as described previously. MSNA in this study was quantified almost similarly to the way Welch et al. did [50]. First, the MSNA peaks were detected for the baseline control conditions (baseline phase), for a particular condition and for a particular subject, using the MSNA peak detection algorithm previously described. Next, the average of all the burst during the baseline phase was calculated and assigned a value of 100 units. Then, MSNA peaks for the breath hold phase was detected in a similar fashion and each of them was normalized to average of all bursts during the baseline phase. For example, if the average of all MSNA peaks during the baseline phase was found to be 0.3 mV, then a MSNA peak of 0.6 mV during the breath hold phase would have a normalized value of 200 units.

Once the normalized value for each MSNA peak during the breath hold phase is calculated, the total of all those calculated values are obtained and termed as the total normalized MSNA. This is then divided by the total breath hold time to obtain the Time Averaged Normalized MSNA which is denoted as M, for each condition and for each subject. The total normalized MSNA was divided by the total breath hold time, as all the subjects could not hold their breath for 30 seconds.

2.5 Statistics

A repeated measures ANOVA (2 factors- experimental condition and subject type) was done to determine the effects of each factor on M. Since the results showed that the two subject types were not significantly different ($p>0.05$) in their effects on M, all 10 subjects were pooled into one group for further analysis of the effects of experimental condition on M. Further, a paired Student's t-test and Wilcoxon signed rank test (two tailed) was used to compare M responses between room air and each experimental condition (when treated exclusive of other conditions). Differences were considered significant at a level of $p<0.05$. All values are presented as Mean ± S.E.

Since experimental conditions were due to three different factors (O_2%, CO_2% and inspiratory efforts), a repeated measures ANOVA was performed to determine the factor effects on M. To compare each experimental condition with others and effect of each experimental condition on MSNA response when treated in combination, an N-way Repeated Measures ANOVA (General Linear Model) was used followed by a multiple comparison test (Tukey's test). Moreover, a multiple linear regression model using stepwise method was built to predict M from the three factors: O_2%, CO_2% and inspiratory efforts. ANOVA statistics and model statistics were also shown for the model. All statistical calculations were made using SPSS 12.0 (SPSS Inc., Chicago, IL). A cube plot and 3D graph was also built for predicted values of MSNA for different conditions from the linear regression model using Design Expert 7.0 (Stat-Ease, Minneapolis, MN).

CHAPTER 3

RESULTS

As discussed in Chapter 2, Muscle Sympathetic Nerve Activity (MSNA) was quantified as Time Averaged Normalized MSNA (M) in Arbitrary Integrated Unit (AIU). Moreover, with two levels of O_2% (21% and 12%), two levels of CO_2% (0% and 3%) and two levels of inspiratory efforts (0 mmHg and 20 mmHg), each intervention can be categorized as shown in Table 3.1. As such there are eight such experimental conditions for which M is calculated.

Table 3.1 List of all Experimental Conditions

Experimental Condition			O_2%	CO_2%	Inspiratory Efforts (mmHg)
Exp. No.	Abbre.	Condition			
1		Room Air	21	0	0
2	HO	Hypoxia	12	0	0
3	HC	Hypercapnia	21	3	0
4	HH	Hypoxic Hypercapnia	12	3	0
5	IE	Inspiratory Efforts	21	0	20
6	HO+IE	Hypoxia + Inspiratory Efforts	12	0	20
7	HC+IE	Hypercapnia +Inspiratory Efforts	21	3	20
8	HH+IE	Hypoxic Hypercapnia + Inspiratory Efforts	12	3	20

The M calculated (using the method discussed in Chapter 2) for each of the experimental condition is as shown in Table 3.2. Mean and Standard Error (S.E.) is also calculated for all 10 subjects.

Table 3.2 M Values (AIU) for each Experimental Condition

Subject	Room Air	HO	HC	HH	IE	HO+IE	HC+IE	HH+IE
Healthy 1	99.2	145.6	151.6	262.1	94.4	139.5	107.6	149.6
Healthy 2	130.2	150.4	153.1	171.5	119.1	126.7	148.8	156.8
Healthy 3	112.2	139.0	145.1	151.3	108.7	131.1	122.2	145.5
Healthy 4	36.3	104.7	106.2	194.8	36.0	101.9	99.1	115.8
Healthy 5	104.7	131.1	131.3	175.1	94.2	113.3	122.3	141.5
Healthy 6	53.1	60.4	102.2	124.8	37.2	49.3	81.2	65.8
Patient 1	81.8	84.3	102.3	118.3	82.9	74.6	95.2	100.5
Patient 2	124.4	127.1	125.5	154.6	98.5	118.3	125.1	145.4
Patient 3	67.9	105.9	104.6	298.8	61.2	78.1	90.8	280.7
Patient 4	75.6	115.5	88.6	136.5	55.7	105.5	73.7	113.3
Mean	88.5	116.4	121.1	178.8	78.8	103.8	106.6	141.5
S.E.	30.9	28.4	23.4	59.0	29.5	28.6	23.0	56.3

3.1 Difference between OSA and Healthy Subjects

Repeated measures ANOVA test was conducted to test the effects of two factors (subject type and experimental condition) on M and the results showed that there was no significant difference (p=0.498) between the two types of subjects (healthy subjects and OSA patients) in the metric M used to measure MSNA responses. Details of the statistical results are provided in Appendix B (Table B.1). As such, the control and normal subjects had the same effects on M across experimental conditions. Details of the statistical results are provided in Appendix B (Table B.1). Since these subject populations were not significantly different from each other, we pooled the two subject populations for further analysis and comparisons. With the pooled subjects sample, the ANOVA was redone to determine the effects of experimental condition on M.

3.2 Relative Contributions of Hypoxia, Hypercapnia and Inspiratory Efforts to MSNA during Breath Hold

Until now, we treated each experimental condition independent of others; however, it would be interesting to observe the effects of hypoxia, hypercapnia and inspiratory efforts when all eight experimental conditions are treated together. To evaluate this, an N-way repeated measures ANOVA (ANalysis Of VAriance) test for three factors (independent variables) was performed, which also accounts for variability in the subjects. The three factors being considered here were O_2%, CO_2% and inspiratory efforts. Each factor had two levels as discussed before, viz. 21% and 12% for factor 'O_2%', 0% and 3% for factor 'CO_2%' and 0 mmHg and 20 mmHg for factor

'Inspiratory Efforts'. The response variable (dependent variable) was 'M'. The results for repeated measures ANOVA showed that:

1. Only one interaction, among the three above mentioned factors were significant ($p<0.05$). $O_2\%$ * $CO_2\%$, $CO_2\%$ * Inspiratory Efforts and $O_2\%$ * $CO_2\%$ * Inspiratory Efforts were insignificant ($p>0.05$). Details of the statistical results are provided in Appendix B (Table B.3). The significant interaction was O_2 * Inspiratory Efforts ($p=0.022$) indicating that the effects of $O_2\%$ on M depends on the levels of inspiratory efforts, and vice versa (Figure 3.1).

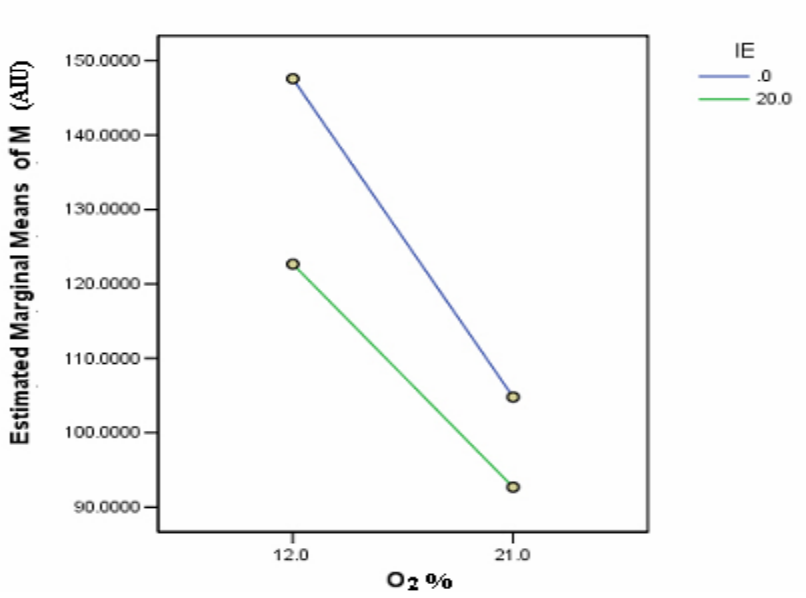

Figure 3.1 Plot of interaction effect ($O_2\%$ * IE) on M

2. Factor '$O_2\%$' had a significant effect on the response variable 'M' ($p=0.007$). M increased significantly from 98.7 ± 4.9 AIU for 21% O_2 to 135.1 ± 8.3 AIU for 12% O_2 ($p=0.007$). That is, hypoxia results in an increase in sympathoexcitation (M) during the

breath hold. Figure 3.2 shows a comparison of estimated marginal means of M for both levels of O_2%.

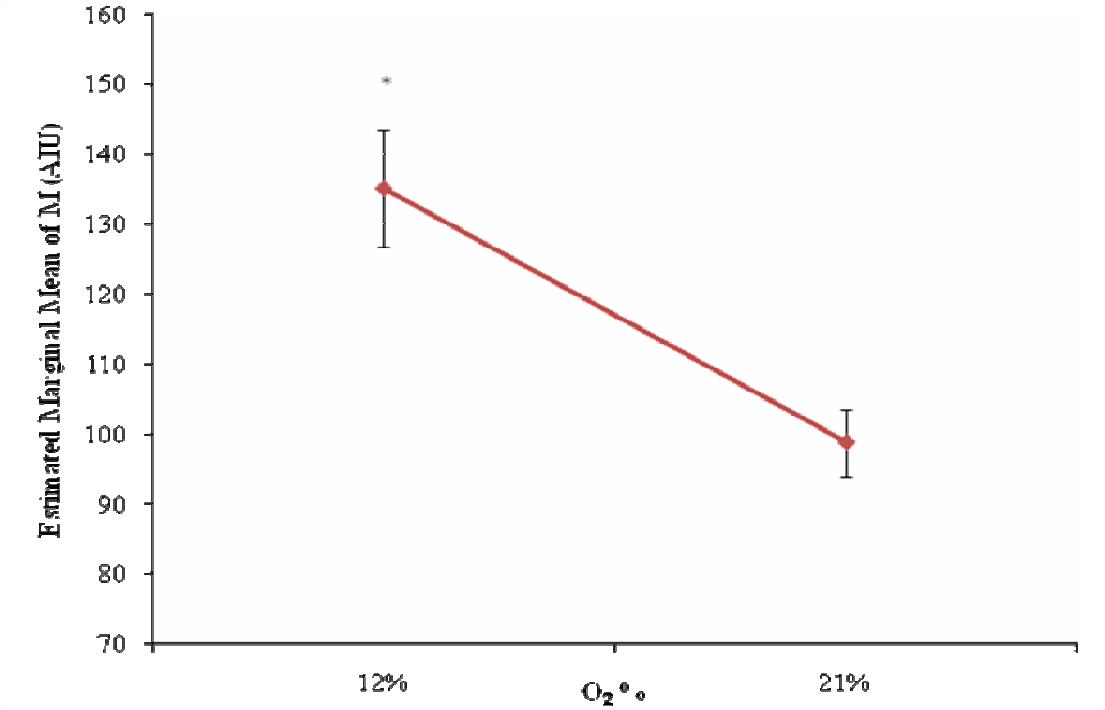

Figure 3.2 Effect of factor 'O_2%' on M. *: $p<0.05$ as compared to 21% O_2. Number of subjects=10.

3. Factor 'CO_2%' had a significant effect on the response variable 'M' ($p=0.002$). M increased significantly from 96.9 ± 5.0 AIU for 0% CO_2 to 137.0 ± 8.0 AIU for 3% CO_2 ($p=0.002$). That is, hypercapnia results in an increase in sympathoexcitation (M) during the breath hold. Figure 3.3 shows a comparison of estimated marginal means of M for both levels of CO_2%.

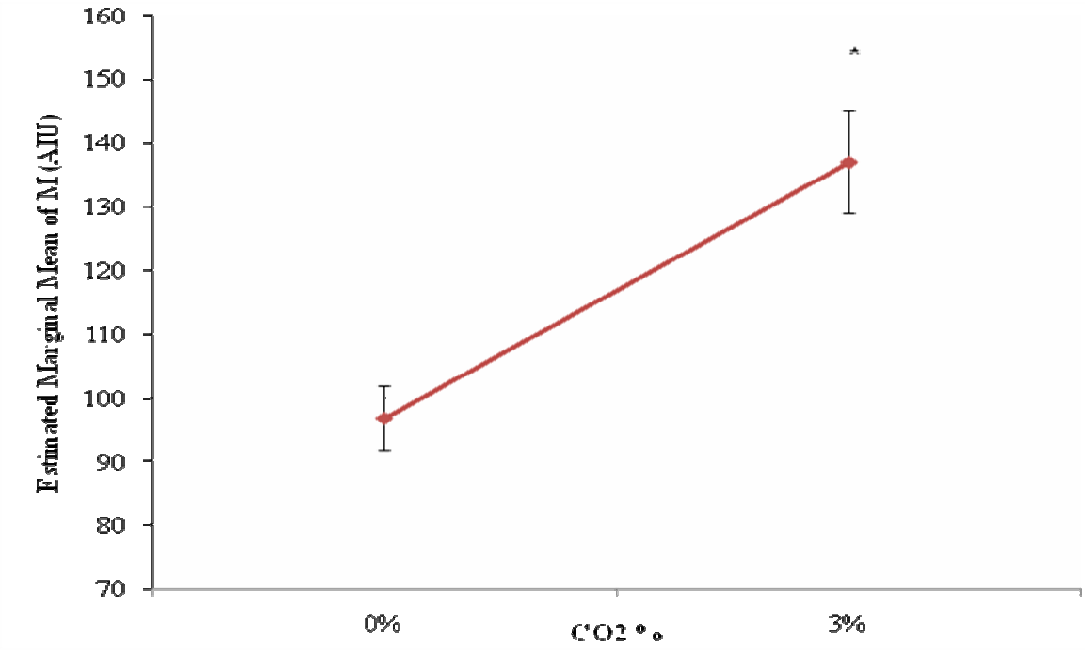

Figure 3.3 Effect of factor 'CO_2%' on M. *: $p<0.05$ as compared to 0% CO_2. Number of subjects=10.

4. Factor 'Inspiratory Efforts' had a significant effect on the response variable 'M' ($p<0.001$). M decreased significantly from 126.2 ± 7.8 AIU for 0 mmHg inspiratory efforts to 107.7 ± 6.6 AIU for 20 mmHg inspiratory efforts ($p<0.001$). That is, inspiratory efforts result in a decrease in sympathoexcitation (or increase in sympathoinhibition) during the breath hold. Figure 3.4 shows a comparison of estimated marginal means of M for both levels of inspiratory efforts.

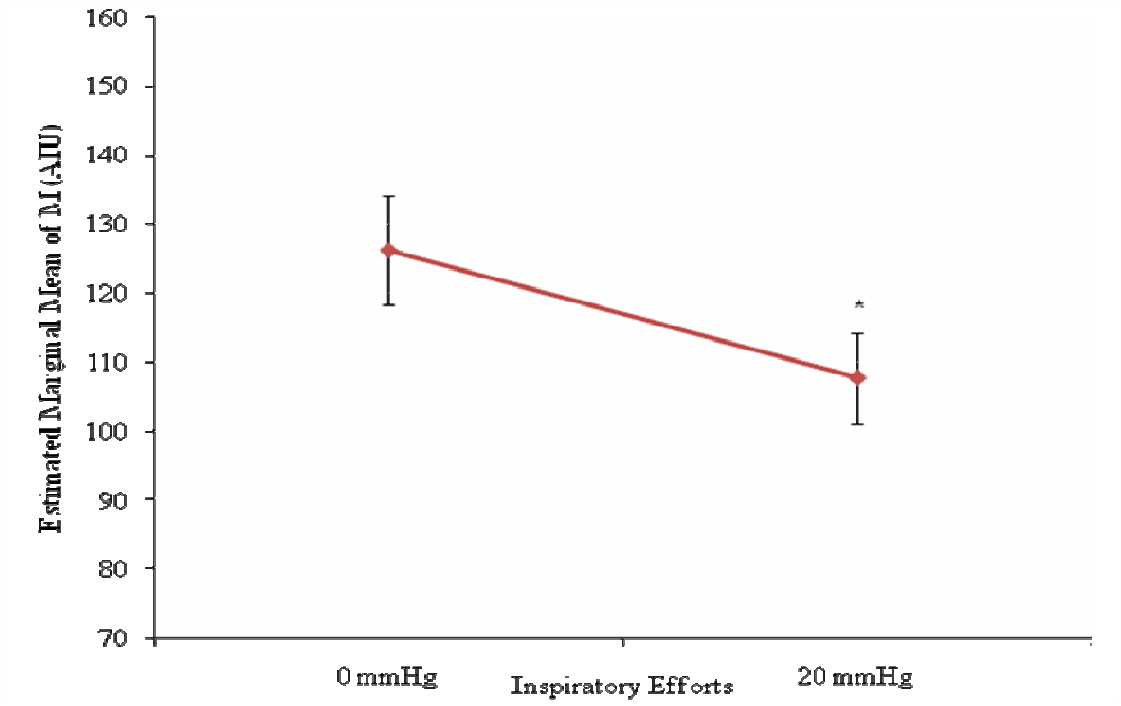

Figure 3.4 Effect of factor 'Inspiratory Efforts' on M. *: $p<0.05$ as compared to 0 mmHg inspiratory efforts. Number of subjects=10.

3.3 Effect of Apnea on MSNA to Hypoxia

Figure 3.5 shows MSNA recordings for breath hold phase for both room air (21% O_2) and hypoxia (12% O_2) for a particular subject. As this sample plot shows in Figure 3.5, MSNA response to hypoxia is much higher as compared to room air during the breath hold.

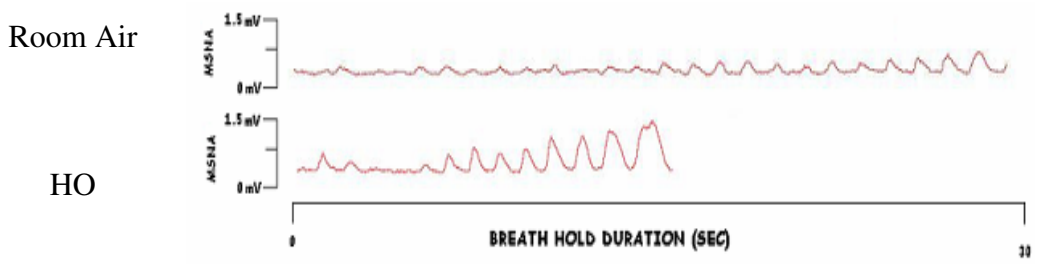

Figure 3.5 Typical MSNA recording during the breath hold phase for room air and hypoxia.

To evaluate the effect of hypoxia alone on MSNA during breath hold phase, we compared the M values for hypoxic conditions with those for room air conditions for all 10 subjects using a paired student's t test (two tailed). It was found that the M significantly increased from 88.5 ± 9.8 AIU for room air conditions to 116.4 ± 9.0 AIU for hypoxic conditions ($p=0.002$). Figure 3.6 shows the comparison of M between room air and hypoxic conditions during the breath hold. As number of subjects is small, nonparametric tests like Wilcoxon signed rank test (two tailed) were also performed and the results of t-test were confirmed ($p=0.005$). Wilcoxon signed rank test is the nonparametric equivalent test of paired student's t test. Thus, hypoxia alone has the effect of increasing sympathoexcitation significantly during simulated apnea.

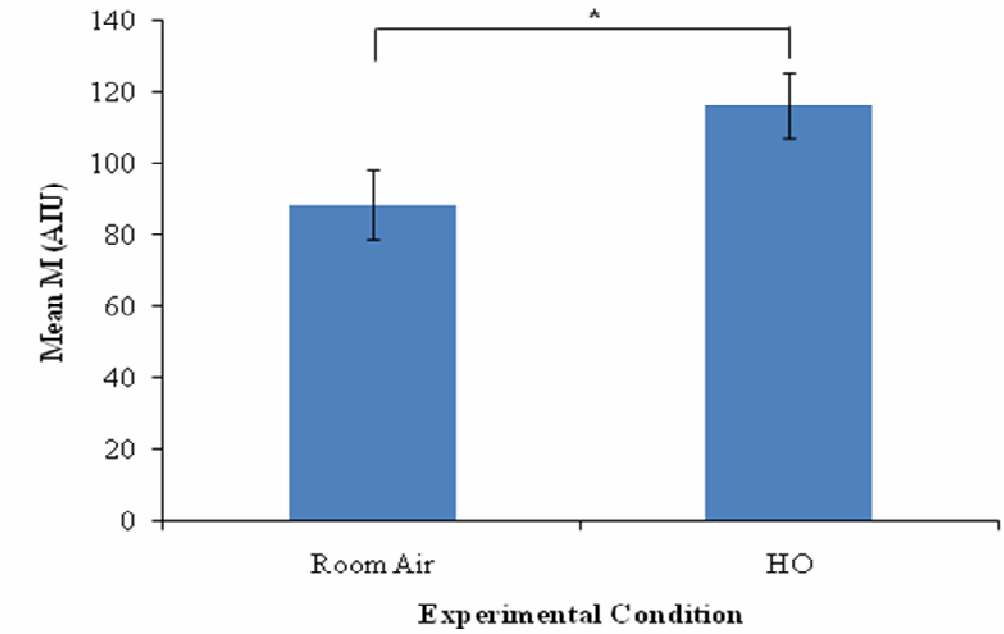

Figure 3.6 Comparison of MSNA responses measured as M between room air and hypoxic conditions during breath hold. *: $p<0.05$ compared to room air. Number of subjects=10. Values are expressed in Means ± S.E.

3.4 Effect of Apnea on MSNA to Hypercapnia

Figure 3.7 shows MSNA recordings for breath hold phase for both room air (21% O_2) and hypercapnia (3% CO_2) for a particular subject. As this sample plot shows in Figure 3.7, MSNA response to hypercapnia is much higher as compared to room air conditions during the breath hold.

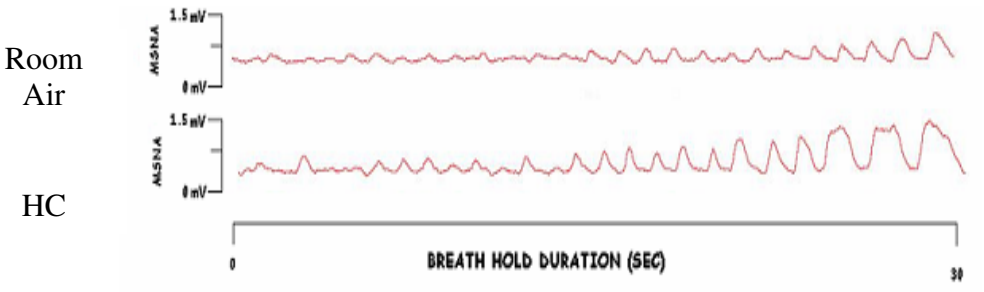

Figure 3.7 Typical MSNA recording during the breath hold phase for room air and hypercapnia.

To evaluate the effect of hypercapnia alone on MSNA during breath hold phase, we compared the M values for hypercapnic conditions with those for room air conditions for all 10 subjects using a paired student's t test (two tailed). It was found that M significantly increased from 88.5 ± 9.8 AIU for room air conditions to 121.1 ± 7.4 AIU for hypercapnic conditions ($p<0.001$). Figure 3.8 shows the comparison of M between room air and hypercapnic conditions during the breath hold. Nonparametric tests like Wilcoxon signed rank test (two tailed) were also performed and the results of t-test were confirmed ($p=0.005$). Thus, hypercapnia alone has the effect of increasing sympathoexcitation significantly during simulated apnea.

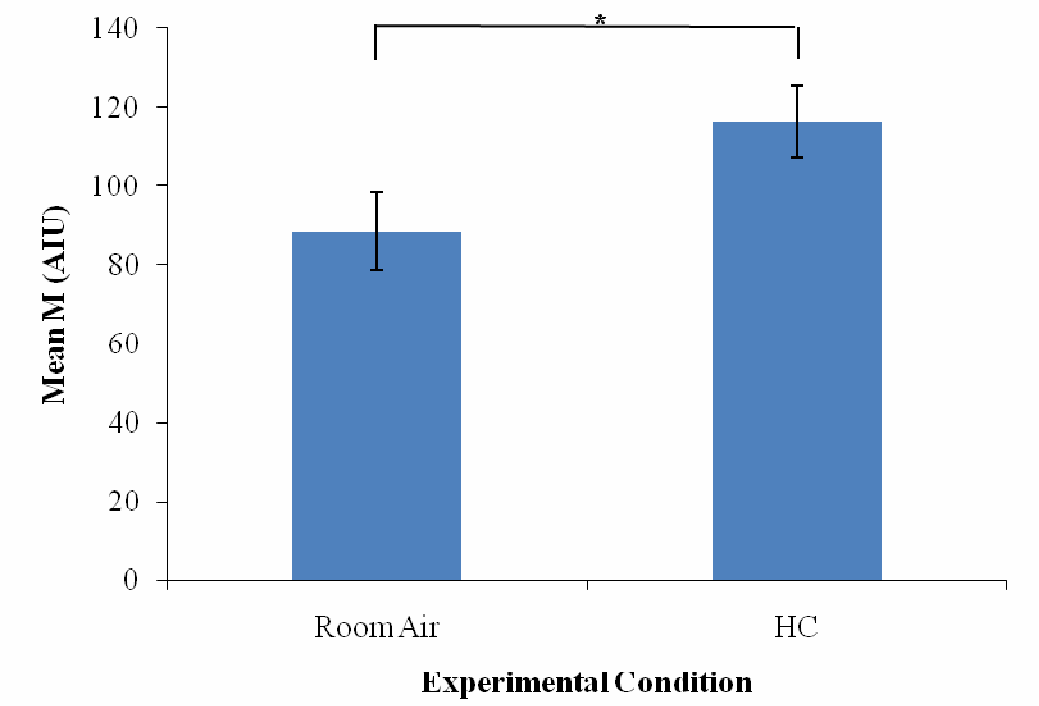

Figure 3.8 Comparison of MSNA responses measured as M between room air and hypercapnic conditions during breath hold. *: $p<0.05$ compared to room air. Number of subjects=10. Values are expressed in Means ± S.E.

3.5 Effect of Apnea on MSNA to Inspiratory Efforts

Figure 3.9 shows MSNA recordings for breath hold phase for both room air (21% O_2) and inspiratory efforts (20 mmHg) for a particular subject. As this sample plot shows in Figure 3.9, MSNA response to inspiratory efforts is much lower as compared to room air conditions during the breath hold.

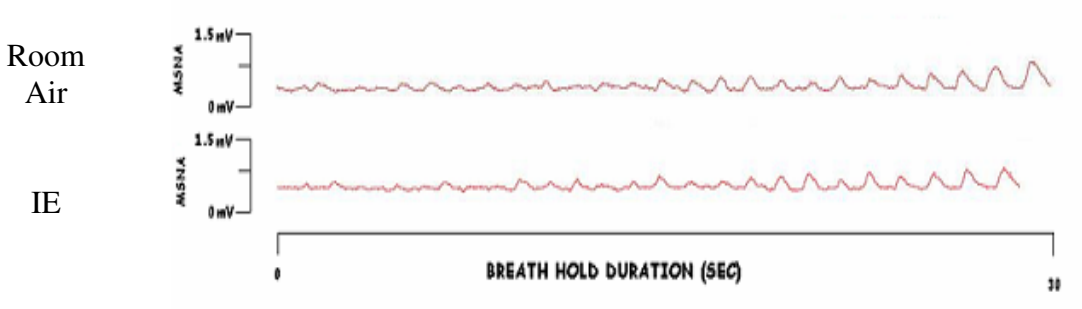

Figure 3.9 Typical MSNA recording during the breath hold phase for room air and inspiratory efforts.

To evaluate the effect of inspiratory efforts alone on MSNA during breath hold phase, we compared the M values for inspiratory efforts conditions with those for room air conditions for all 10 subjects using a paired student's t test (two tailed). It was found that M significantly decreased from 88.5 ± 9.8 AIU for room air conditions to 78.8 ± 9.3 AIU for inspiratory efforts conditions ($p=0.006$). Figure 3.10 shows the comparison of M between room air and IE conditions during the breath hold. Nonparametric tests like Wilcoxon signed rank test (two tailed) were also performed and the results of the t-test were confirmed ($p=0.009$). Thus, inspiratory efforts alone has the effect of reducing sympathoexcitation significantly during simulated apnea.

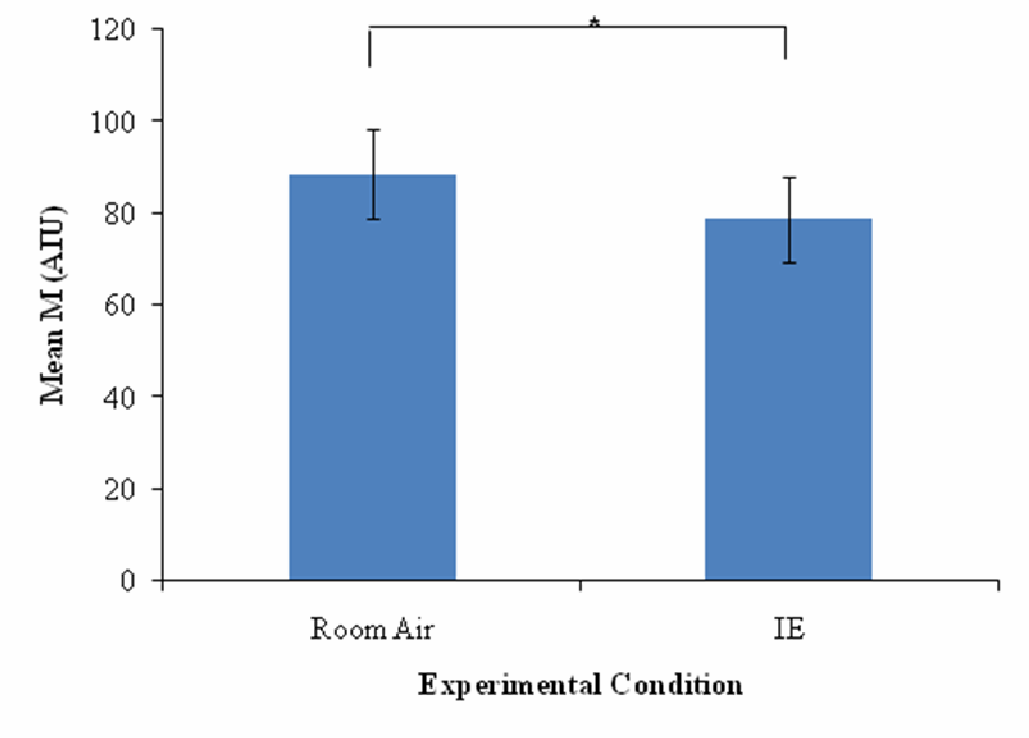

Figure 3.10 Comparison of MSNA responses measured as M between room air and inspiratory efforts conditions during breath hold. *: $p<0.05$ compared to room air. Number of subjects=10. Values are expressed in Means ± S.E.

3.6 Effect of Apnea on MSNA for each Experimental Condition

As we discussed earlier, there are eight different experimental conditions. We have already discussed three of them: HO, HC and IE. Now in this section, we would evaluate the effects of those experimental conditions, when two or more factors are involved: HH, HO+IE, HC+IE and HH+IE (Table 3.1). Figure 3.11 shows MSNA recordings for breath hold phase for room air, HH, HO+IE, HC+IE and HH+IE experimental conditions for a particular subject. To evaluate the effect of each experimental condition on MSNA during breath hold phase, we compared M values for

each experimental condition with those for room air conditions for all 10 subjects using a paired student's t test (two tailed).

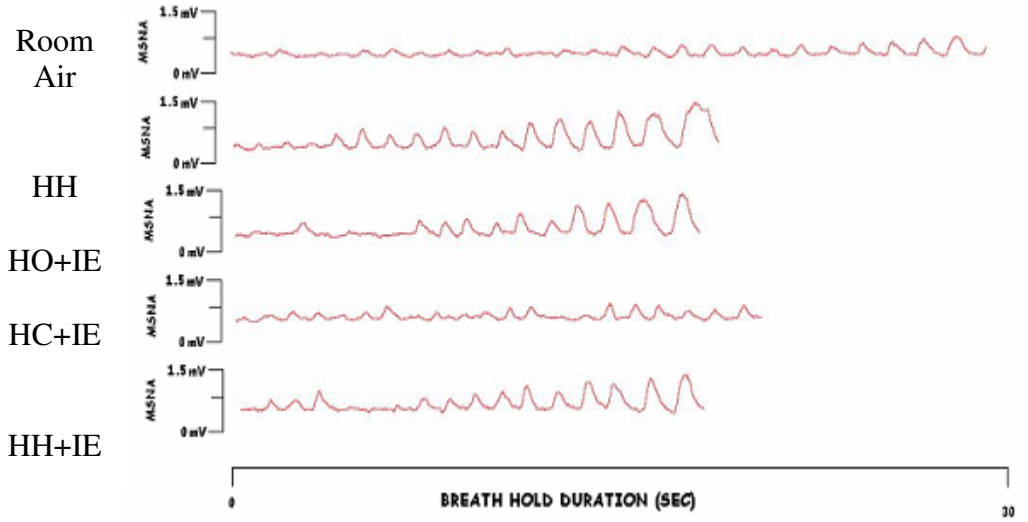

Figure 3.11 Typical MSNA recording during the breath hold phase for room air, HH, HO+IE, HC+IE and HH+IE experimental conditions.

The effect of three experimental conditions: HO, HC and IE have already been discussed. It was found that M significantly increased from 88.5 ± 9.8 AIU for room air conditions to 178.8 ± 18.7 AIU for hypoxic hypercapnia condition (p=0.003), 106.6 ± 7.3 AIU for hypercapnia + inspiratory efforts condition (p=0.012) and 141.5 ± 17.8 AIU for hypoxic hypercapnia + inspiratory efforts condition (p=0.019). However, M increased from 88.5 ± 9.8 AIU for room air conditions to 103.8 ± 9.0 AIU for hypoxia + inspiratory efforts condition, but was not significant (p=0.074). Figure 3.12 shows the comparison of M between room air and all the experimental conditions during the breath hold. Nonparametric tests like Wilcoxon signed rank test (two tailed) were also

performed and the results of t-test were confirmed ($p<0.05$). Details of the statistical results are provided in Appendix B (Table B.2).

Thus, hypoxic hypercapnia, hypercapnia + inspiratory efforts, hypoxic hypercapnia + inspiratory efforts have the effect of increasing sympathoexcitation significantly during simulated apnea.

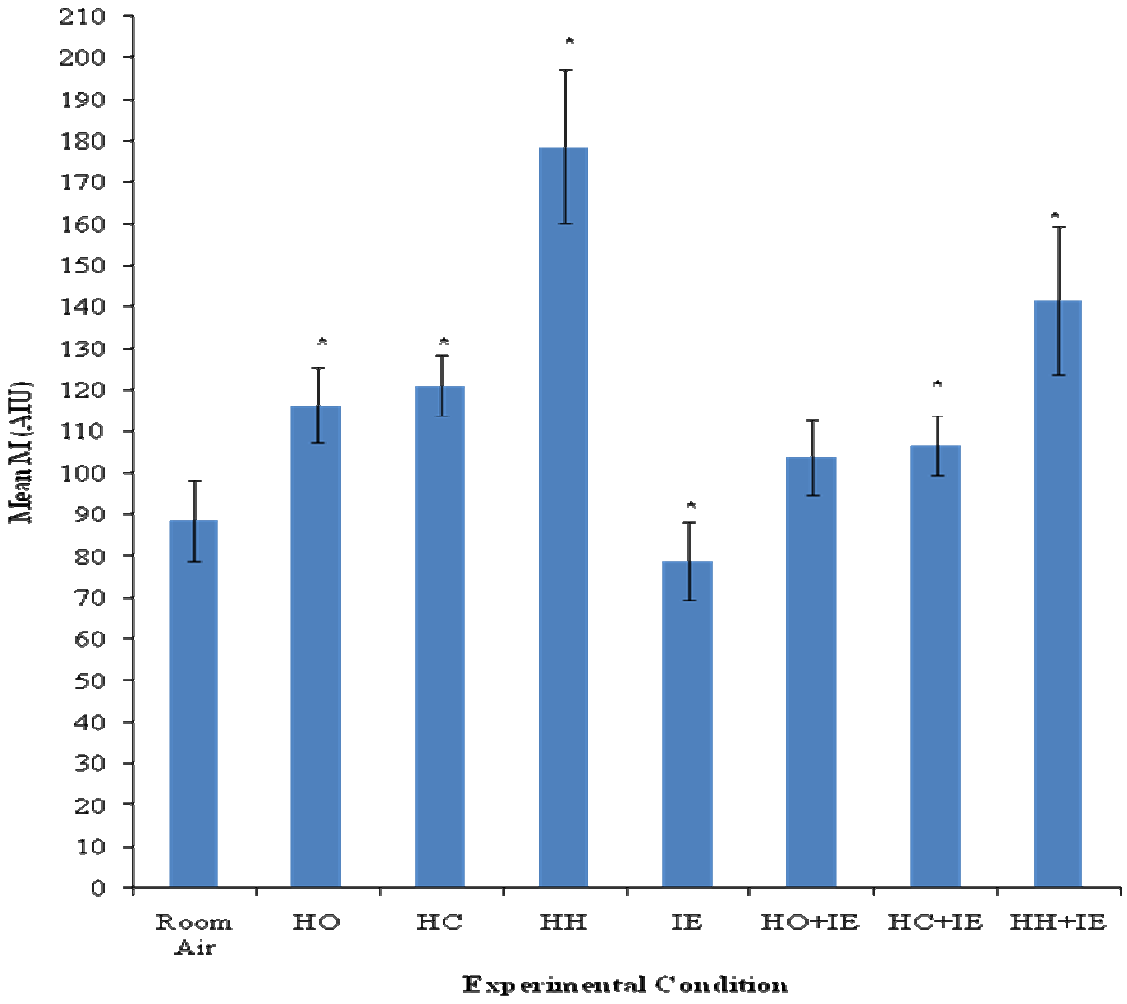

Figure 3.12 Comparison of MSNA responses measured as M between room air and all seven experimental conditions during breath hold. *$p<0.05$ compared to room air. Number of subjects=10. Values are expressed in Means ± S.E. Each experimental condition is treated exclusively.

3.7 Relationship between each Experimental Condition

A multiple comparisons test called Tukey's test was performed after ANOVA to determine which of the eight experimental conditions differed from each other. Table 3.3 shows comparisons among the experimental conditions. It can be observed that HH is significantly different from the rest of the conditions, except HH+IE. Thus, MSNA response to hypoxic hypercapnia is significantly greater that MSNA response to room air ($p<0.001$, Tukey's test), hypoxia ($p=0.010$, Tukey's test) and hypercapnia ($p=0.024$, Tukey's test). Details of the statistical results are provided in Appendix B (Table B.4).

Table 3.3 Multiple Comparisons Test for Each Experimental Condition. *: p<0.05 for Tukey's Test, Number of subjects = 10

Condition	Room Air	HO	HC	IE	HH	HO+IE	HC+IE	HH+IE
Room Air					*			
HO								
HC					*			
IE			*					*
HH		*		*			*	
HO+IE					*			
HC+IE								
HH+IE	*							

N/A Repeat condition Tukey's test insignificant (p<0.05) * Tukey's test significant

3.8 Multiple Linear Regression Model for M

A multiple linear regression model was built with O_2%, CO_2%, inspiratory efforts and interaction terms as possible predictor variables (independent variables) and M as the response variable (dependent variable). A stepwise regression approach was used for determining the linear regression equation after elimination of insignificant predictor variables. Before the stepwise procedure, tests for the assumptions of normality and constancy of variance in the errors were conducted and these tests indicated no violation of the assumptions. Hence, there was no need to transform the dependent variable.

The Model 3 shown in Table 3.4 was the best model. This table shows models with the main factors only, since no interaction factor was significant. The final predictive equation was:

(M) = 172.845 - 4.042 * (O_2%) + 13.364 * (CO_2%) - 0.926 * (IE) : Eq(2)

For comparisons, the best one variable and the best two variable models are also shown in Table 3.4. The model coefficients are shown in Table 3.5.

Table 3.4 ANOVA Results for the Stepwise Multiple Linear Regression Model

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	32149.398	1	32149.398	18.126	<0.001
	Residual	138342.200	78	1773.618		
	Total	170491.598	79			
2	Regression	58622.881	2	29311.440	20.175	<0.001
	Residual	111868.717	77	1452.840		
	Total	170491.598	79			
3	Regression	65481.017	3	21827.006	15.797	<0.001
	Residual	105010.581	76	1381.718		
	Total	170491.598	79			

Dependent Variable: (M)
Model 1 Predictors: (Constant), CO_2 %
Model 2 Predictors: (Constant), CO_2 %, O_2 %
Model 3 Predictors: (Constant), CO_2 %, O_2 %, Inspiratory Efforts (mmHg)

Table 3.5 Parameter Estimates of the Stepwise Multiple Linear Regression Model

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics
		B	Std. Error	Beta			VIF
1	(Const.)	96.885	6.659		14.550	<0.001	
	CO_2%	13.364	3.139	0.434	4.258	<0.001	1.000
2	(Const.)	163.586	16.748		9.768	<0.001	
	CO_2%	13.364	2.841	0.434	4.704	<0.001	1.000
	O_2%	-4.042	0.947	-0.394	-4.269	<0.001	1.000
3	(Const.)	172.845	16.853		10.256	<0.001	
	CO_2%	13.364	2.771	0.434	4.824	<0.001	1.000
	O_2%	-4.042	0.924	-0.394	-4.377	<0.001	1.000
	IE	-0.926	0.416	-0.201	-2.228	0.029	1.000

Dependent Variable: (M)
Model 1 Predictors: (Constant), CO_2 %
Model 2 Predictors: (Constant), CO_2 %, O_2 %
Model 3 Predictors: (Constant), CO_2 %, O_2 %, Inspiratory Efforts (mmHg)

R Squared values for a model measures the strength of relationship between the independent variables and dependent variable. It is interpreted as the percent of

variance in the dependent variable explained by the independent variables. R Squared value for model 3 was found out to be 0.384 (Table 3.6). This indicates that the independent variables explain 38.4% of the variance in the dependent variable. Further, Adjusted R Squared values were also calculated, which is an adjustment to penalize for the possibility that, with many independents, some of the variance may occur due to chance. Adjusted R Squared value for Model 3 with three independent variables was found out to be 0.360 (Table 3.6)

Table 3.6 Stepwise Multiple Linear Regression Model Parameters

Model	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics		
				Adjusted R Square Change	F Change	Sig. F Change
1	0.189	0.178	42.114	0.178	18.126	<0.01
2	0.344	0.327	38.116	0.149	18.222	<0.01
3	0.384	0.360	37.171	0.033	4.963	<0.029

Dependent Variable: (M)
Model 1 Predictors: (Constant), CO_2 %
Model 2 Predictors: (Constant), CO_2 %, O_2 %
Model 3 Predictors: (Constant), CO_2 %, O_2 %, Inspiratory Efforts (mmHg)

Adjusted R Square value penalizes for addition of insignificant independent variables in the model. For model 3, we found that the difference was small (0.033). Table 3.5 shows the parameter estimates (coefficients) of the stepwise multiple linear regression model. The theoretical regression equation for three independent variables and one dependent variable is as described.

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} \qquad \textbf{: Equation 1}$$

Here, Y_i is the dependent variable; X_{i1}, X_{i2} and X_{i3} are the independent variables, β_0 is the Y intercept, β_1, β_2 and β_3 are the unstandardized coefficient estimates (slopes) for the independent variables. Variance Inflation Factor (VIF) is calculated for the model and should be near to 1.0 for low multicollinearity. VIF measures how much the variances of the estimated regression coefficients are inflated as compared to when the predictor variables are not linearly related. The results showed no multicollinearity in the model (all three VIF's equal 1.0 in model 3).

The results of the sequence of steps in the stepwise regression is shown in Table 3.5. It begins with just one independent variable and gradually works up its way to the largest number of significantly important independent variables. However, each time a variable is added all variables in the model are examined to see if any should be eliminated at that step. Model 3 was used as the model for predicting M and further analysis. The best equation for M can now be written as described below in equation 2, where IE is Inspiratory Efforts.

$(M) = 172.845 - 4.042 * (O_2\%) + 13.364 * (CO_2\%) - 0.926 * (IE)$: Eq(2)

As discussed before, the VIF for each factor is 1.0 and the interaction factors are insignificant. It can be assessed how important and in which direction each factor is, in predicting or influencing M. From Table 3.5 and equation 2, it can be clearly seen that $CO_2\%$ has the largest influence on M (B=13.364) than $O_2\%$ (B=-4.042) and inspiratory efforts (B=-0.926). Moreover, it can be seen from Table 3.6 that the Adjusted R Square change is also the highest (0.178) when $CO_2\%$ is added

as compared to O_2 % (0.149) and inspiratory efforts (0.033), which indicates that CO_2% is the best predictor of the dependent variable M.

Unstandardized regression coefficients (β_1, β_2 and β_3) indicate that with a unit increase in the predictor variable X_k, the response variable is expected to change by β_k units, when all other predictor variables in the regression model are held constant. Thus, it would be incorrect to compare the unstandardized coefficients as the units of the three independent variables are different. It would be like comparing apples with oranges. To solve this problem, we incorporate standardized regression coefficients, which are calculated by multiplying the unstandardized regression coefficients with a ratio of standard deviation of independent variable and dependent variable. The standardized coefficients (beta coefficients) now indicate that with a unit change of one standard deviation in the predictor variable, it is expected that there will be a change of beta in the dependent variable. Thus, higher the standardized regression coefficient, the greater is the impact of predictor variable on the response variable. As the regression coefficients are now standardized, we can compare the relative importance of each independent variable in predicting the dependent variable.

From the regression model as shown in Table 3.5, it can be inferred that the effect of CO_2% (beta=0.434) is greater than the effect of O_2% (beta=-0.394) and inspiratory efforts (beta=-0.201). However, it would be interesting to see if the effect of CO_2% is significantly ($p<0.05$) greater than the effect of O_2% and inspiratory efforts. To test this hypothesis, we have the null hypothesis H_0: $\beta_1 = \beta_2$ and alternate hypothesis H_a: $\beta_1 \neq \beta_2$, we would create a reduced model (Eq 3)

$$Y_i = \beta_0 + \beta_c\,(X_{i1} + X_{i2}) + \beta_3 X_{i3}$$ **: Equation 3**

Here, β_c denotes the common coefficient for β_1 and β_2 under H_0 and $X_{i1} + X_{i2}$ is the corresponding new X variable. We then use the general F* test statistic with 1 and n-4 degrees of freedom as follows.

$$F^* = \frac{SSE(R) - SSE(F)}{df_R - df_F} \div \frac{SSE(F)}{df_F}$$

$$df_F = n - 4$$

$$df_R - df_F = (n-3) - (n-4) = 1$$

Here SSE is the error sum of squares (or residual sum of squares) and df is the degree of freedom. R indicates the reduced model and F indicates the full model described by equation 1 discussed previously. We found that, the effect of CO_2% on M was significantly greater than the effect of O_2% on M ($p<0.001$) and inspiratory efforts on M ($p<0.001$). Further, effect of O_2% on M was significantly greater than the effect of inspiratory efforts on M ($p<0.001$). Moreover, positive coefficients for CO_2%, and negative coefficients for O_2% and inspiratory efforts indicate the following:

1. With an increase in CO_2%, M also increases. Thus, hypercapnia contributes significantly to cause an increase in M.

2. With a decrease in O_2%, M also increases. Thus, hypoxia contributes significantly to cause an increase in M.

3. With a decrease in inspiratory efforts, M also increases. Thus, inspiratory efforts contribute significantly to cause an increase in M.

Thus, hypercapnia influences M more than hypoxia and inspiratory efforts does. In other words, hypercapnia causes a much greater increase in M as compared to the increase caused by hypoxia and decrease caused by inspiratory efforts. Figure 3.13 shows a cube plot of predicted M values with O_2%, CO_2% and inspiratory efforts being the independent factors.

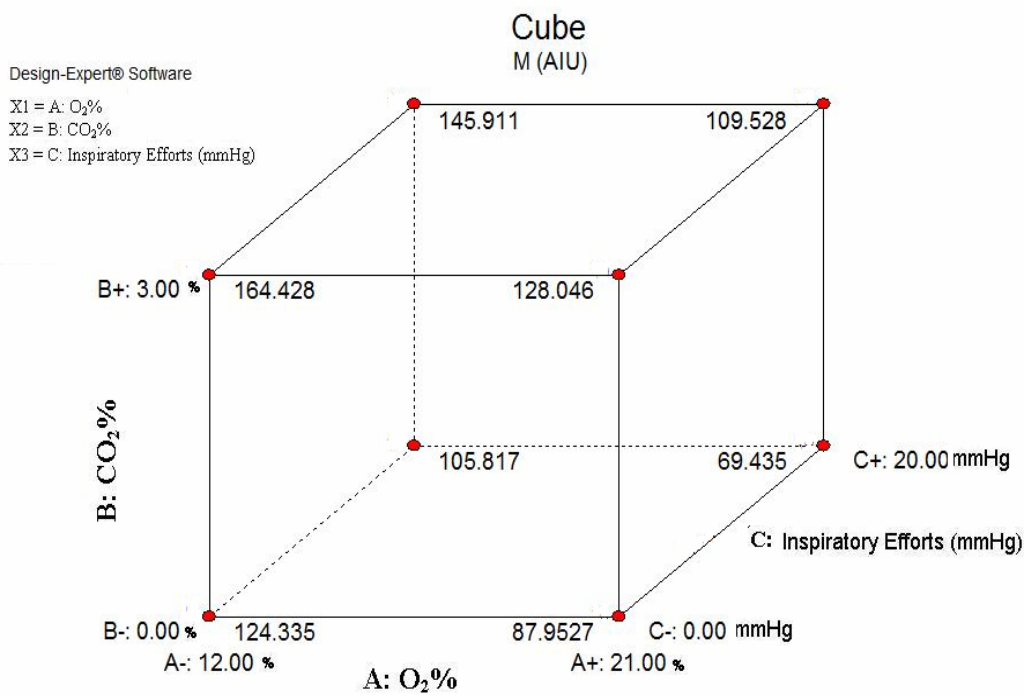

Figure 3.13 Cube plot of predicted M values (AIU).

Figure 3.14 and Figure 3.15 show a 3D surface graph of predicted M values for 0 mmHg and 20 mmHg inspiratory efforts, respectively. These 3D surface graphs clearly show the effects of all the three independent factors on M and the relative importance of each one of them. These graphs have been made using Design Expert Software. However, this regression model would hold good only if the values of O_2% is between 21% and 12%, CO_2% is between 0% and 3% , and inspiratory efforts is between 0 mmHg and 20 mmHg.

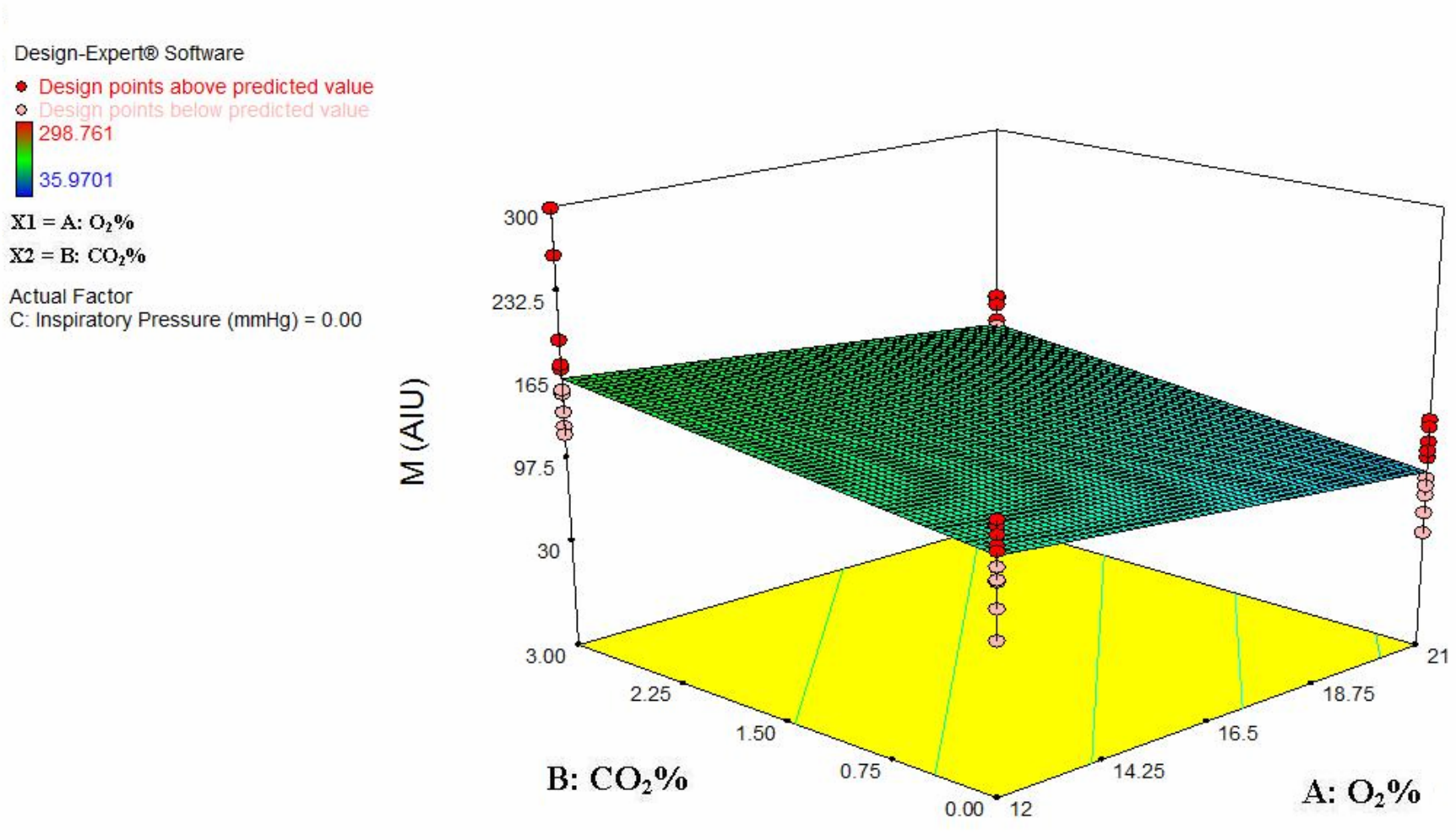

Figure 3.14 3D plot for predicted M values for inspiratory efforts = 0 mmHg.

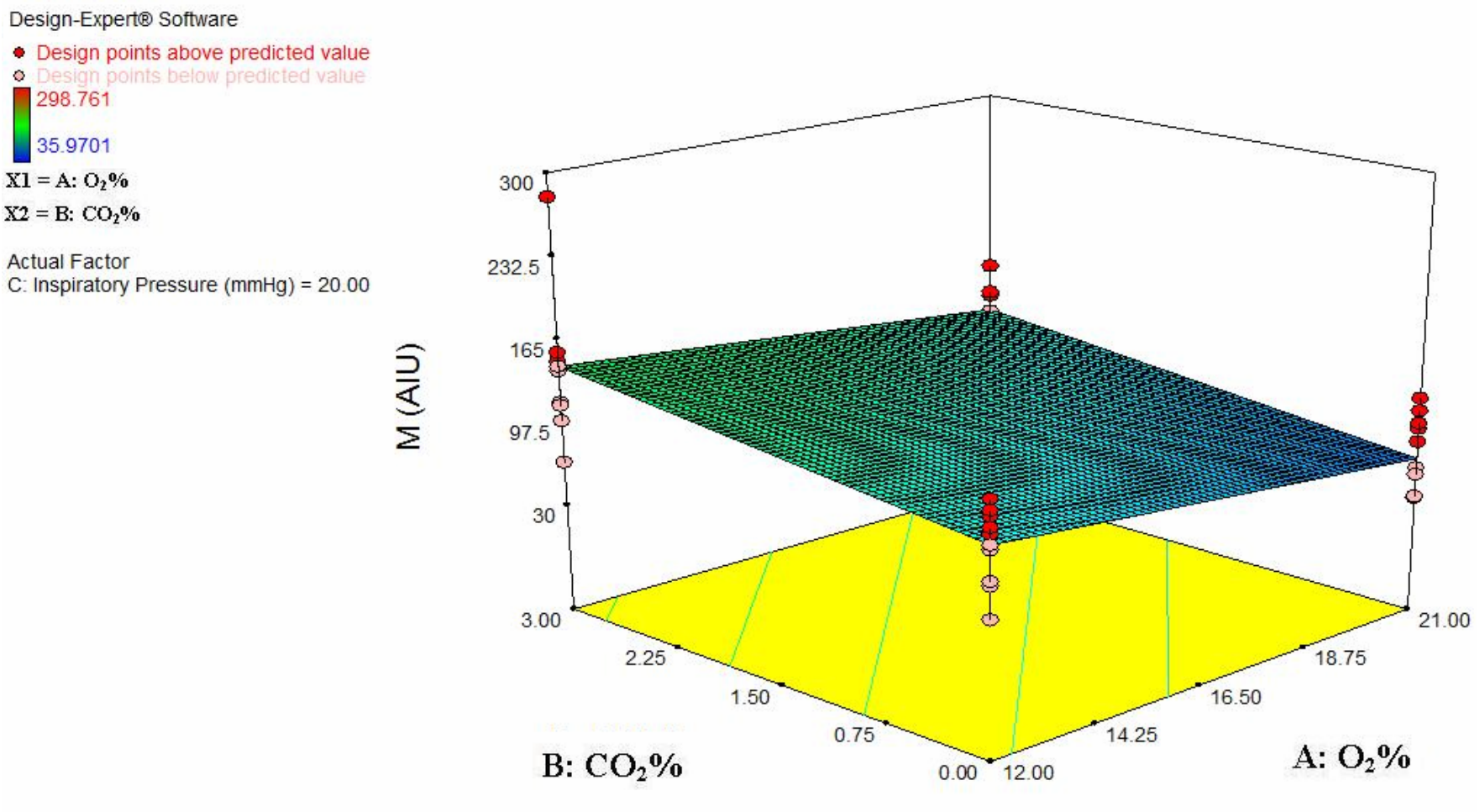

Figure 3.15 3D plot for predicted M values for inspiratory efforts = 20 mmHg.

CHAPTER 4

DISCUSSION AND CONCLUSION

The major new finding in this study was the greater effect of hypercapnia on MSNA as compared to hypoxia and inspiratory efforts during apnea. It was also found that hypoxia and hypercapnia had the effect of increasing MSNA during apnea as compared to MSNA response for breathing room air prior to apnea. However, the synergism of hypoxia and hypercapnia was not observed during apnea. This could be because the effect of apnea, hypercapnia and hypoxia causes the MSNA to reach maximal levels (saturation) and cannot increase beyond that level and hence not showing any synergism. Moreover, the inspiratory efforts resulted in a decreased MSNA response during apnea compared to MSNA response for breathing room air prior to apnea. Additionally, Somers et al. found that effect of apnea, whether during room air or hypoxic and/or hypercapnic gas increased MSNA [32]. Further, each apneic episode is accompanied by periods of sympathoexcitation, increased arterial blood pressure and heart rate which return toward room air after apnea termination. Increase in blood pressure causes an increase in parasympathetic activity, which in turn lowers the heart rate and blood pressure [32]. Effects of hypoxia, hypercapnia and inspiratory efforts are discussed in the following sections.

4.1 Effect of Hypoxia during Apnea on MSNA

Our data suggest that hypoxia during apnea (at 12% O_2) increases MSNA significantly compared to apnea after breathing room air (21% O_2). This can be explained by the fact that hypoxia tends to activate peripheral chemoreceptors, thus increasing sympathoexcitation, as a result of chemoreflex responses. In a similar study in which healthy subjects underwent voluntary apnea after breathing room air and hypoxic gas (14% O_2), it was found that apnea potentiated the sympathetic responses to hypoxic gas to a greater extent than it potentiated sympathetic responses to room air [60]. Further, Somers et al. suggest that there may be a threshold of hypoxia for discernible sympathetic activation [60].

4.2 Effect of Hypercapnia during Apnea on MSNA

Our findings indicate that hypercapnia during apnea produces a significantly greater response in MSNA than MSNA response to apnea after breathing room air. This can be attributed to activation of central chemoreceptors due to hypercapnia, which consecutively increases sympathoexcitation as a result of chemoreflex responses. Further, we also found that MSNA response to apnea after breathing a combination of hypoxic and hypercapnic gas was significantly greater than the MSNA response to apnea after breathing room air, or hypoxic gas, or hypercapnic gas. Thus, hypoxia during apnea, hypercapnia during apnea and hypoxic hypercapnia during apnea all increase MSNA significantly indicating that both peripheral chemoreceptors and central

chemoreceptors act individually as well as together to increase sympathoexcitation in both healthy subjects and OSA patients.

4.3 Effect of Inspiratory Efforts during apnea on MSNA

An inspiratory effort against a collapsed upper airway is physiologically similar to a mueller maneuver. The mueller maneuver leads to a reduction in negative intrathoracic pressures which lead to initial increases in venous return, diastolic pressure followed by an abrupt cessation of venous return due to collapse of veins in the thoracic cavity [61, 62]. This in turn, activates the baroreceptors (mechanoreceptors) causing sympathoinhibition. Our data shows clearly that inspiratory efforts result in a decreased muscle sympathetic nerve activity during apnea. This is in good agreement with Somers et al., who showed that baroreflex activation achieved with intravenous infusion of phenylepinephrine (vasoconstrictor), significantly increased MSNA [31].

4.4 Contrasting Effects of Hypoxia, Hypercapnia, Inspiratory Efforts on MSNA during Apnea

A study showing which of the effects: hypercapnia during apnea, hypoxia during apnea and inspiratory efforts during apnea has a greater influence on MSNA for both healthy subjects and OSA patients, has not yet been done. Our study indicates that hypercapnia during apnea (at 3% CO_2) has a significantly stronger effect on MSNA response than hypoxia during apnea (at 12% O_2) and inspiratory efforts during apnea (at 20 mmHg). Thus, indicating that central chemoreceptors have a stronger effect on sympathetic activity than peripheral chemoreceptors and baroreceptors during apnea. It

is interesting to speculate why this happens. One possible cause could be because of the selective effect of apnea to both hypoxia and hypercapnia on increasing sympathoexcitation while greatly reducing the effect to inspiratory efforts. Another possible cause could be attributed to the close proximity of peripheral chemoreceptors and baroreceptors at the solitary nucleus in brainstem, thus cancelling out the effect of each other. The repeated measures ANOVA also showed that the interaction between factors O_2% and inspiratory efforts was significant indicating that they may cancel out the effect of each other on MSNA response. This was also consistent with the paired t test results which indicate that HO+IE (hypoxia + inspiratory efforts) was not different from room air conditions (p=0.070). Also, nonparametric test like Wilcoxon signed rank test showed that HO+IE was not different from room air conditions (p=0.074). Moreover, Tukey's test also showed that HO+IE was not different from room air conditions (p=0.942)

4.5 Limitations and Future Research

Inherent shortcomings of the multiunit recordings exist as they are entirely dependent on the site of correct electrode placement within the peroneal nerve. The accuracy of these recordings also deteriorates with the movements of leg. As such, one needs to make sure there is absolutely no or minimum movements of leg during the experiment. Moreover, the recording also depends on the number of firing units in the vicinity of the electrodes placed. Further, the position of the electrodes may vary from subject to subject thus resulting in intersubject variations. Moreover, peak detection of

MSNA recordings is also dependent on observer's ability to correctly identify MSNA peaks manually following computerized peak detection. A robust peak detection method using wavelets can be formulated to detect MSNA peaks accurately.

There might be some kind of threshold associated with hypoxia, hypercapnia and inspiratory efforts during apnea after which some discernible effects on MSNA can be observed. This necessitates the need for performing this study at numerous levels of severity of hypoxia, hypercapnia and inspiratory efforts. Also, evaluating the effect of each of these afferents on MSNA during the breathing phase and recovery phase could provide for better understanding.

Though this study did not show any differences between the healthy subjects and OSA patients, but obviously there is a difference between them, because of the airway collapse during sleep for OSA patients. Also, there is a need for identifying a factor that could be accounted for differences between the OSA patients and healthy subjects and hence serve as an indicator of OSA. One might be able to use other simultaneous measurements used in this study to possibly derive models that are predictive of MSNA. Primary stimulus like arterial oxygen saturation could be used to predict MSNA which would bypass the need to measure MSNA, as microneurographic technique is a very tedious and complicated procedure.

4.6 Conclusion

In conclusion, our data demonstrate that both hypoxia and hypercapnia result in increased sympathoexcitation during apnea, while inspiratory efforts result in increased sympathoinhibition during apnea. Moreover, this study also shows that hypoxia, hypercapnia and inspiratory efforts contribute significantly to predict MSNA. However, the influence of hypercapnia on muscle sympathetic nerve activity was significantly greater than the effect of hypoxia and inspiratory efforts during apnea. Moreover, this study also showed that there is no significant difference in MSNA responses between healthy subjects and OSA patients during each experimental condition. Also, when simulated apnea involves inspiratory efforts following hypoxic gas breathing, MSNA response was not significantly different from MSNA response when apnea was simulated after breathing room air. However, there was a significant increase in MSNA response to simulated apnea which involves inspiratory efforts following hypercapnic gas as compared to MSNA responses to simulated apnea following room air breathing.

APPENDIX A

MATLAB PROGRAM FOR MSNA PEAK DETECTION

```
% %%% MATLAB program for MSNA Peak Detection
clc
clear all
close all

 % To read .wdq files (clipped phases) into MATLAB using Activex control commands
readdataqfile1 = actxcontrol('DATAQFILE.ReadDataqFileCtrl.1',[10,10,5,5])

%Register the ReadDataqFile Events
readdataqfile1.registerevent({'FileError','dataqfileerror';'EndofFile','endoffile';'ControlEr
ror','controlerror'})
[filename, pathname] = uigetfile('*.wdq', 'Pick a .wdq file');
a=strcat(pathname,filename);
%Select the WinDaq file that you want to read from
set(readdataqfile1, 'FileName', a)

%Open the WinDaq file
readdataqfile1.Open
points=readdataqfile1.TotalDataPoints;

%Display data on the screen, and put into matrix dqAnalysis
dqAnalysis = readdataqfile1.GetData(points, 1);
[dqChannels,dqDataPts] = size(dqAnalysis);

%Create a time vector, dqTime, of integers from 1 to dqDataPts.
dqTime = 1:dqDataPts;
sna=dqAnalysis(1,:);
ECGClip=dqAnalysis(2,:);
Fs=readdataqfile1.SampleRate
Totallength=points/Fs
samplerate=1/readdataqfile1.SampleRate;
t=0:samplerate:((points-1)*samplerate);
plot(t,ECGClip)
figure; plot(t,sna)

% % % ECG Peak Detection

[Peaks] = QRSHilbert(ECGClip, Fs, 1, 0, 0, 0); % using sridhar's program for peak
detection [57]

% calculating the center for each window which would be 1.1 seconds from each R
Peak of ECG

starttime=Peaks+round((1.2)*Fs);
```

```
% This part uses a logic that is MSNA(n)<= MSNA(n+1) >=MSNA(n+2). And then
% from those sna(n+1) points which obey the above rule, select the max of
% those points and this point is the peak for that particular window
Windwidth=round(0.3*Fs);
for i=1:(length(starttime)-2)
            snawindow=sna((starttime(i)-Windwidth):(starttime(i)+Windwidth ));
      w(:,i)=snawindow; % w contains all the points from the sna signal with no. of
rows= window length and no of column=no. of windows
end
   a=size(w);u=a(1,2);v=a(1,1); % u= no. of columns of w  and  v= no. of rows of w
   tpeak=zeros(v,u);
 % finding those points in w which follow this rule and put into a different matrix tpeak
for i=1:u
   for k=1:(length(snawindow)-2)
        if w(k,i)<=w(k+1,i) && w(k+1,i)>=w(k+2,i)
        tpeak(k+1,i)=w(k+1,i); % putting those values of w into tpeak which follow the
described rule for each window
        end
   end
end

% Calculating the max of tpeak for each window
for i=1:u
      [s(i),Ind(i)]=max(tpeak(:,i)); % s is the value of the those detected peak for each
window and Ind is the sample no associated with it
      Ind(i)=(starttime(i)-Windwidth+Ind(i)-2)/Fs; % converting Ind from sample
domain to time domain
end
% Plotting the original  MSNA signal with the detected peaks
figure;plot(t,sna); hold on; plot(Ind,s,'r*'); hold off
th=input('enter the threshold in %')
threshold=th/100;
threshold=threshold*max(s);
f=find(s>=threshold);
figure;plot(t,sna); hold on; plot(Ind(f),s(f),'r*'); hold off
Finalpeaks=s(f);
Finalpeaktime=Ind(f);
Finalpeaks=Finalpeaks';% Contains the amplitude of MSNA Peaks
Finalpeaktime=Finalpeaktime';% Contains the time instant at which MSNA peaks occur
[X,Y,BUTTON] = GINPUT   % To manually select the peak using the click of mouse
```

Figure A.1 shows a sample plot of a peak detected MSNA signal during the breath hold phase using the MSNA peak detection algorithm described above.

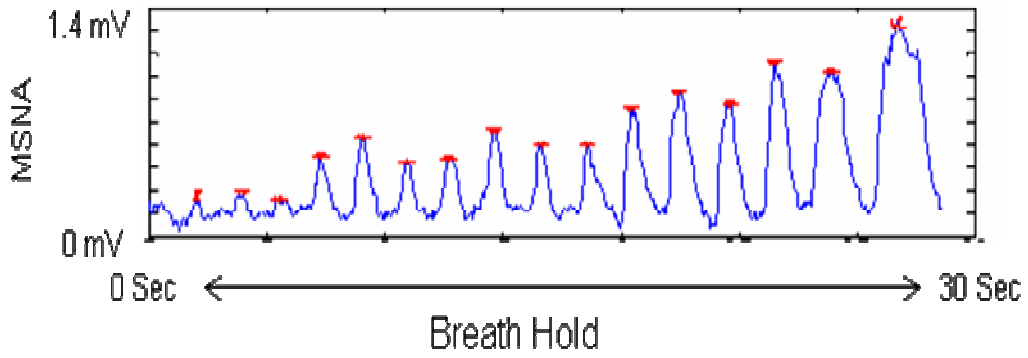

Figure A.1 Sample plot of peak detected MSNA signal during breath hold using MSNA peak detection algorithm. * indicates peaks of MSNA signal.

To clip the breath hold phase and baseline phase from the original raw .wdq file, we first open the raw .wdq file. Event markers were placed by the physician during the experiment. These event markers can be accessed by using Ctrl + <ArrowKeys>. First, the beginning of the breath hold phase is located using Ctrl + Right arrow key. Next, the time marker is enabled at this instant (using F4 key). This would reset the time marker to 0 seconds. Then, the end of breath hold is located using Ctrl + Right arrow key. The file is then decompressed using F7 and then decompressing it to a factor of 1. This part is then clipped using save as option (Ctrl + v) with file type as .calc and the file is stored in an appropriate directory. This procedure is summarized in Figure A.2. These steps are then repeated for the baseline phase of the same file.

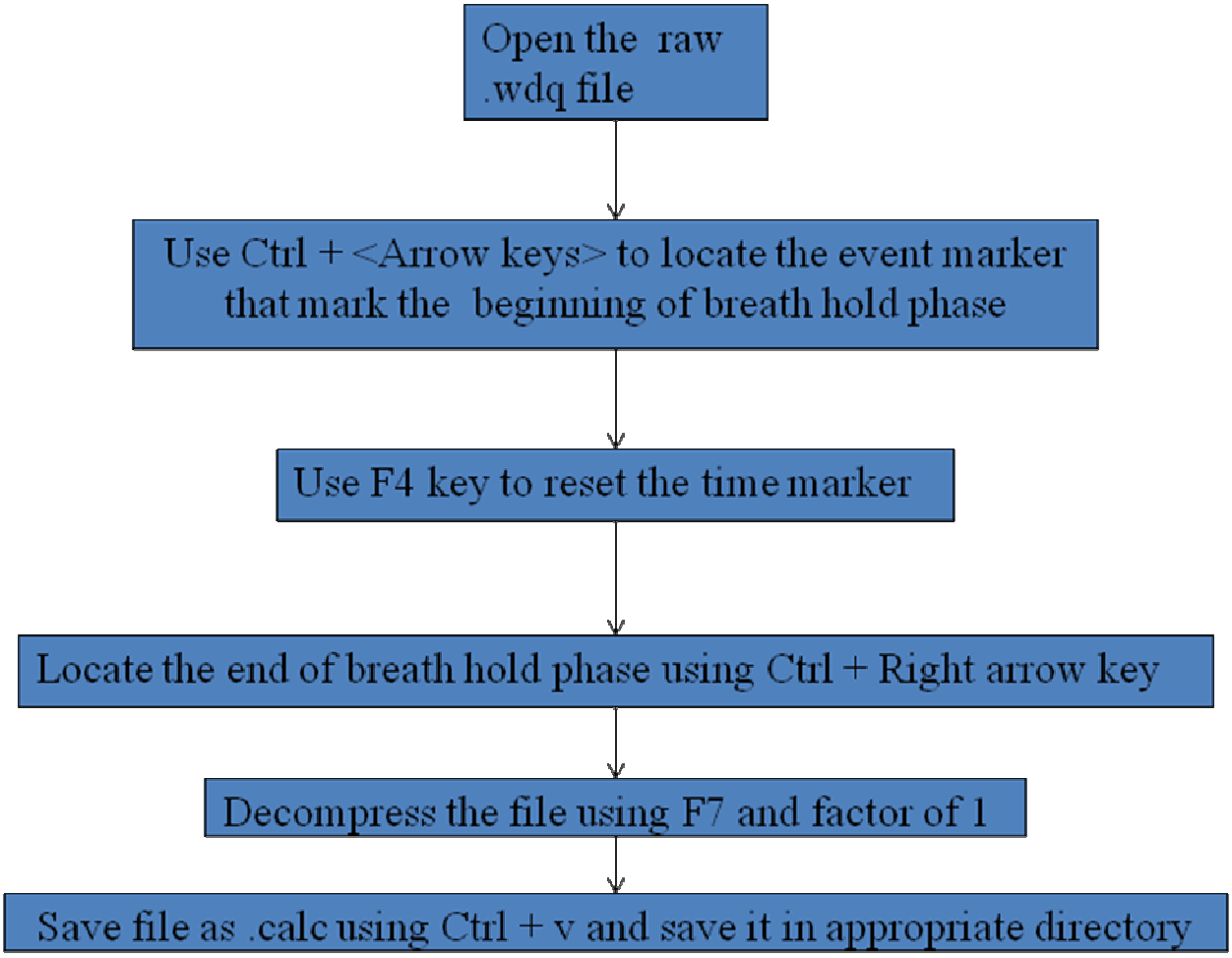

Figure A.2 Steps to clip the breath hold phase from the raw .wdq file.

APPENDIX B

STATISTICAL RESULTS

Table B.1 Results for Comparison of M Values between Healthy Subjects and OSA Patients. Significant Difference at $p<0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Subject Type	4645.126	1	4645.126	1.948	0.257
Experimental Condition	62794.715	7	8970.674	11.312	<0.001

Table B.2 Results for Comparison of M values between Room Air and Each Experimental Condition. Significant Difference at $p<0.05$

Conditions	Paired t test p values	Wilcoxon Signed Rank Test p values
Room Air-HO	0.002	0.005
Room Air- HC	<0.001	0.005
Room Air- IE	0.006	0.009
Room Air- HH	0.003	0.005
Room Air- HOIE	0.070	0.074
Room Air- HCIE	0.012	0.009
Room Air- HHIE	0.02	0.005

Table B.3 Test of Between-Subjects Effects (Repeated Measures ANOVA). Dependent variable: M. Significant Difference at $p<0.05$

Source	Type III Sum of Squares	df	F	Sig.
Intercept	1093840.829	1	216.778	<0.001
O_2	26473.482	1	12.192	0.007
CO_2	32149.398	1	17.712	0.002
IE	6858.136	1	34.629	<0.001
O_2 * CO_2	1970.912	1	1.426	0.263
O_2 * IE	822.217	1	7.562	0.022
CO_2 * IE	1080.539	1	3.245	0.105
O_2 * CO_2 * IE	499.608	1	3.906	0.080
Error	100637.305	72		
Total	1264332.427	80		

Table B.4 Results for Multiple Comparison Tests (Tukey's test) for M Values. Significant Difference at $p<0.05$

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.
HC	HCIE	14.5	13.2	0.956
	HH	-57.7	13.2	0.001
	HHIE	-20.4	13.2	0.781
	HO	4.6	13.2	1.000
	HOIE	17.2	13.2	0.895
	IE	42.3	13.2	0.043
	Room Air	32.5	13.2	0.234
HCIE	HC	-14.5	13.2	0.956
	HH	-72.2	13.2	0.000
	HHIE	-34.9	13.2	0.163
	HO	-9.8	13.2	0.995
	HOIE	2.8	13.2	1.000
	IE	27.8	13.2	0.426
	Room Air	18.1	13.2	0.870
HH	HC	57.7	13.2	0.001
	HCIE	72.2	13.2	0.000
	HHIE	37.3	13.2	0.109
	HO	62.4	13.2	0.000
	HOIE	74.9	13.2	0.000
	IE	100.0	13.2	0.000
	Room Air	90.2	13.2	0.000

Table B.4 – Continued

HHIE	HC	20.4	13.2	0.781
	HCIE	34.9	13.2	0.163
	HH	-37.3	13.2	0.109
	HO	25.1	13.2	0.559
	HOIE	37.7	13.2	0.102
	IE	62.7	13.2	0.000
	Room Air	53.0	13.2	0.004
HO	HC	-4.6	13.2	1.000
	HCIE	9.8	13.2	0.995
	HH	-62.4	13.2	0.000
	HHIE	-25.1	13.2	0.559
	HOIE	12.6	13.2	0.980
	IE	37.6	13.2	0.103
	Room Air	27.9	13.2	0.423
HOIE	HC	-17.2	13.2	0.895
	HCIE	-2.8	13.2	1.000
	HH	-74.9	13.2	0.000
	HHIE	-37.7	13.2	0.102
	HO	-12.6	13.2	0.980
	IE	25.0	13.2	0.562
	Room Air	15.3	13.2	0.942
IE	HC	-42.3	13.2	0.043
	HCIE	-27.8	13.2	0.426
	HH	-100.0	13.2	0.000
	HHIE	-62.7	13.2	0.000
	HO	-37.6	13.2	0.103
	HOIE	-25.0	13.2	0.562
	Room Air	-9.8	13.2	0.995
Room Air	HC	-32.5	13.2	0.234
	HCIE	-18.1	13.2	0.870
	HH	-90.2	13.2	0.000
	HHIE	-53.0	13.2	0.004
	HO	-27.9	13.2	0.423
	HOIE	-15.3	13.2	0.942
	IE	9.8	13.2	0.995

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BIOGRAPHICAL INFORMATION

Vikram Rao was born in Bangalore, India in July 1984. He graduated with a Bachelor of Engineering degree in Biomedical & Instrumentation Engineering from U.V. Patel College of Engineering, North Gujarat University, India in June 2006. He then received a Deans Fellowship at the Bioengineering Department at University of Texas at Arlington in August, 2006, which gave him an impetus to pursue Masters in Bioengineering at University of Texas at Arlington. He found it interesting how integrating different disciplines in science and engineering contribute to the improvement of human life. He was bent on doing Research in the field of Biomedical Signal Processing and sleep apnea which geared him up to work with Dr. Khosrow Behbehani. He feels that sleep apnea is still not taken very seriously in many countries. His undergraduate knowledge and work experience gave him a better understanding of different disciplines in science and engineering. He also served as a Graduate Teaching Assistant for two years for subjects like Biological Digital Signal Processing and Process Control in Biotechnology. His research interests include Biological Statistical Analysis, Algorithm Development and Digital Signal Processing.