THE EFFECTS OF OBSTRUCTIVE SLEEP APNEA ON BLOOD PRESSURE

by

Dina Brooks

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of the Institute of Medical Sciences,
University of Toronto

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The Effects of Obstructive Sleep Apnea on Blood Pressure

Dina Brooks, PhD (1997)

Institute of Medical Sciences
University of Toronto

Several epidemiological studies have identified obstructive sleep apnea (OSA) as a risk factor for systemic hypertension, but a direct etiologic link between the two disorders has not been established definitively. Furthermore, the specific physiological mechanisms underlying the association between OSA and systemic hypertension have not been identified. The purpose of this study was to use a canine model of OSA to systematically examine the effects of OSA on daytime and night-time blood pressure (BP). In addition, this model was used to determine the long-term effects of sleep apnea on the acute responses to airway occlusion during sleep. Four dogs were studied during a control period before induction of OSA, during a period of OSA (of 83-133 days), and following cessation of OSA. After completion of the OSA protocol, the dogs were re-studied on a sleep fragmentation protocol to determine the impact of sleep disruption without OSA on the acute responses to airway occlusion. OSA resulted in acute transient increases in night-time BP to a maximum of 13.0±2.0 mm Hg (mean±S.E.); and eventually produced sustained daytime hypertension to a maximum of 15.7±4.3 mm Hg. In contrast, recurrent arousal from sleep without airway occlusion caused night-time hypertension but did not result in daytime hypertension. In addition, OSA and sleep fragmentation both resulted in progressive lengthening of the time to arousal in response to acute airway occlusion (all p values <0.02), and in greater arterial oxygen desaturation (p<0.05), peak inspiratory pressures (p<0.003), and surges in maximum systolic and diastolic BP during airway occlusion (p<0.01).
There were no differences between the changes observed during OSA and during sleep fragmentation. Thus, the impact of OSA on the acute responses to airway occlusion during sleep is primarily the result of the associated sleep fragmentation. The demonstration that OSA can lead to the development of sustained hypertension has considerable importance, given the high prevalence of both disorders in the population.
This Thesis is dedicated to my family
ACKNOWLEDGEMENTS

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<td>number of apneas plus hypopneas per hour of sleep</td>
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<tr>
<td>AI</td>
<td>apnea index</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ANP</td>
<td>atrial natriuretic peptide</td>
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<td>BP</td>
<td>blood pressure</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CO</td>
<td>cardiac output</td>
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<td>CPAP</td>
<td>continuous positive airway pressure</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<td>EMG</td>
<td>electromyogram</td>
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<td>EOG</td>
<td>electrooculogram</td>
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<td>HR</td>
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<td>i.m.</td>
<td>intramuscular</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
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<td>MABP</td>
<td>mean arterial blood pressure</td>
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<tr>
<td>nREM</td>
<td>non-rapid eye movement</td>
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<tr>
<td>OSA</td>
<td>obstructive sleep apnea</td>
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<tr>
<td>PAP</td>
<td>pulmonary artery pressure</td>
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<tr>
<td>P_TR</td>
<td>tracheal pressure</td>
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<tr>
<td>P_EtCO₂</td>
<td>end-expiratory partial pressure of carbon dioxide</td>
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<tr>
<td>PCO₂</td>
<td>partial pressure of carbon dioxide; P_aCO₂, refers to arterial levels; P_ACO₂, refers to alveolar levels</td>
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<td>RR</td>
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<td>SaO₂</td>
<td>percent saturation of haemoglobin with oxygen in arterial blood</td>
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<td>SD</td>
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<td>SE</td>
<td>standard error</td>
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1.1.4. Pathophysiology of OSA

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PART I: INTRODUCTION AND BACKGROUND

The clinical syndrome of obstructive sleep apnea (OSA) is characterized by repeated episodes of upper airway occlusion during sleep, resulting in recurrent asphyxia and disruption of sleep. Epidemiological studies have identified OSA as a risk factor for hypertension, myocardial infarction, stroke, and sudden death, but the physiological mechanisms underlying these associations have not been defined. Progress in studying the long-term complications of OSA has been hampered by the fact that, by the time patients with OSA come to clinical attention, the disorder and its possible long-term sequelae have often been present for several years. Furthermore elucidation of the possible underlying mechanisms that link OSA with the development of hypertension and cardiovascular disease requires that several physiological measurements (some of which are invasive) be made over extended period of time, a requirement that is not feasible in patients with the disorder.

Given the limitations in studying disease mechanisms in patients with OSA, we have developed a canine model of this syndrome. The canine species was used because of the similarities between the human and canine respiratory control systems, and the canine model of OSA and the human condition (Kimoff et al., 1994). Several stimuli that characterize OSA may contribute to the relationship between OSA and hypertension, including repetitive episodes of hypoxia and hypercapnea, disruption of sleep architecture, and fluctuations in intrathoracic pressure during the occluded respiratory efforts (6). This thesis specifically addresses the question of whether OSA leads to sustained systemic hypertension in this canine model and investigates the stimuli and mechanisms that may be involved. Sustained hypertension, in the context of this research project, is defined as elevation in blood pressure that persists during periods of uninterrupted breathing, i.e., in the absence of acute airway occlusions.
In this introduction (Part I), the background and rationale for this work will be discussed. An overview of the two disorders, OSA and hypertension, will be given. The epidemiological and clinical data that point to an association between OSA and cardiovascular morbidity and mortality will be detailed. Natural and induced animal models of OSA will also be described.
Chapter 1: Introduction and Background

1.1. Obstructive Sleep Apnea: An Overview

1.1.1. Historical Review and Definition of Obstructive Sleep Apnea (OSA)

The occurrence of disordered breathing during sleep was first described more than a century ago (Broadbent, 1877), but the specific characteristics of OSA were not identified until 1965 by Gaustaut and co-workers. These investigators classified the different periods of cessation of breathing observed during sleep on polysomnographic recordings into either obstructive, central or mixed apneas (Gaustaut et al., 1966). Over the next 2 decades, awareness of sleep-disordered breathing increased, with numerous studies published on the pathogenesis, pathophysiology and therapeutic management of this disorder. Nevertheless, sleep apnea remained poorly appreciated by the medical community. For example, in 1989, Kryger stated:

'... in the large recent two-volume (3792 pages) edition of the Oxford Textbook of Medicine, there are 17 pages devoted to syphilis [whose incidence has declined tremendously to 10 per 100,000 population] and less than four pages to the entire topic of sleep-related disorders of breathing.'

Over the past decade, considerable research has focused on the prevalence of sleep-disordered breathing and the potential dangerous consequences of these disorders (Young et al., 1993). For instance, patients with sleep-disordered breathing have increased risk of automobile crashes and of cardiovascular morbidity and mortality (Findley et al., 1989; Shepard, 1992). Substantial insights have emerged into the hazards of sleep apnea, so much so that, in 1993, the National Commission on Sleep Disorders Research identified disorders of sleep as a major public health burden that affects the lives of millions of Americans; and called attention to the impact of these disorders on the health and welfare of society.
Polysomnography is the accepted standard for the diagnosis of sleep disordered breathing (Martin et al., 1985; ATS, 1989). There are two broad categories of sleep disordered breathing: central and obstructive sleep apnea. Central sleep apnea is identified by cessation of airflow in the absence of respiratory efforts, resulting from transient withdrawal of central drive to the muscles of respiration (Bradley and Phillipson, 1992). Conversely, obstructive sleep apnea - which is the focus of this thesis- is characterized by repetitive episodes of upper airway obstruction during sleep, resulting in cessation (apnea) or marked reduction (hypopnea) of airflow despite persisting respiratory efforts (Phillipson, 1993). The apneas or hypopneas typically result in increasing asphyxia and are terminated by a brief arousal from sleep and restoration of upper airway patency (Phillipson, 1988). The repeated obstructive apneas are often associated with symptoms of functional impairment such as daytime hypersomnolence (Phillipson et al., 1993).

1.1.2. Prevalence and Risk Factors

The best estimate of the prevalence of sleep disordered breathing is the study by Young and colleagues (1993) in men and women between the ages of 30 and 60 years. Previous studies have been limited by either the use of a small sample, restriction of the subjects to males, or the use of home-based recordings rather than full sleep studies (Lavie, 1983; Gislason et al., 1988; Stradling and Crosby, 1991; Ancoli-Israel et al., 1991).

Young and co-workers (1993) extrapolated their findings in 602 subjects to the general population and reported that 4% of women and 9% of men experienced 15 or more episodes of apnea or hypopnea per hour of sleep. However, the nature of the disorder (i.e. obstructive or central apneas) was not defined. Based on these results, if the threshold for sleep apnea is reduced to 5 apneas/hypopneas or more per hour of sleep, the estimate of sleep disordered
breathing in the general population increases to 9% in women and 24% in men. When the most conservative criteria for the diagnosis of sleep-disordered breathing are used, specifically an apnea-hypopnea score of 15 or higher and self-reported hypersomnolence, sleep disordered breathing is estimated to occur in 2% of women and 4% of men (Phillipson, 1993).

The risk factors for OSA include gender, age, genetics, obesity, and alcohol intake (Deegan and McNicholas, 1995). The greater prevalence of OSA in males may be due to the higher pharyngeal and supraglottic resistance in normal males compared to females (White et al., 1985). Similarly, pharyngeal resistance increases with age in normal men (White et al., 1985) and may result in greater prevalence of OSA in the older age group (Ancoli-Israel, 1985). However, the association between age and OSA may be influenced by confounding factors such as obesity and has not been well established (Young et al., 1993). Conversely, the association between obesity and OSA has been well documented (Grunstein et al., 1993; Strohl et al., 1994), but the mechanisms responsible for this link remain unclear (Deegan and McNicholas, 1995). Also, some reports describe several members of a family who were afflicted with OSA, suggesting a genetic disposition (Strohl et al., 1978; Manon-Espaillat et al., 1988). Finally, ethanol ingestion, as little as 3 oz. before sleep, increases the severity of sleep apnea, possibly through its depressant effects on upper airway muscle tone, arousability and chemoreceptor activity (Scrima et al., 1982; Issa and Sullivan, 1982; Guilleminault et al., 1981).

Several risk factors for OSA often co-exist in patients and may collectively affect the severity of OSA. Future research is needed to determine the independent and combined contribution of these risk factors in the pathogenesis and progression of OSA. The specific weight (above ideal) at which OSA is induced, the identification of a genetic marker (if any)
for OSA, and the effect of the different combinations of risk factors are just a few of the issues that remain to be resolved.

1.1.3. Pathogenesis of OSA

Obstructive apneas are caused by collapse of the pharyngeal airway, with half the obstructions occurring at the palatal level and half at the hypopharyngeal level (Chaban et al., 1988; Douglas and Polo, 1994). The size of the airway, mechanical factors, action of upper airway muscles and reflexes, and central factors may contribute to the obstruction (Deegan and McNicholas, 1995).

Although some authors have reported an overall narrower upper airway in patients with OSA compared to normal, others found that only certain sections of the upper airway, mainly in the region posterior to the soft palate and at the base of the tongue, were considerably narrower than normal (Suratt et al., 1983; Haponik et al., 1983). The upper airway of patients with OSA is also more compliant during wakefulness and sleep, and therefore more collapsible than normal (Brown et al., 1985; Gleadhill et al., 1991). In addition, the shape of the airway in these patients is circular compared to elliptical in normal subjects (Rodenstein et al., 1990). The shape and size of the airway in patients with OSA may be a consequence of fat deposits around the airway (Horner et al., 1989). In a small proportion of patients with OSA, anatomical abnormalities such as retroposition of the mandible or cranio-facial anomalies are responsible for the narrowing of the upper airway (Schafer, 1982).

The activity of the pharyngeal dilator muscles are synchronized to breathing (Strohl, 1981; Onal et al., 1981) and control the upper airway caliber by acting to support or distend the collapsible upper airway during inspiration. The activity of the upper airway muscles in patients with OSA is greater than in normal subjects since these patients are breathing through a
narrower more collapsible lumen (Suratt et al., 1988). However, sleep results in a decrease in muscle tone throughout the body including that of the upper airway muscles, and may therefore result in narrowing of the airway (Suratt et al., 1988). Abnormalities in upper airway responses and asynchrony between the upper airway and other respiratory muscle activity may predispose to upper airway collapse (Brouillette and Thach, 1980; Onal et al., 1982; Issa and Sullivan, 1983).

In addition to upper airway muscles, upper airway reflexes which are sensitive to changes in airway pressure may influence patency (Horner et al., 1991; Mathew et al., 1982). Abnormal upper airway reflexes may lead to the development of obstructions due to imbalance in transmural airway pressure and contraction of the upper airway dilator muscles (Deegan and Nicholas, 1995). Finally, defects in respiratory control may also contribute to the pathogenesis of OSA (Onal and Lopata, 1982; McNicholas et al., 1984). These abnormalities may be manifested as impaired detection of a resistive load or periodic breathing representing instability of respiratory control (Onal and Lopata, 1982; McNicholas et al., 1984).

The onset of upper airway occlusion interrupts ventilation, resulting in progressive hypoxia and hypercapnea, despite persistent respiratory efforts by the patients. Apneas are usually terminated by arousal from sleep, which is accompanied by activation of upper airway dilator muscles and restoration of upper airway patency (Bradley and Phillipson, 1985). The primary stimulus producing arousal from sleep during an apneic event is the degree of effort to breathe produced by the patient (Kimoff et al., 1994a). Chemical stimuli from progressive hypoxia and hypercapnea may indirectly influence arousal through effects on ventilatory effort (Kimoff et al., 1994a). The arousal response do not usually lead to full awakening from sleep and often the patient may be unaware of the obstructive events; nevertheless, the fragmentation
of sleep from the recurrent arousals play an important role in the clinical complications of OSA (Bradley and Phillipson, 1985).

1.1.4. Pathophysiology of OSA

The clinical features arise from the physiological events that characterize OSA. The frequency of symptoms that accompany sleep apnea is shown in Table 1.

Table 1: Frequency of symptoms in Sleep Apnea*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>Loud snoring</td>
<td>94%</td>
</tr>
<tr>
<td>Excessive daytime sleepiness</td>
<td>78%</td>
</tr>
<tr>
<td>Restless sleep (increased motor activity)</td>
<td>100%</td>
</tr>
<tr>
<td>Decreased intellectual capacity</td>
<td>58%</td>
</tr>
<tr>
<td>Personality change</td>
<td>48%</td>
</tr>
<tr>
<td>Sexual impotence</td>
<td>42%</td>
</tr>
<tr>
<td>Morning headaches</td>
<td>36%</td>
</tr>
<tr>
<td>Frequent nocturnal enuresis</td>
<td>30%</td>
</tr>
<tr>
<td>Choking Sensations with abrupt awakening</td>
<td>not known</td>
</tr>
<tr>
<td>Disturbed sleep (frequent awakening)</td>
<td>not known</td>
</tr>
</tbody>
</table>

* modified from Martin et al., 1985

The neuropsychological complications of sleep apnea, in particular hypersomnolence, may be a consequence of sleep disruption, and possibly the ‘cerebral metabolic disturbances’ that accompany hypoxemia (Weil et al., 1987). However, excessive daytime sleepiness is not directly related to the severity of sleep disruption, which suggests that differences in the degree of arousal may be important in causing this symptom (Cheshire et al., 1992). Regardless of the primary cause, the neuropsychological complications of sleep apnea can have detrimental effects on the quality of life of patients with OSA. In particular, excessive daytime sleepiness can be a disabling and dangerous, and may be responsible for the higher prevalence of automobile crashes in patients with severe OSA (Weil et al., 1987; Findley et al., 1989).
In addition to the neuropsychological and behavioral consequences, OSA is associated with increased risk of cardiovascular morbidity and mortality (Shepard, 1992). These complications will be discussed in detail in later sections of this chapter (see 1.4, 1.5 and 1.8).

1.1.5. Treatment of OSA

Until the advent of nasal continuous positive airway pressure (CPAP), the only treatment for obstructive sleep apnea was tracheostomy (Polo et al., 1994). In 1981, Sullivan and colleagues (1981) described how nasal CPAP, through its action as a pneumatic splint, abolishes airway obstructions during sleep. Today, nasal CPAP is considered the ‘first line therapy’ for the management of OSA (Polo et al., 1994).

Nasal CPAP, when set at an adequate pressure (5-20 cm H2O), maintains the patency of the upper airway, thus abolishing the abnormalities in blood gases and the changes in hemodynamics that accompany obstructive apneas (Polo et al., 1994). These nocturnal effects of CPAP lead to improvement in the daytime neuropsychological function of patients with OSA, and long-term use of CPAP results in lower mortality rates (Engleman et al., 1994; He et al., 1988).

In the presence of specific upper airway anomalies (e.g., enlarged tonsils and adenoids, bony abnormalities), surgery is indicated to correct the defects (Kryger, 1992). Other treatment options that may be used in patients with OSA (without upper airway anomaly) are uvulopalatopharyngoplasty, drug therapy, dental appliances and weight loss (conservative or surgical). The effectiveness of these treatment techniques has not been established in large groups of patients (Kryger, 1992). Nevertheless, these therapies may provide an alternative if CPAP is not effective or not tolerated by the patient.
1.2. Hypertension: Overview

1.2.1. Background and Definitions

Cardiovascular disease is the primary cause of death in industrialized countries (Thom et al., 1992). Hypertension is a recognized risk factor for cardiovascular disease and its management is critical when attempting to minimize the burden of cardiovascular disease (Whelton, 1994).

There is no universally accepted definition of hypertension. The World Health Organization (WHO) defines hypertension as follows:

<table>
<thead>
<tr>
<th>Type</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotension</td>
<td>Systolic BP ≤140 mm Hg and Diastolic BP ≤90 mm Hg</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Systolic BP ≥160 mm Hg and/or Diastolic BP ≥95 mm Hg</td>
</tr>
<tr>
<td>Borderline Hypertension</td>
<td>Systolic BP = 140-159 mm Hg and/or Diastolic BP = 90-94 mm Hg</td>
</tr>
</tbody>
</table>

The Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure has further subdivided the normotension and hypertension categories. Based on an average of at least two readings on at least two occasions, optimal BP is defined as a systolic BP of less than 120 mm Hg and diastolic of less than 80 mm Hg. ‘High Normal’ corresponds to systolic BP of 130-139 mm Hg and diastolic BP of 85-89 mm Hg. Hypertension is subdivided into 4 subgroups as shown in Table 3.
Table 3: Definition of Hypertension based on the Joint National Committee on Detection, Evaluation and Treatment of High BP (1993)

<table>
<thead>
<tr>
<th>Type</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>Systolic BP=140-159 mm Hg &amp; Diastolic BP=90-99 mm Hg</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Systolic BP=160-179 mm Hg &amp; Diastolic BP=100-109 mm Hg</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Systolic BP=180-209 mm Hg &amp; Diastolic BP=110-119 mm Hg</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Systolic BP≥210 mm Hg &amp; Diastolic BP≥120 mm Hg</td>
</tr>
</tbody>
</table>

Although these guidelines are arbitrary and are not based on biological rationale, they provide a common basis for discussion of hypertension among health care professionals.

There are two main categories of hypertension, essential and secondary. In secondary hypertension, the cause of the hypertension is self-evident, such as renal vascular disease, tumor of the adrenal gland or coarctation of the aorta (Swales, 1995). Essential hypertension is best regarded as a 'category of convenience', and refers to elevation in BP which cannot be attributed to a single discrete cause (Swales, 1995).

1.2.2. Epidemiology of Hypertension

Using the definition provided by the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (1993), the prevalence of hypertension in the general U.S. population ranges from 4% in individuals between 18 and 29 years of age and 65% in those older than 80 years of age (Working Group on Primary Prevention of Hypertension, 1993).

Effects of age on BP have been documented in different populations of industrialized nations (Whelton, 1994). Systolic and diastolic blood pressures increase throughout childhood,
adolescence and adulthood but the rate of increase is much less for diastolic BP, resulting in a wider pulse pressure with advancing age (Whelton, 1994). In contrast, some authors report no effect of age on BP in remote societies, but note a rise in BP with adaptation to western lifestyle (Carvalho et al., 1989). This finding suggests a role for environmental factors and diet in the pattern of change in BP with age that is observed in industrialized nations (Carvalho et al., 1989).

Gender, ethnicity and socio-economic status are other factors that influence BP. The difference in BP between the sexes is apparent in young and middle aged adults but is considerably reduced in the elderly (Whelton, 1994). Certain ethnic subgroups such as African-Americans have higher BP than age and gender-matched whites (Roberts et al., 1977). The type and level of employment may also influence BP (Marmot, 1985). Other risk factors that may predispose to essential hypertension are obesity (especially central obesity), excessive sodium intake, alcohol consumption, smoking, insufficient physical activity, stress and general environmental factors (Working Group on Primary Prevention of Hypertension, 1993; Joint National Committee, 1993).

1.2.3. Etiology and Pathophysiology of Hypertension

Arterial BP is dependent on total peripheral resistance and cardiac output. Cardiac output is affected by changes in cardiac rate or stroke volume. An increase in any of these variables, if not compensated by a decrease in one of the other variables, results in increased BP. BP is primarily regulated by the autonomic nervous system and the kidneys, which control blood volume. The autonomic nervous system controls BP primarily by changing total peripheral resistance and cardiac output.
The etiology is evident in most forms of secondary hypertension. For instance, various pathologies result in renal ischaemia and hypertension, such as atheromatous renal artery stenosis and fibromuscular dysplasia (Swales, 1995). Overactivity of the adrenal cortex causes mineralocorticoid or glucocorticoid hypertension, and tumors of the renal medulla results in excess catecholamine secretion (phaeochromocytomata) causing hypertension (Swales, 1995). Other causes of secondary hypertension include thyroid disease and acromegaly, coarctation of the aorta and lesions in the central nervous system (Swales, 1995).

Essential hypertension has three main etiologic factors: genetic predisposition, environmental factors, and structural adaptation of the cardiovascular system (Folkow, 1993). The environmental factors are often related to lifestyle such as diet. Genetic predisposition and environmental factors may be the early triggers of hypertension through increased ‘central neurohumoral activity’ that manifests as increased cardiac output, with little change in total peripheral resistance (Folkow, 1993). The structural adaptations of the cardiovascular system may play an important role in the later stages of hypertension and consist of wall thickening and stiffening in the cardiovascular system, upward resetting of pressor sensors, and increased total peripheral resistance, while cardiac output is normalized (Folkow, 1983).

Metabolic disorders, such as abnormalities in metabolism of glucose, insulin and lipoprotein, are prevalent in patients with hypertension (Reaven et al., 1996) and may play a role in the pathogenesis of hypertension. It is estimated that at least 50% of patients with essential hypertension present with insulin resistance and hyper-insulinemia and this relationship is independent of obesity (Pollare et al., 1990; Zavaroni et al., 1992). Insulin resistance is defined as the resistance of peripheral tissues to glucose uptake when stimulated
by insulin (Pollare et al., 1990; Reaven, 1991). The prevalence of these metabolic disorders in patients with essential hypertension, and evidence from rodents showing increases in BP after insulin infusion support the possibility that these metabolic abnormalities are linked to the pathogenesis of essential hypertension (Brands et al., 1991; Reaven et al., 1996). The link between metabolic disorders and hypertension is thought to be through the involvement of sympathetic nervous system, although further evidence is needed to establish a cause and effect relationship (Reaven et al., 1996).

There is also evidence of an association between serum lipids and blood pressure, after confounding variables such as age and obesity are controlled (Goode et al., 1995). Successful cholesterol reduction results in a significant reduction in blood pressure (Goode et al., 1995). The positive relationship between serum lipids and blood pressure may be through the direct effects of serum lipids on endothelial function (Cohen et al., 1988; Harrison, 1989). Low density lipoprotein (LDL) appears to be primarily responsible for these effects on endothelial function (Goode et al., 1995).

Several factors may play a role in the structural changes in hypertension. These include genetic predisposition, renal dysfunction, changes in blood flow and pulse pressure, growth promoting factors and the sympathetic nervous system (Lee et al., 1995). These factors result in vessel wall hypertrophy, mainly an increase in media to lumen size, smooth muscle hyperplasia and vascular remodeling (Lee et al., 1995). These alterations are considered primary since they occur before considerable elevations in BP are observed (Lee and Smeda, 1985; Lee, 1985). Endothelial cell necrosis, hypertrophy of smooth muscle cells and increases in adventitial collagen are secondary adaptive changes to hypertension (Lee et al., 1995).
Endothelial dysfunction may be associated with essential hypertension. It has been hypothesized that the lack of endogenous vasodilator nitric oxide (likely identical with endothelium-derived relaxant factor) may result in an increase in total peripheral resistance (van Zwieten et al., 1995). Evidence for this hypothesis comes from studies that have shown lower levels of nitric oxide production in forearm vascular beds of patients with hypertension (Linder et al., 1990). However, other authors have challenged this hypothesis on the role of endothelial dysfunction in essential hypertension based on the fact that the majority of the evidence from animal studies is based on data from large conduit arteries, particularly the aorta (Angus and Lew, 1992). In humans, recent evidence by Cockcroft and co-workers (1994) further discounts a role for endothelial dysfunction in hypertension; these authors found that endothelium-dependent vasodilatation in the forearm vasculature is the same in normotensive and hypertensive patients. Thus, although endothelial dysfunction in large conduit arteries has been established in hypertensive animals and humans, the presence of such abnormalities in smaller resistance vessels remains controversial (van Zwieten et al., 1995).

1.2.4. Cardiovascular Consequences of Hypertension

Coronary heart disease, stroke and congestive heart failure are recognized consequences of hypertension (Collins et al., 1990a; Stamler et al., 1993). In a 10 year follow up of individuals with no history of coronary heart disease, the risk of stroke was ten times higher for individuals with a mean overall diastolic BP of 105 compared to those with mean diastolic BP of 76 mm Hg (MacMahon et al., 1990). A 5 mm Hg reduction in diastolic BP is associated with a four-fifths decrease in the risk of coronary heart disease (Collins et al., 1990b). Effective management of hypertension appears to reduce the prevalence of stroke to a greater degree than
the prevalence of coronary heart disease (Collins et al., 1990a). Similarly, in a 34 year follow-up, the risk of congestive heart failure was at least 2 to 4 times greater for those who were hypertensive at entry to the study compared to normotensives (Kannel and Belanger, 1991). This increased risk of cardiovascular disease with hypertension is apparent in both men and women and in different ethnic subgroups; both systolic and diastolic BP are correlated with increased risk of cardiovascular disease although systolic BP is a better predictor of cardiovascular risk (Whelton, 1994).

An association between hypertension and hypertrophy of the left ventricle has also been established (Jern, 1992). Evidence of left ventricular hypertrophy in hypertension is associated with increased risk of myocardial infarction, stroke, arrhythmia, angina and sudden cardiovascular death independent of prevailing BP or other factors (Jern, 1992; Devereux et al., 1994a). Left ventricular hypertrophy using echocardiography allows classification of left ventricular geometry based on mass and relative wall thickness (Devereux et al., 1994b). Patients with increased left ventricular mass can be divided into those with concentric hypertrophy (i.e., increased relative wall thickness) or eccentric hypertrophy (i.e., normal wall thickness); patients with normal left ventricular mass can be separated into those with normal left ventricular geometry or concentric remodeling (Devereux et al., 1994a). These geometric patterns are associated with different pathological presentation and predictions of prognosis (Devereux et al., 1994a). Patients with left ventricular hypertrophy present with high blood pressure and total peripheral resistance, little change in cardiac output and have the greatest risk of cardiovascular morbidity and mortality (Devereux et al., 1994a). Patients with eccentric hypertrophy have elevated BP, cardiac output and plasma volume but normal total peripheral resistance (Devereux et al., 1994a). Patients with concentric left ventricular remodeling have
mild hypertension, elevated total peripheral resistance but low cardiac output; finally, patients with normal geometry have the lowest risk of cardiovascular morbidity and mortality and present with mild hypertension, slightly increased total peripheral resistance but normal cardiac output (Devereux et al., 1994a). In addition to total peripheral resistance and volume overload, other factors such as genetics, humoral/metabolic factors, vascular compliance and blood viscosity may play a role in the cardiac hypertrophic changes associated with hypertension (Jern, 1992).

1.2.5. Baroreceptor Function in Hypertension

The baroreceptor reflex is a negative feedback system by which the central nervous system controls BP (Sved and Gordon, 1994). The reflex consists of different components, mainly the sensors with stretch receptors located in the cardiovascular and other tissues, the afferent input to the central nervous system and an efferent component consisting of the autonomic innervation of the vasculature and heart (Sved and Gordon, 1994). BP changes stimulate the peripheral mechanoreceptors that are sensitive to changes in stretch or transmural pressure (Taylor, 1994). There are three types of baroreceptors: the arterial baroreceptors in the carotid sinus and aortic arch, the cardiopulmonary receptors in the great veins, right atrium and possibly the lungs and pleura, and the cardiac sinus baroreceptors in the ventricles of the heart (Taylor, 1994). Peripheral and central chemoreceptors also provide afferent input into the brainstem and may interact with baroreceptor input in modulating autonomic activity (Somers et al., 1991).

A variety of methods can be used to indirectly stimulate or inhibit the baroreflex and characterize the cardiovascular responses to autonomic reflex activation. Methods used to study the arterial baroreceptors consist of pharmacological or physiologic maneuvers that
cause a sudden change in BP (Taylor, 1994). These include: (1) neck pressure/suction that activate/deactivate the carotid baroreceptors; (2) infusion or bolus injection of vasoactive agents (e.g., phenylephrine/angiotensin that leads to stimulation of the arterial baroreflexes versus nitroprusside/nitroglycerin that leads to their inhibition; (3) the valsalva maneuver, which reduces venous return to the heart and unloads the arterial baroreceptors; (4) passive tilt or standing which results in transient hypotension; and (5) computer analyses that discover ‘baroreflex engagement’ in records of spontaneous fluctuations of R-R intervals and arterial pressures (Eckberg and Sleight, 1992; Taylor, 1994).

Cardiopulmonary baroreceptors located in the lungs, pleura and the heart are primarily sensitive to changes in blood volume (Taylor, 1994). Central venous pressure is often measured as the index of blood volume (Taylor, 1994). Maneuvers used to indirectly stimulate or inhibit the cardiopulmonary baroreflexes include: (1) lower body negative or positive pressure which decreases and increases central venous pressure respectively, and deactivates or activates the baroreceptors; and (2) fluid volume expansion, head down tilt, and passive leg raising, that alter central venous pressure and activate these baroreceptors (Taylor, 1994).

Hemodynamic parameters, primarily blood pressure and heart rate, are monitored to assess the effects of these maneuvers on the autonomic nervous system. Direct measurements of sympathetic activity using microneurography techniques, measures of systemic and regional nor-epinephrine spillover rates, or measures of plasma catecholamine concentrations also provide insight into sympathetic activity (Taylor, 1994).

The relationship between pressure and R-R interval is sigmoid and includes a threshold (i.e., the minimum pressure at which a response is observed), a linear portion, and a
saturation portion (i.e., the pressure at which the maximum response is observed) (Eckberg and Sleight, 1992). The concept of resetting of the baroreflex may describe changes in a number of components. Peripheral resetting represents the adaptation of the peripheral baroreceptor whereas central resetting represents adaptation in the central nervous system (Chapleau et al., 1989).

Central resetting has been defined as a change in the relationship between baroreceptor activity and efferent sympathetic and parasympathetic activity and occurs with acute and chronic hypertension (Chapleau and colleagues, 1989). Central adaptation has been demonstrated using electrical stimulation of baroreceptor afferents; after the initial rapid inhibition of sympathetic and excitation of parasympathetic activity, the response decreases over time, despite constant baroreceptor input to the central nervous system (Chapleau et al., 1989).

Peripheral resetting consists of ‘decreased pressure-sensitivity’ of the baroreceptors, i.e., the baroreceptor discharge is less at any given level of pressure (Chapleau et al., 1989). Acute resetting that occurs within minutes and stabilizes within 5-15 minutes consists of decreased baroreceptor activity for the same pressure, without a change in gain (Chapleau et al., 1989). Thus, the sigmoid relationship between R-R interval and BP shifts to the right (on the pressure axis curve) with no change in its slope. With chronic resetting, baroreceptor activity is further reduced for a given pressure and there is a decrease in the gain of the relationship between baroreceptor activity and BP (Chapleau et al., 1989). In other words, the baroreceptor is less sensitive to a change in pressure (Sleight, 1991). Thus, in moderate and late hypertension, the slope of the sigmoid relationship between R-R interval and BP decreases [i.e., sensitivity or gain is reduced (Eckberg and Sleight, 1992)]. Mechanisms that
have been implicated in the peripheral resetting are: (1) changes in the mechanical properties of the vessel wall (e.g., reduced compliance); (2) altered ionic mechanisms (e.g., changes in permeability of the membrane to specific ions); or (3) secondary effects of substances released from the endothelium during vascular stretch (e.g., prostacyclin and endothelium derived relaxing factors) (Sleight, 1991; Chapleau et al., 1989).

There is a strong interaction between baroreceptor and chemoreceptor activities that influence sympathetic output. In animal studies, elevation of blood pressure and activation of baroreceptors in the dog inhibit the vasoconstriction that accompanies stimulation of the peripheral chemoreceptor (Mancia, 1975). In humans, baroreflex activation with phenylephrine selectively abolishes the sympathetic nerve activity response to hypoxia but not to hypercapnea (Somers et al., 1991). Furthermore, baroreflex activation inhibits the ventilatory response to stimulation of the peripheral chemoreceptors whereas deactivation of the baroreflex has the opposite effects (Heistad et al., 1975). These interactions may be a function of interneuronal connections in the medulla (Somers et al., 1991).

1.2.6. Central Control of BP

There are three centers in the medulla that are responsible for the control of blood pressure: the nucleus tractus solitarius in the dorsomedial medulla, the caudal ventrolateral medulla and the rostral ventral medulla (Chalmers et al., 1992). There are two main subgroups of bulbospinal sympathoexcitatory neurons in the rostral ventral medulla: the rostral ventrolateral and the rostral ventromedial medulla (Cox and Brody, 1989 a & b). The nucleus tractus solitarius and the caudal ventrolateral medulla are depressor centers in the baroreceptor reflex pathway, whereas the rostral ventrolateral medulla is the major pressor center in the baroreceptor reflex arc (Chalmers et al., 1992). An acute increase in BP
stimulates the baroreceptors in the aortic arch and carotid sinus. Baroreceptor afferent neurons synapse into the nucleus tractus solitarius center, which sends the signal to inhibitory neurons in the caudal ventrolateral medulla. These neurons project to rostral ventrolateral medulla and synapse with bulbospinal sympathoexcitatory neurons (Chalmers and Pilowsky, 1991). In addition, these centers may be activated by changes that do not involve baroreceptor function (Chalmers et al., 1992). Neurophysiological studies have demonstrated that carotid baroreceptor and chemoreceptor neurons are distributed in close proximity in the nucleus tractus solitarius and in the rostral medulla, and interneurons may facilitate interaction between the cardiovascular and respiratory systems (Somers et al., 1991).

1.3. Hemodynamics During Sleep in Normal Subjects

It is important to understand the cardiovascular effects of normal sleep in order to fully appreciate the abnormal patterns observed in OSA. Sleep represents a period of reduced physiological workload on the cardiovascular system (Parish and Shepard, 1990). This reduction in workload is manifested by changes in blood pressure, heart rate and cardiac output.

1.3.1. Blood Pressure

Normal sleep is associated with a decrease in BP as first reported by Brush and Fayerweather in 1901. These earlier observations have been confirmed both by direct (Khatri and Fries, 1967; Coccagna et al., 1971) and indirect measurements of BP (Snyder et al., 1964; Richardson et al., 1964). Although there is agreement that BP decreases during sleep, the degree of fall in arterial pressure is controversial as shown in Table 4. The discrepancy among studies may be a function of the method of analysis, such as whether the BP values were pooled hourly or in 20 minute intervals (Floras et al., 1978).
Table 4. Variations in the degree of BP reduction during sleep

<table>
<thead>
<tr>
<th>Study</th>
<th>Greatest decrease in BP with sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richardson et al., 1964</td>
<td>19% in systolic and diastolic BP in normotensives</td>
</tr>
<tr>
<td></td>
<td>12% in systolic and diastolic BP in hypertensives</td>
</tr>
<tr>
<td>Khatri and Fries, 1967</td>
<td>6.5-8.5% in mean arterial BP</td>
</tr>
<tr>
<td>Bevan et al., 1969</td>
<td>5% in systolic and 2% in diastolic BP in hypertensives</td>
</tr>
<tr>
<td></td>
<td>10% in systolic and 17% in diastolic BP in normotensives</td>
</tr>
<tr>
<td>Bristow et al., 1969</td>
<td>7-12% drop in mean arterial BP</td>
</tr>
<tr>
<td>Coccagna et al., 1971</td>
<td>23% in systolic and diastolic BP</td>
</tr>
</tbody>
</table>

With development of technology for portable, fully automated, ambulatory BP monitoring, many investigators have examined the BP profile over 24-hours and their findings support the earlier data that BP is normally higher during the daytime than the night-time (Littler et al., 1975; Millar-Craig et al., 1978; Richard et al., 1986; Degaute et al., 1991). These day-night variations may be caused by an endogenous circadian rhythm and/or the direct effects of sleep onset (Clark et al., 1987; Van den Meiracker et al., 1988; Chau et al., 1989). The latter hypothesis is supported by evidence showing that sleep is a stronger predictor of the blood pressure change than time of day and estimations that 65-75% of the nocturnal decline in BP is the result of recumbence and sleep (Clark et al., 1987; Degaute et al., 1991). Conversely, a study in shift workers has revealed that factors other than the subject’s activity level contribute to 24 hour profile, suggesting the effects of an endogenous circadian rhythm (Chau et al., 1989).

In addition to stimuli such as behavior that influence 24-hour BP variability, several mechanisms may be involved in the modulation of 24-hour BP and HR, specifically, humoral factors (e.g. plasma catecholamines, renin-angiotensin system and vasopressin), the sympathetic nervous system, respiration and the arterial baroreflex (Parati et al., 1995). Evidence of the latter mechanism has been obtained from studies showing an inverse relationship between
baroreflex sensitivity and 24-hour blood pressure variability (Mancia and Zanchetti, 1986; Floras et al., 1988).

When the specific sleep stages are considered, BP declines 5-10% in stages 1 and 2 of n-REM sleep and 10-15% in stages 3 and 4 (Khatri and Fries, 1967; Coccagna et al., 1971). REM sleep is associated with greater variability in BP and values may return to levels observed during wakefulness (Somers et al., 1993; Khatri and Fries, 1967; Bristow et al. 1969; Coccagna et al., 1971). The BP rises during REM are sudden, irregular and may be associated with clusters of eye movements and irregular breathing.

Sympathetic nerve activity has been shown to parallel the changes in BP mentioned above with a decrease in muscle sympathetic activity during n-REM sleep (Hornyak et al., 1991; Somers et al., 1993). This suppression in sympathetic activity and the finding of both a lower blood pressure and heart rate during n-REM sleep suggest a substantial change in baroreceptor function (Somers et al., 1993). This change may be the result of resetting of the baroreceptors to lower blood pressure values although the effect of sleep on baroreflex sensitivity remains controversial (Bristow et al., 1969; Smyth et al., 1969; Conway et al., 1983). During REM sleep, sympathetic nerve activity increases above levels recorded during wakefulness (Hornyak et al., 1991; Somers et al., 1993), and the values of BP and HR returns to wakefulness levels (Somers et al., 1993). REM-sleep twitches are associated with surges in BP and abrupt cessation of sympathetic activity, possibly due to restoration of muscle tone and baroreceptor-reflex-mediated inhibition of sympathetic activity in response to increase in BP (Somers et al., 1993). The mechanisms responsible for the general increase in sympathetic activity during REM sleep have not been defined.
1.3.2. Heart Rate

Heart rate declines by 10-25% during sleep compared to wakefulness (Snyder, 1964; Khatri and Freis, 1967) and this reduction is thought to be vagally mediated (Kirby and Verrier, 1989). Slow wave sleep is characterized by a reduction in HR and an increase in respiratory sinus arrhythmia (Zemaityte et al., 1984). REM is associated with high variability in HR compared to n-REM sleep (Coccagna and Lugaresi, 1995; Kirby and Verrier, 1989), and HR during REM may increase to waking levels (Somers et al., 1993). One confounding variable is the change in respiration that is associated with the change in sleep-wake state and that influences HR. In studies where respiration was maintained constant with mechanical hyperventilation, the higher HR observed during wakefulness compared to n-REM sleep was mainly due to increase in sympathetic activity (Horner et al. 1995). In contrast, spontaneous arousal from sleep is associated with an increase in HR that is higher than the HR observed during quiet wakefulness; this difference was due to a decrease in parasympathetic activity and an increase in sympathetic activity (Horner et al., 1995). It has been suggested that the 24-hour HR pattern, in contrast to BP patterns, may reflect an endogenous circadian rhythm, since falling asleep accounts for only 50% of the nocturnal decline in HR (Degaute et al, 1991).

1.3.3. Cardiac Output and Total Peripheral Resistance

Cardiac output is calculated as the product of heart rate and stroke volume and is determined by changes in cardiac function and venous return. Any changes in these variables may therefore result in a change in cardiac output. During n-REM sleep, cardiac output has been reported by several authors to either remain unchanged or to decrease during n-REM sleep (table 5). The decreases in cardiac output are the result of reductions in HR and, possibly, reductions in stroke volume (Khatri and Fries, 1967; Bristow et al., 1969). The changes in
cardiac output during REM are variable (Khatri and Fries, 1967; Bristow et al., 1969; Miller et al., 1976). The degree of reduction in cardiac output compared to the reduction in systemic pressure provides insight into the changes in total peripheral resistance with sleep. For instance, Bristow and colleagues (1969) reported that total peripheral resistance decreased during sleep, on the basis of decreased pressure with no significant change in cardiac output. The discrepancies between studies on whether total peripheral resistance increases or decreases during sleep may be a function of the definition of sleep. Somers and colleagues (1993) reported that although sympathetic nerve activity was decreased during nREM sleep, REM sleep was associated with sympathetic-nerve activity that was above wakefulness levels. In addition, K complexes during nREM sleep were accompanied with increased sympathetic activity. Table 5 summarizes the findings to date on the effects of sleep on cardiac output and total peripheral resistance in human and animal studies.

1.4. Hemodynamic Changes during Apneic Events in OSA

The hemodynamic changes associated with normal sleep have been described and will be compared to the changes associated with apneas of disordered sleep. Airway occlusion in OSA results in four main consequences that are of considerable hemodynamic importance: (1) progressive hypoxia which is dependent on apnea duration, lung volume and baseline oxygen concentration (Bradley et al., 1985b; Shepard, 1985); (2) progressive hypercapnea which is considerably less severe than the hypoxia, due to the buffering ability of the body for CO₂ (Parish and Shepard, 1990); (3) negative intrathoracic pressure generated by the futile respiratory efforts by the patient against the closed airway; and (4) arousal from sleep, which results in restoration of upper airway patency and resumption of ventilation.
Table 5: Cardiac output and total peripheral resistance during sleep

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Cardiac Output</th>
<th>Total Peripheral Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lund-Johansen, 1967</td>
<td>human, hypertensives</td>
<td>decreased</td>
<td>unchanged</td>
</tr>
<tr>
<td>Khatri and Fries, 1967</td>
<td>human, normotensive</td>
<td>decreased</td>
<td>unchanged</td>
</tr>
<tr>
<td>Bristow et al., 1969</td>
<td>human, normotensive and hypertensive</td>
<td>unchanged</td>
<td>decreased</td>
</tr>
<tr>
<td>Khatri and Fries. 1969</td>
<td>human, hypertensive</td>
<td>decreased</td>
<td>unchanged</td>
</tr>
<tr>
<td>Miller and Horvath. 1976</td>
<td>human, normotensive</td>
<td>decreased</td>
<td>increased</td>
</tr>
<tr>
<td>Coote, 1982</td>
<td>human, normotensive</td>
<td>decreased</td>
<td></td>
</tr>
<tr>
<td>Takagi, 1986</td>
<td>human, normotensive</td>
<td>decreased</td>
<td>decreased</td>
</tr>
<tr>
<td>Mehta et al., 1987</td>
<td>human, hypertensive</td>
<td></td>
<td>increased</td>
</tr>
<tr>
<td>Mori. 1990</td>
<td>hypertensive</td>
<td>decreased</td>
<td>increased</td>
</tr>
<tr>
<td>Imai et al., 1990</td>
<td>normotensive and hypertensive</td>
<td>decreased</td>
<td>unchanged</td>
</tr>
<tr>
<td>Kumazawa et al., 1967</td>
<td>cats</td>
<td>unchanged</td>
<td>decreased</td>
</tr>
<tr>
<td>Smith et al. 1987</td>
<td>rats</td>
<td>decreased</td>
<td>increased</td>
</tr>
<tr>
<td>Engel and Talman. 1987</td>
<td>monkey</td>
<td>decreased</td>
<td>increased</td>
</tr>
</tbody>
</table>

(Table modified from Imai et al., 1990)

1.4.1. Effects of Apnea on BP

Arterial systolic and diastolic BP increase as much as 15-50 mm Hg in response to apneas during all phases of sleep (Gastaut et al., 1966; Tilkian et al., 1976; Girling, 1972; Coccagna et al., 1972a; Shepard, 1985; Motta et al., 1978). At the start of an apnea, there is a transient decrease in BP, possibly as a result of recurrent falls in intrathoracic pressure; later in the apnea, BP increases progressively with the peak rise occurring when ventilation resumes at the point of apnea termination and arousal from sleep (Shepard, 1985). After this peak, BP remains elevated for 10-15 seconds before returning slowly to normal levels (Shepard, 1985).
The main stimuli to the BP surges with apneas are hypoxia, hypercapnea and arousal. Several studies support the role of hypoxia. First, the rise in BP before arousal correlates with the degree of oxygen desaturation, with each percent of desaturation resulting in 0.4–4.4 mm Hg increase in BP (Shepard, 1985). The peak blood pressure, usually occurring after arousal, correlates with apnea duration (Jennum and Borgeson, 1990). Second, administration of supplemental oxygen prevents the post-apneic increase in BP in humans and dogs (Iwase et al., 1992; van den Aardweg et al., 1992). In addition, hypercapnea augments the cardiovascular effects of hypoxia during breath-holding (Lin et al., 1983). In contrast to these findings, other researchers have not found a close relationship between hypoxia and elevations in BP. Ringler and co-workers (1990) examined the effects of hypoxia on the hypertensive response by administering supplemental oxygen to patients during apnea at levels that maintained SaO2 greater than 90%. They found that the increase in BP associated with apnea was not diminished and concluded that other factors (e.g. arousal from sleep, changes in intrathoracic pressure or resumption of breathing) may be involved in the BP surge. In addition, the effect of arousal on BP has been examined using graded acoustic stimuli during sleep (Davies et al., 1993). Acoustic stimuli result in an increase in BP during sleep, in the presence and absence of an EEG arousal (Ringler et al., 1990; Davies et al., 1993). Furthermore, delay of the release of occlusion for 6-12 seconds after arousal in normal subjects results in BP elevations that peaked at arousal not with resumption of ventilation (Ringler et al., 1994). This latter finding suggests that BP surges at the termination of apnea are attributable more to arousal than resumption of breathing. In contrast, in the dog, the acute changes in BP associated with an apnea are only partially a function of arousal, since termination of apneas prior to arousal results in a significant BP
surge (O'Donnell et al., 1996). These acute changes in BP in the dog are due to activation of
the autonomic nervous system rather that mechanical redistribution of blood volume
(O'Donnell et al., 1996). Finally, sleep stage may also alter the BP response to obstructive
apneas during sleep (Garpestad et al., 1995).

During spontaneous breathing, the observed response to hypoxemia is increased
ventilation, vasodilatation in the systemic circulation and vasoconstriction in the pulmonary
circulation (Levinson and Millman, 1991). Hypoxia and hypercapnea activate the arterial
peripheral chemoreceptors in the carotid and aortic bodies (Heistad and Abboud, 1980; Daly
and Scott, 1962; Pelletier, 1972). This stimulation results in signals being sent to the
respiratory and cardiovascular centers in the medulla. Stimulation of the cardiovascular
center causes an increase in sympathetic and parasympathetic outflow leading to
vasoconstriction of systemic circulation, a surge in BP and bradycardia (Heistad and
Abboud, 1980). However, the respiratory center activation causes increased ventilation
which stimulates lung stretch receptors and inhibits parasympathetic and sympathetic outflow
(Heistad and Abboud, 1980; Daly and Scott, 1962; Rowell and Blackman, 1987; Vatner and
Rutherford, 1981; Angell James and Daly, 1969). In patients with OSA, obstruction of the
upper airway prevents an increase in ventilation and stimulation of the lung stretch receptors,
resulting in lasting vasoconstriction and bradycardia until the resumption of breathing. The
further increase in BP at the end of the apnea may be the result of a rebound increase in HR
and the concomitant effects of arousal from sleep and hypoxia (Guilleminault et al., 1986).

There is direct and indirect evidence suggesting that the final mechanism causing the
increase in BP associated with apneic events is through sympathetic activity. Obstructive
apneas are associated with a progressive increase in sympathetic neural activity recorded
directly from the peroneal nerve in patients with OSA, followed by a sudden reduction at apnea termination (Hedner et al., 1988; Somers et al., 1995). This reduction at the termination of the apnea occurs before the peak in BP (Carlson et al., 1993b). In addition, patients with Shy-Drager syndrome (i.e. Parkinson’s disease with autonomic insufficiency) and OSA do not demonstrate the surges in BP associated with apnea (Schroeder et al., 1978; Briskin et al. 1978). In normal subjects, voluntary apneas amplify the increase in muscle sympathetic neural activity induced with hypoxia (Somers et al., 1988a). This finding suggests that the increase in sympathetic activity is the result of hypoxia and the lack of inhibitory effects from the pulmonary afferents.

A role for the sympathetic nervous system in the surges in BP in OSA has been obtained from studies showing high levels of urinary norepinephrine during sleep in such patients (Fletcher et al., 1987) and transient surges in plasma norepinephrine during severe apneas (Vitiello et al., 1982). Plasma norepinephrine levels are dependent on the degree of oxygen desaturation and the severity of OSA (Eisenberg et al., 1990). In contrast, Jennum and colleagues (1989) reported no difference in serial overnight measures of plasma norepinephrine in patients with OSA before and after treatment with nasal CPAP. However, they noted a significant reduction in whole-night plasma epinephrine which was correlated with the drop in BP observed with application of CPAP. More recently, Baylor and coworkers (1995) found that, in patients with OSA, the levels and variability of plasma norepinephrine increase with more severe desaturation, without a change in the variability of plasma epinephrine. These findings suggest that the episodic hypoxia that accompanies apneas may be responsible for the changes in norepinephrine. Norepinephrine is the main neurotransmitter for the postganglionic sympathetic nervous system (Baylor et al., 1995) and
the rate at which norepinephrine spills into plasma is proportional to the rate of sympathetic nerve firing (Esler et al., 1990). However, several additional factors influence the plasma level of norepinephrine, such as transmitter re-uptake, re-utilization, and metabolism, regional blood flow and capillary permeability (Esler et al., 1990). These factors may result in incongruity between sympathetic nerve firing and plasma levels of norepinephrine (Esler et al., 1990) and may explain the differences in findings in patients with OSA.

Additional evidence regarding the role of the sympathetic nervous system during apneas comes from studies that quantify blood flow during apneas in preterm infants (Suichies et al., 1989; Storrs, 1977). Apneas are associated with decreases in skin flow to the forehead which is not correlated with the concurrent drop in HR, suggesting an increase in sympathetic activity to skin (Suichies et al., 1989). Similarly, Stors (1977) reported a decrease in limb blood flow by 43% during apneas in pre-term infants as a result of reduction in cardiac output and increased peripheral vasoconstriction (Storrs, 1977).

1.4.2. Effects of Apnea on Heart Rate (HR)

Apnea results in a decrease in HR, which is proportional to the degree of hypoxemia and the duration of the apnea (Zwillich et al., 1982). This bradycardia is the result of increased vagal parasympathetic activity and is reduced or abolished with atropine (Zwillich et al., 1982). Resumption of breathing is associated with tachycardia from the combined effects of decreased vagal parasympathetic activity and increased sympathetic activity from hypoxemia and arousal (Shepard, 1992; Guilleminault et al., 1984; Tilkian et al., 1976; 1978).

Hypoxia and cessation of breathing may be two stimuli that contribute to the bradycardia during apneas (Zwillich et al., 1982). Hypoxic hypercapnea in the presence of
spontaneous ventilation does not result in bradycardia and administration of supplemental oxygen reduces the heart rate changes associated with an apnea (Zwillich et al., 1982; Guilleminault et al., 1984). In lambs, arousal from sleep without release of the obstruction does not alter HR whereas resumption of breathing rapidly alters HR, suggesting a significant role of lung inflation (Baker and Fewell, 1988). From studies in normal subjects during wakefulness, hypercapnea may slightly offset the bradycardia induced by apnea and hypoxia (Lin et al., 1983). The effect of apnea on HR may be mediated through peripheral baroreceptors or central mechanisms (Findley et al., 1985; Bonsignore et al., 1994). In addition, activation of receptors at the site of the upper airway occlusion may contribute to the change in heart rate (Andreas et al., 1992). Regardless of the stimulus, the autonomic nervous system is responsible for mediating the changes in HR since patients with autonomic dysfunction do not demonstrate changes in HR during OSA (Guilleminault et al., 1984; Coccagna et al., 1985).

Apneas are often associated with sinus brady-tachyarrhythmia (Guilleminault, 1984) and sometimes accompanied by heart rates of less than 30 beats/minute, or by second-degree atrioventricular block (Tilkian et al., 1977; Miller, 1982; Shepard, 1985; Guilleminault et al., 1983). In addition, patients with OSA may have an increased incidence of ventricular ectopic beats during sleep (Tilkian et al., 1978; Shepard et al., 1985). Ventricular arrhythmias appear to be influenced considerably by hypoxemia (Miller, 1982; Guilleminault et al., 1983), since a three-fold increase in frequency of these events has been reported at desaturations below 60% (Shepard et al., 1985).
1.4.3. Effects of Apnea on Cardiac Function

During breath hold experiments, cardiac output either increases slightly, remains unchanged or decreases (Lin et al., 1983; Hong et al., 1971; Kawakami et al., 1967; Paulev et al., 1968). HR decreases but stroke volume progressively increases during breath-hold (Lin et al., 1983) and the balance between these changes determine the change in cardiac output. However, caution must be used when extrapolating the findings from breath-holding experiments to the apneic state because of substantial differences between these two conditions, primarily voluntary control and state.

During OSA, some authors have reported a decrease in cardiac output with apnea as measured with thermodilution techniques and impedance cardiography (Guilleminault et al., 1986; Tolle et al., 1983). These reductions were the result of a decrease in HR (Guilleminault et al., 1986; Tolle et al., 1983) and stroke volume (Tolle et al., 1983). Other authors have reported no systematic changes in cardiac output during apneas using the same technique (Schroeder et al., 1978; Tilkian et al., 1976). However, thermodilution techniques and impedance cardiography may not be sensitive enough to determine rapid changes in cardiac output during and after apneas (Shepard, 1986; Garpestad et al., 1992a). Nevertheless, the application of beat by beat methods by other authors have also revealed no changes in cardiac output (Stoohs and Guilleminault, 1992; Garpestad et al., 1992a). However, late in the apnea (i.e. the last two respiratory efforts), cardiac output decreases (Garpestad et al., 1992a). With arousal and resumption of breathing, despite the increase in HR, cardiac output decreases because of the substantial decrease in stroke volume (Garpestad et al., 1992a). This drop in cardiac output coincides with the surge in BP, suggesting a marked increase in peripheral vascular resistance (Garpestad et al., 1992a). Supplemental oxygen that maintains oxygen
saturation above 90% does not change the response of cardiac output to obstructive apnea suggesting that hypoxia is not the primary stimulus contributing to the changes (Garpestad et al., 1994).

The decrease in intrathoracic pressure induced by the Mueller maneuver (i.e., voluntary inspiration against a closed glottis) reduces right atrial pressure, increases venous return to the right heart and affects left ventricular performance and outflow (Scharf et al., 1979; Buda et al., 1979). The increase in right ventricular volume may result in a shift of the intraventricular septum to the left, and lead to decreased compliance and left ventricular stroke volume (Taylor et al., 1967; Shiomi et al., 1991, Tolle et al., 1983; Scharf et al., 1979). However, the rise in venous return to the right heart is limited by collapse of the great veins at the entrance of the thoracic cavity (Natori et al., 1979; Condos et al., 1987; Guyton and Adkins, 1954). Right ventricular afterload may also increase from hypoxic pulmonary vasoconstriction. In addition, the lower intrapleural pressure during the respiratory efforts increases the transmural pressure gradient of the right and left ventricles and increases their afterload (Buda et al., 1979; Marrone et al., 1989). Thus, the increase in afterload and the decrease in compliance of the left ventricle may lead to an increase in left ventricular end-diastolic pressure and left atrial pressure (Parrish and Shepard, 1990), as supported from evidence of increased pulmonary capillary wedge pressure during apneas (Buda et al., 1981). These hemodynamic changes result in an increase in intra-thoracic blood volume, which increases the heart size as seen by direct fluoroscopic evidence (Lugaresi et al., 1984).

The negative intrapleural pressure during obstructive apneas also increases the preload of the right side of the heart, resulting in distention of the right atrium and the release of atrial natriuretic peptides (ANP). Mean plasma levels of ANP are correlated with the
degree of hypoxemia and the negative pleural pressure generated and decrease with CPAP treatment (Krieger et al., 1991). ANP causes natriuresis (Shepard, 1992) and could be responsible for the nocturnal diuresis (i.e. increases in urine and sodium excretions) that is seen in patients with OSA (Krieger et al., 1988; Warley and Stradling, 1988).

Finally, obstructive apneas may affect coronary blood flow and vascular resistance. In a porcine model of OSA, coronary blood flow and coronary vascular resistance increases at the termination of apnea, (Pinto et al., 1993). In humans, Shepard (1986) analyzed myocardial oxygen supply and demand and concluded that at the release of the apnea, coronary blood flow reserve is decreased which may compromise myocardial tissue oxygen delivery. Oxygen desaturation has been implicated in these changes (Shepard, 1986).

1.4.4. Effects of Apneas on Pulmonary Artery Pressure

Apneas are associated with cyclical changes in pulmonary artery pressure that parallel the changes in blood gases (Coccagna et al., 1972a; Schroeder et al., 1978). The pulmonary artery pressure associated with apneas exceeds the levels observed during quiet wakefulness and the pulse pressure is widened (Coccagna et al., 1972a).

During an apnea, since intrathoracic pressure becomes more negative during an inspiratory effort against a closed airway, the absolute value of pulmonary artery pressure decreases (Parrish and Shepard, 1990). However, transmural pulmonary pressure, which is the difference between pulmonary artery and pleural pressures increases (Marrone et al., 1989), reflecting an increased afterload on the right ventricle (Parrish and Shepard, 1990). At the termination of apnea, pulmonary artery pressure surges, possibly due to the increase in HR and the effects of hypoxia (Parrish and Shepard, 1990). However, after the first unobstructed breath, Marrone and co-workers (1989) noted a decline in transmural
pulmonary artery pressure while HR was still increasing, suggesting a role of hypoxia in the changes.

Hypoxia and hypercapnea result in pulmonary vasoconstriction and pulmonary hypertension and may contribute to the surge in pulmonary pressure (Parrish and Shepard, 1990). A significant correlation between transmural pulmonary artery pressure and the decrease in oxygen saturation has been reported (Marrone et al., 1989). Administration of oxygen reduces the pulmonary hypertensive peaks associated with apneas in patients with OSA and in an animal model (Iwase et al., 1992; Marrone et al., 1992). The above evidence suggests that hypoxia may play a major role in the surges in pulmonary arterial pressure, although other authors have attributed the changes mainly to alterations in intrathoracic pressure (Podszus et al., 1990).

1.4.5. Effects of Apneas on Intracranial Pressure and Cerebral Blood Flow

Transient elevations in intracranial pressure similar to the changes in BP occur during apnea and have been hypothesized to cause an increase in sympathetic outflow (Jennum and Borgeson, 1990). A strong correlation exists between intracranial pressure and duration of apnea (Jennum and Borgeson, 1990). Cerebral blood flow and mean flow velocity increase during apneic events (Siebler and Nachtmann, 1993; Klingelhofer et al., 1992; Balfors and Franklin, 1992), with maximal changes observed in REM sleep (Klingelhofer et al., 1992). The increase in mean flow velocity may be a consequence of carbon dioxide and blood pressure increases that are associated with apneas (Klingelhofer et al., 1992). Immediately following apnea termination, mean arterial BP and cerebral blood flow velocity reached maximum levels compared to baseline (Balfors and Franklin, 1992). However, 20 seconds after termination of apnea, mean arterial BP and cerebral blood flow velocity decreased to a
minimum (-8 and -23% respectively). This decrease in cerebral blood flow velocity was more prominent during repeated episodes of apneas. There was a strong correlation between mean arterial BP and cerebral blood flow velocity, suggesting that the mechanisms of cerebral autoregulation do not protect the brain completely from the effects of rapid systemic pressure changes (Balfors and Franklin, 1994). The significant increases in mean flow velocity during and immediately following termination of apnea may predispose patients with OSA to cerebral vascular incidents from increased strain (Klingelhofer et al., 1992). The reduced cerebral blood velocity 20 seconds after termination of apnea may increase the risk of nocturnal cerebral ischemia in patients with OSA (Balfors and Franklin, 1994).

1.5. Evidence of an Association between OSA and Hypertension

Accumulating epidemiological and clinical evidence has described an association between OSA and systemic hypertension. The research studies that have investigated this association can essentially be divided into three groups: studies of the prevalence of hypertension in patients with OSA; studies of the prevalence of OSA in hypertensive patients; and studies of the effect of treatment of OSA on BP.

1.5.1. Hypertension in Patients with OSA

There is extensive literature on the prevalence of hypertension among patients with OSA and studies in selected patients with OSA have estimated the prevalence to be greater than 50%, compared to 22% in the general population (Guilleminault et al., 1981; Burack, 1984; Shepard et al., 1985; Kaplan, 1986). However, the rates reported by these investigators may have been influenced by several variables including obesity, age, insulin levels, smoking or alcohol intake (Carlson et al., 1993a).
Over the last 5 years, several investigators have estimated the prevalence of hypertension in OSA while statistically controlling for confounding variables such as obesity. While some studies show that sleep apnea is independently associated with hypertension (Hla et al., 1994; Carlson et al., 1994; Strohl et al., 1994; Grunstein et al., 1993), other studies fail to show a relationship between these disorders, once body weight is considered (Millman et al., 1991; Jennum and Sjol, 1993; Davies et al., 1994). In a cross-sectional community-based study of 147 patients (ages 30-60 years), sleep apnea was significantly associated with diurnal hypertension in a dose-response fashion (Hla et al., 1994). After controlling for obesity, age and gender, the odds ratio for this association was significant and ranged from 2.0 to 5.0. Similarly, Carlson and co-workers (1994) examined 377 consecutive patients and found that the relative risk of hypertension with sleep apnea, independent of age and weight, was 2.1. Two other cross-section studies in large samples of men (261 and 1464 respectively) concluded that the degree of sleep apnea was independently related to morning blood pressure measured by sphygmomanometer (Strohl et al., 1994; Grunstein et al., 1993), but not evening blood pressure (Grunstein et al., 1993).

Three epidemiological studies found no independent association between sleep apnea and hypertension. Millman and co-workers (1991) reported that 45% of patients studied (n=206) presented with daytime hypertension. Stepwise regression, however, revealed that sleep apnea was not an independent predictor of hypertension and concluded that the high prevalence was related to age and excess obesity. A larger scale cross-sectional study in 748 patients (Jennum and Sjol, 1993) reached essentially the same conclusion, i.e., a lack of a direct association between sleep apnea and blood pressure. Finally, using well-matched controls (i.e., matched for age, sex, body mass index, smoking and alcohol intake), Davies and co-workers
(1994) found that daytime BP was not increased in patients with obstructive sleep apnea compared to control.

In summary, there is disagreement in the literature on an independent association between OSA and hypertension. This discrepancy may be secondary to several confounding correlates, particularly obesity, smoking and alcohol intake, and may be dependent on the time of measurement of BP.

1.5.2. Prevalence of OSA among Hypertensive Patients

In contrast to the studies that have reported a high prevalence of hypertension in patients with OSA is a group of studies that examined the prevalence of OSA in hypertensive patients. Essentially eight studies investigated whether there are occult cases of sleep apnea among patients initially diagnosed with hypertension (table 6). Five studies reported a high prevalence (i.e., 22-56%) of sleep apnea among hypertensive patients (Kales et al., 1984; Lavie et al., 1984; Fletcher et al., 1985; Williams et al., 1985). Kales and colleagues (1984) reported that 30% of hypertensives had sleep apnea, which was relatively mild with an average apnea index of 22.4. None of the control subjects exhibited evidence of sleep apnea. However, the control and hypertensive groups were not weight-matched and the hypertensive patients were on a variety of anti-hypertensive medications.

Lavie and colleagues (1984) studied 16 patients with a history suggesting sleep apnea from a group of 50 hypertensive patients; they found that 8 patients had evidence of obstructive apneas and 3 presented with central or mixed apneas. There was no control group in this study and all hypertensive patients were receiving medications that may have affected sleep pattern and apnea activity (Weichler et al., 1991). Fletcher and colleagues (1985) reported a greater prevalence of sleep apnea in hypertensive men (30%) as compared to control (9%). The hypertensive men with apnea were generally asymptomatic but tended to
<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Study</th>
<th>Sample Size</th>
<th>Blood Pressure (mm Hg)</th>
<th>Matched for Age?</th>
<th>Matched for Weight?</th>
<th>Matched for Gender?</th>
<th>Criteria for sleep apnea</th>
<th>Percentage of subjects with Sleep Apnea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kales et al., 1984</td>
<td>case control</td>
<td>H: 50</td>
<td>H:S=154</td>
<td>Yes</td>
<td>not specified</td>
<td>Yes (males &amp; females)</td>
<td>&gt;30 apneas/night apnea: &gt;10s duration</td>
<td>30% in H 0% in N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 50</td>
<td>D=88</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N:S=131</td>
<td></td>
<td></td>
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<td>D=81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavie et al., 1984</td>
<td>observational</td>
<td>H: 50</td>
<td>H: S&gt;160</td>
<td>no control group</td>
<td>no control group</td>
<td>no control group</td>
<td>AI&gt;10 apneas&gt; 10s. duration</td>
<td>22% in H no control group</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 0</td>
<td>D&gt; 95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fletcher et al., 1985</td>
<td>case control</td>
<td>H: 46</td>
<td>H: S=143</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (males only)</td>
<td>AI&gt;10 apneas&gt; 10s. duration</td>
<td>30% in H 9% in N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 34</td>
<td>D=94</td>
<td></td>
<td></td>
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<td>D=79</td>
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<td></td>
</tr>
<tr>
<td>Williams et al., 1985</td>
<td>case control</td>
<td>H: 23</td>
<td>not specified</td>
<td>Yes</td>
<td>Yes</td>
<td>not specified</td>
<td>AI&gt;5 apneas&gt; 10s. duration</td>
<td>48% in H 13% in N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGinly et al., 1988</td>
<td>case control</td>
<td>H: 13</td>
<td>MABP: H: 120</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (all men)</td>
<td>AI&gt;12 apneas&gt; 10s. duration</td>
<td>62% in H 62% in N</td>
</tr>
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<td></td>
<td>N: 13</td>
<td>N: .94</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Warley et al., 1988b</td>
<td>case control</td>
<td>H: 30</td>
<td>H:S=152</td>
<td>Yes</td>
<td>Yes</td>
<td>not specified</td>
<td>based on changes in SaO₂ not apnea events</td>
<td>SₐO₂ the same in H and N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 30</td>
<td>D=101</td>
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<td>D=79</td>
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<td></td>
</tr>
<tr>
<td>Hirshkowitz et al., 1989</td>
<td>case control</td>
<td>H: 38</td>
<td>H:S=141</td>
<td>Yes</td>
<td>No</td>
<td>Yes (all men)</td>
<td>AI&gt;10 apneas&gt; 10s. duration</td>
<td>11% in H 11% in N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 53</td>
<td>D=96</td>
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<tr>
<td></td>
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<td>D=80</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Isaksson et al., 1991</td>
<td>case control</td>
<td>H: 16</td>
<td>H:S=168</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (all men)</td>
<td>periodic of obstructive type; &gt;45% oxygen desaturation index &gt;6</td>
<td>56% in H 19% in N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N: 16</td>
<td>D=102</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>N:S=147</td>
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<td></td>
<td></td>
<td></td>
<td>D=87</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Legend: H = hypertensives, N = normotensives; S = systolic blood pressure; D = diastolic blood pressure; MABP = mean arterial blood pressure; Sa = sleep apnea; AI = apnea index i.e., number of apneas per hour of sleep.
be heavier and slightly older than the hypertensive men without apnea. Williams and co-workers (1985) evaluated 23 hypertensive patients (all taking medications) during a 3 hour morning nap, following a night of sleep deprivation. Sleep apnea was found in 11 of the hypertensive patients, in 8 of whom the apneas were of the obstructive type. Hypertensives with apneas were heavier than the hypertensive patients with no apneas. Sleep deprivation, lack of control of alcohol consumption, differences in weight and hypertensive medications may have influenced the prevalence rates.

The highest prevalence rate of obstructive sleep apnea among hypertensive patients (56% versus 19% in control) was reported by Isaksson and co-workers (1991). These authors used static charge sensitive beds, oximetry (and polysomnography in borderline cases) to investigate the presence of obstructive sleep apnea among 16 therapy resistant hypertensive patients. The high prevalence rates reported in this study may have been a function of the criteria used and differences in alcohol intake and medications between the two groups. A similar high prevalence was reported by Rajala and colleagues (1991) who reported an odds ratio of 5.2 for OSA among hypertensive patients in 27 morbidly obese patients (body mass index ≥ 40).

The remaining 3 studies examining the prevalence of sleep apnea found no significant differences between hypertensive and normotensive patients (McGinty et al., 1988; Warley et al., 1988b; Hirskowitz et al., 1989). In all three studies, the hypertensive patients were not on medications. The prevalence of sleep apnea in the study of McGinty and colleagues was high among both normotensives and hypertensives (table 6), possibly due to the older age of these patients (mean of 64 years). Warley and colleagues (1988b) matched the hypertensive and normotensive groups for age, height, weight, smoking habit and alcohol consumption and studied
the subjects with oximetry in their homes. Despite the differences in BP between the hypertensive and control groups, there were no differences in the degree of hypoxemia. However, these authors did not perform polysomnographic studies. The last study by Hirskowitz and colleagues (1989) found no relationship between BP and severity of sleep apnea as determined from full sleep studies in the untreated hypertensive and control groups. However, the group of patients with high BP, persistent even with anti-hypertensive medications, had the highest prevalence of sleep apnea. This latter finding suggests that concurrent treatment with anti-hypertensive medications may influence the prevalence of sleep apnea and may have distorted the rates observed in other studies (Stradling, 1989).

In summary, the results of the preceding studies indicate that there is a disagreement on the prevalence of OSA among hypertensive patients. Potential sources for the differences among study findings are: the definition of sleep-disordered breathing, the presence of confounding variables such as obesity and age and the pharmacological management of hypertension which may influence the severity of sleep apnea (Weichler et al., 1991).

1.5.3. The Effect of Treatment of OSA on BP

There are numerous reports of reduction in blood pressure after treatment of OSA with a variety of therapies (table 7). Pharmacological management with a tricyclic antidepressant, specifically protriptyline (Fletcher et al., 1985) and uvulopalatopharyngoplasty (Fletcher et al., 1985; Lund-Johansen and White, 1990) resulted in significant decreases in resting awake and sleeping blood pressures. These studies must be interpreted cautiously due to the use of small sample size (2 in total for uvulopalatopharyngoplasty and 7 for protriptyline), and the secondary effects of surgery or medications on weight and/or sleep.
Effective treatment of OSA with tracheostomy has been documented to eliminate nocturnal elevations in BP and to decrease the severity of diurnal hypertension (Coccagna et al., 1972a; Burack, 1984; Guilleminault et al., 1981; Motta et al., 1978). Coccagna and colleagues (1972a) reported a significant drop in systemic blood pressure from 170/97 to 133/66 in 5 patients with OSA following tracheostomy. Burack (1984) confirmed these changes and observed a drop from 157/110 to 124/74, one week post-tracheostomy in 10 patients. Two other groups of investigators found significant reductions in BP during wakefulness (Guilleminault et al., 1981; Motta et al., 1978) and sleep (Motta et al., 1978) after a variable period following tracheostomy (up to 6 years in Guilleminault et al., 1981). Tracheostomy also effectively eliminated cardiac arrhythmias associated with obstructive episodes (Tilkian et al., 1977; Coccagna et al., 1972a; Guilleminault et al., 1981). However, the results from these studies are inconclusive due to a number of confounding factors which include the associated weight loss, which averaged 15 kg in one study (Guilleminault et al., 1981), the small sample sizes, the variable follow-up periods, the lack of a control group and, in some studies, the use of single sphygmomanometric BP measurements.

Several studies have described a decrease in night-time, morning and daytime BP in patients with OSA following the use of nasal CPAP. In 14 patients, a significant drop in awake morning and nocturnal BP was observed 7 days after introduction of CPAP therapy (Jenum et al., 1989). Similar reductions in nocturnal and daytime BP were observed 6 months after initiation of treatment (Mayer et. al., 1991). In contrast, Davies and co-workers (194) reported a reduction in night-time systolic BP but not daytime BP in 11 patients, three months after starting CPAP therapy. The effects of CPAP on daytime and night-time blood pressure were addressed by two studies that reported nasal CPAP to be effective in improving circadian BP profiles in hypertensives patients.
Table 7: Studies of BP before and after treatment of OSA

<table>
<thead>
<tr>
<th>Source</th>
<th>Treatment</th>
<th>Sample Size</th>
<th>Time period after intervention</th>
<th>Method of BP measurement</th>
<th>Time of day (or state) during measurements of BP</th>
<th>Mean BP before treatment</th>
<th>Mean BP after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burack, 1984</td>
<td>Tracheostomy</td>
<td>10</td>
<td>7 days</td>
<td>non-invasive</td>
<td>awake</td>
<td>S:157; D:110</td>
<td>S:124; D:74</td>
</tr>
<tr>
<td>Fletcher et al., 1985</td>
<td>UVPP (1) Protripyline (7)</td>
<td>8</td>
<td>&gt; 2 months</td>
<td>non-invasive</td>
<td>awake</td>
<td>S:149; D:95</td>
<td>S:139; D:90</td>
</tr>
<tr>
<td>Lund-Johansen et al., 1990</td>
<td>UVPP</td>
<td>1</td>
<td>6 weeks</td>
<td>intra-arterial and sphygmomanometer</td>
<td>awake</td>
<td>S:145; D:98</td>
<td>S:126; D:86</td>
</tr>
<tr>
<td>Coccagna et al., 1972a</td>
<td>Tracheostomy</td>
<td>5</td>
<td>not stated</td>
<td>intra-arterial and sphygmomanometer</td>
<td>awake</td>
<td>S:170; D:97</td>
<td>S:133; D:66</td>
</tr>
<tr>
<td>Motta et al., 1978</td>
<td>Tracheostomy</td>
<td>6</td>
<td>not stated</td>
<td>intra-arterial</td>
<td>awake: peak sleep:</td>
<td>S:135; D:82</td>
<td>S:123; D:73</td>
</tr>
<tr>
<td>Guillemiault et al., 1981</td>
<td>Tracheostomy</td>
<td>25</td>
<td>9 months-6 years</td>
<td>non-invasive</td>
<td>awake</td>
<td>a drop of 15 to 18 mm Hg</td>
<td>S:133; D:79</td>
</tr>
<tr>
<td>Jennum et al., 1989</td>
<td>CPAP</td>
<td>15</td>
<td>6 days</td>
<td>intra-arterial</td>
<td>a.m. awake</td>
<td>S:148; D:84</td>
<td>S:122; D:71</td>
</tr>
<tr>
<td>Mayer et al., 1991</td>
<td>CPAP (33) and weight loss(27)</td>
<td>60</td>
<td>512 days</td>
<td>non-invasive</td>
<td>awake</td>
<td>drop in BP in 58% treated with weight loss and 29% treated with CPAP</td>
<td>S:140; D:89</td>
</tr>
<tr>
<td>Rauscher et al., 1993</td>
<td>CPAP</td>
<td>4 H 5N</td>
<td>≥ 5 weeks</td>
<td>non-invasive</td>
<td>daytime night-time</td>
<td>S:152; D:92</td>
<td>S:141; D:84</td>
</tr>
<tr>
<td>Suzuki et al., 1993</td>
<td>CPAP</td>
<td>19</td>
<td>8 weeks</td>
<td>non-invasive</td>
<td>24-hour</td>
<td>S:141; D:85</td>
<td>S:134; D:85</td>
</tr>
<tr>
<td>Wilcox et al., 1993</td>
<td>CPAP</td>
<td>11</td>
<td>&gt; 3 months</td>
<td>non-invasive</td>
<td>daytime night-time</td>
<td>S:113; D:76</td>
<td>S:111; D:76</td>
</tr>
<tr>
<td>Davies et al., 1994</td>
<td>CPAP</td>
<td>12</td>
<td>20.5 months</td>
<td>non-invasive</td>
<td>24-hour</td>
<td>S:106; D:72</td>
<td>S:100; D:67</td>
</tr>
<tr>
<td>Hedner et al., 1995</td>
<td>CPAP</td>
<td>12</td>
<td>20.5 months</td>
<td>non-invasive</td>
<td>24-hour</td>
<td>S:140; D:89</td>
<td>S:149; D:89</td>
</tr>
</tbody>
</table>

Legend: UVPP=uvulopalatopharyngoplasty; H= hypertensives, N= normotensives; S= systolic blood pressure; D= diastolic blood pressure.
with OSA (Suzuki et al., 1993; Wilcox et al., 1993). These changes were independent of changes in body weight, medications or alcohol consumption, but were dependent on good compliance with nasal CPAP (Wilcox et al., 1993). In an attempt to determine the role of sympathetic activity in these changes in BP, Hedner and colleagues (1995) studied 12 patients before and after 14-26 months of CPAP. These authors found no overall reduction in BP during that time period despite reductions in biochemical markers of sympathetic activity.

The fall in BP that accompanies the use of CPAP may be attributed to other variables such as weight loss or the direct effects of CPAP on the cardiovascular system (Wilcox et al., 1993; Rauscher et al., 1993). However, weight loss was not a factor in all the CPAP trials outlined. The question that remains to be answered is whether the effects of CPAP on BP are the result of the elimination of obstructive events or the direct effects of CPAP on the cardiovascular system.

1.6. Pathogenesis of sustained systemic hypertension in OSA

1.6.1. During sleep

Section 1.4.1. described the BP changes associated with apneas, and discussed the potential stimuli and mechanisms responsible for these acute changes in BP that accompany apneas. The major stimuli that are thought to be involved in the BP changes associated with apneas are hypoxia and arousal, but there is considerable controversy as to the most important stimulus. Some authors maintain that hypoxia is the main stimulus while others have determined a major role for arousal (Shepard, 1985; Iwase et al., 1992; van den Aardweg et al., 1992; O’Donnell et al., 1996; Ringler et al., 1990 and 1994; Davies et al., 1993). The conflicting evidence may be the result of differences in experimental conditions among studies, such as the degree of hypoxia and the type of arousals produced, or may
indicate a role for both these stimuli in the acute BP changes associated with apneas. Regardless of the stimulus, the final mechanism responsible for the BP surges appears to be an increase in sympathetic activity (as outlined in section 1.4.1).

Although the BP surge that accompanies apneas is well established, the relationship between OSA and daytime hypertension remains questionable. Some authors have postulated potential mechanisms and stimuli that may be responsible for translating the acute events that accompany apneas during sleep into daytime awake sustained hypertension. This section will discuss the mechanisms that are postulated to play a role in the association between OSA and hypertension.

1.6.2. During Wakefulness

Stimuli

Although investigation of the stimuli responsible for the surges in BP that accompany apneas have received considerable attention, there are relatively few studies investigating the stimulus that may link OSA to daytime hypertension. The primary stimuli implicated in the pathogenesis of hypertension are the recurrent exposures to asphyxia (primarily hypoxia) and the arousals from sleep. In an attempt to determine which of these stimuli plays the major role, information must be extrapolated from other diseases that are characterized by recurrent hypoxia or arousals. When such data are examined, hypoxia by itself does not seem to account for hypertension. For example, the prevalence of hypertension in patients with restrictive or obstructive lung disease and comparable nocturnal desaturation to those with OSA is lower than in patients with OSA (Shiner et al., 1990).

In contrast, in a rat model of episodic hypoxia that mimics the hypoxia of OSA, diurnal hypertension is observed within 20-35 days of exposure (Fletcher et al., 1992a). The
increase in BP is through the activation of the carotid chemoreceptors (Fletcher et al., 1992b) and is mediated through increased sympathetic activity (Fletcher et al., 1992c). The discrepancy between this finding and the data from patients with chronic lung disease may be a function of the episodic nature of hypoxia that may have different effects on BP than chronic exposure to hypoxia.

There are no reports in the literature on the effects of recurrent arousals, without airway obstructions, on daytime BP. In rats, 48-hours of REM deprivation altered the changes in BP that normally occur with sleep (Mion and Krieger, 1988). However, REM sleep deprivation caused systemic hypertension only in rats with a genetic predisposition to hypertension (Neves et al., 1992). It is difficult to extrapolate these findings to OSA since patients with OSA experience sleep fragmentation and not sleep deprivation. Finally, in a case report of a patient with narcolepsy and periodic leg movement but no sleep apnea or hypoxemia, substantial increases in systolic BP (i.e., 23% rise) were observed (Ali et al., 1991). It remains to be determined whether these acute surges in BP contribute to daytime hypertension.

Mechanisms

As discussed earlier, confounding variables, particularly obesity, may be the link between OSA and hypertension. However, recent epidemiological studies have shown that sleep apnea is significantly associated with diurnal hypertension, after statistically controlling for obesity, age and gender (Hla et al., 1994; Carlson et al., 1994). Other mechanisms may therefore play a role in the association between OSA and hypertension. It has been postulated that the paroxysmal sympathetic discharge and BP surges associated with apneas during sleep may eventually lead to daytime hypertension, but there is currently no evidence to support this
hypothesis (Levinson and Millman, 1991). There are, however, data showing that patients with OSA have a higher level of waking sympathetic activity, recorded by microneurography, than normal subjects (Somers et al., 1995; Hedner et al., 1988). Sympathetic activity is also increased during sleep in patients with OSA, and treatment of OSA with the application of CPAP decreases sympathetic activity (Somers et al., 1995).

Additional evidence regarding the role of the sympathetic nervous system during apneas comes from studies showing high levels of plasma and/or urine catecholamines in patients with OSA (Clark et al., 1980; Fletcher et al., 1987; Dimsdale et al., 1995). There is a direct correlation between plasma norepinephrine levels at rest and muscle sympathetic nervous activity (Wallin et al., 1981). Clark and colleagues (1980) reported that urinary and plasma catecholamine levels are elevated by 67 and 47% respectively in patients with OSA compared to normal subjects. Both arterial and venous plasma norepinephrine are elevated in patients with OSA compared to controls (Carlson et al., 1993b). Plasma norepinephrine levels are dependent on the severity of OSA (Eisenberg et al., 1990). Treatment of OSA by tracheostomy results in reductions in urinary catecholamine levels (Fletcher et al., 1987). Jennum and colleagues (1989) reported a significant reduction in whole-night plasma epinephrine that is correlated with the drop in BP observed in patients with OSA before and after treatment with nasal CPAP. Finally, Mills and co-workers (1995) examined the relationship between sleep apnea and β-adrenergic receptor characteristics. They found a 25% reduction in sensitivity of β2-adrenergic receptors [as determined by isoproterenol-stimulated cyclic adenosine 5'-monophosphate production in lymphocytes] but no change in postreceptor components, which supports the role of sympathetic nervous system, at least acutely, in the BP changes.
Further support for the role of sympathetic activity comes from Fletcher and co-workers' model of episodic hypoxia (Fletcher et al., 1992c). Pharmacologically sympathectomized rats did not develop the hypertension produced by exposure of rats with intact sympathetic nervous system to episodic hypoxia for 35 consecutive days. This finding suggests a role for the sympathetic nervous system in the pathogenesis of hypertension with recurrent episodic hypoxia. In contrast, rats that were sleep deprived for 21 days showed no changes in levels of plasma epinephrine and nor-epinephrine levels in the first half of the study but small changes emerged in the second half (Bergmann et al., 1989). Thus, sleep deprivation may not have profound effects on sympathetic nervous activity.

The changes in the sympathetic activity in patients with OSA may be due to changes in baroreflex function. In the study by O'Donnell and co-workers (1994), sleep-deprived dogs subjected to 12 hours of airway obstructions had a significant increase in mean arterial BP but no decrease in heart rate. These findings led the authors to suggest that a night of repetitive airway obstructions increases the set point of the baroreflex that may contribute to maintaining an elevated BP after cessation of obstructions. However, Ziegler and co-workers (1995) found no statistically significant difference in baroreflex slope between apneics and non-apneics.

Abnormalities in chemoreceptor function have also been postulated to play a role in the pathogenesis of hypertension (Trzebski, 1992). Hypoxia, hypercapnea and asphyxia cause an increase in sympathetic nerve activity (Morgan et al., 1995) which further increases when apnea is imposed (Somers et al., 1988a; Hardy et al., 1994). The asphyxia induced increase in sympathetic activity persists after return to normoxic normocapnea (Morgan et al., 1995). Borderline hypertensive patients demonstrate 'an exaggerated sympathetic nerve response to
hypoxia'. During apnea and hypoxia in these patients, the increase in sympathetic nerve activity is twelve-fold that recorded in normotensives (Somers et al., 1988b). In normal subjects, baroreceptor activation abolishes the increase in sympathetic nerve activity that accompanies hypoxia (Somers et al., 1991). Baroreflex impairment in hypertensive patients may result in loss of this inhibitory influence of the baroreceptors and thus result in exaggerated response to hypoxia. In addition, Hedner and co-workers (1992) reported an exaggerated pressor response to eucapneic hypoxia during wakefulness in patients with OSA compared to control subjects. These excessive chemoreceptor mediated vasoconstriction responses were present in both normotensive and hypertensive patients and were correlated with the severity of OSA. Thus, in patients with OSA and hypertension, the surges in sympathetic nerve activity and BP during apneas may be exaggerated and may potentially lead to daytime hypertension (Somers and Abboud, 1993). Additional evidence for the role of chemoreceptors comes from the model of episodic hypoxia in the rat (Fletcher et al., 1992b). Carotid body denervation prevented the increase in diurnal BP that was observed in rats with intact peripheral chemoreceptors after 35 days of exposure to episodic hypoxia (Fletcher et al., 1992b).

Another mechanism that may be implicated in the pathogenesis of daytime hypertension in OSA are mediators that influence vasodilatation. Krieger and colleagues (1991b) reported reduced overnight urinary ratio of prostacyclin to thromboxane A₂ in patients with OSA, and the ratio increased after management with CPAP. Prostacyclin has vasodilating effects whereas thromboxane A₂ causes vasoconstriction. The authors concluded that a reduced ratio may indicate increased vasoconstriction. In addition, the negative intrapleural pressures generated during obstructed breathing increases preload on the right
side of the heart, resulting in distention of the right atrium and the release of atrial natriuretic peptides (ANP). ANP levels decrease with effective management of OSA with nasal CPAP (Krieger et al., 1991a). However, ANP cause natriuresis and have sympathoinhibitory effects, and would therefore prevent the rise in BP. Similarly, some patients with OSA have decreased activity of the renin-angiotensin-aldosterone system (Follenius et al., 1991), indicating that this system is not involved in the increase in BP.

In contrast to the data from patients with OSA, O'Donnell and co-workers (1994) investigated, in the dog, the effects of 12 hours of repeated airway occlusions during sleep on mean arterial pressure, and on plasma levels of renin and atrial natriuretic peptides. These authors reported a 12 mm Hg increase in the overall mean arterial pressure over the 12 hours of occlusions that was maintained during 2 hours of recovery. Plasma levels of renin and ANP measured at 2 hour intervals remained unchanged. The differences between these findings and those in humans may be a function of species differences, or the effects of CPAP, applied in the human studies on the cardiovascular and respiratory systems (O'Donnell et al., 1994).

1.6.3. Could Essential Hypertension Predispose Patients to Sleep Apnea?

A discussion of the mechanisms postulated in the pathogenesis of systemic hypertension in patients with OSA would not be complete without reference to the hypothesis that hypertension may also play a role in the development of sleep apnea. Przybylski and co-workers (1986) postulate the involvement of arterial chemoreceptors in the association between hypertension and sleep disordered breathing. Their hypothesis suggests that arterial chemoreceptor stimulation occurs in essential hypertension, resulting in augmented tonic drive and 'instability of the feedback control of the respiratory system' that may lead to
frequent apneas. This interaction may create a vicious cycle between hypertension and OSA (Trzebski, 1992).

Other lines of evidence that suggest a role for hypertension in aggravating sleep-disordered breathing is provided by Garpestad and co-workers (1992b). They found that pharmacologically induced acute increases in BP in normal subjects result in decreases in genioglossus electromyogram activity, without changes in ventilatory parameters. Other authors have also reported a selective effect of acute elevation in BP, produced by mechanical or pharmacological stimuli, on upper airway activity in animals (Salamone et al., 1983; Marks and Harper, 1987). The BP surges differentially inhibited upper airway activity relative to diaphragm activity (Marks and Harper, 1987). These interactions may have clinical implications in patients with OSA. The BP surges at the termination of apnea could contribute to decreased upper airway tone in these patients and predispose them to consequent apneas.

1.7. **Association between Snoring and Cardiovascular Disease**

In addition to the association between OSA and hypertension, there is some epidemiological evidence showing an association between snoring and cardiovascular disease. Snoring is an inspiratory sound occurring during sleep from partial inspiratory obstruction of the upper airway (Lugaresi et al., 1975). There are several epidemiological studies of the prevalence and risk factors of snoring in different populations (Lugaresi et al., 1980; Koskenvuo et al., 1985; Partinen and Palomaki, 1985; Norton and Dunn, 1985; Bloom et al., 1988; Koskenvuo et al., 1987; Gislason et al., 1987). The prevalence of heavy or habitual snoring in these studies has been reported to range from 4-31% in men and 3-29% in women. However, in a recent population study of 294 Australian men aged 40 to 65 years,
80% snored more than 10% of the night and 22% for more than half the night (Bearpark et al., 1995). The large variation in prevalence among studies may be a function of the definition and method of detection of snoring. For instance, major discrepancies exist between subjective and objective reports of snoring (Hoffstein et al., 1994). In addition, age and obesity are recognized risk factors for snoring while nasal obstruction, alcohol intake and cigarette smoking are also linked with snoring (Lugaresi et al., 1975; Hoffstein et al., 1988; Bloom et al., 1988).

The prevalence of obstructive sleep apnea in snorers ranges from 35 to 64% (Koskenvuo et al., 1987; Hoffstein et al., 1988; Block et al., 1987). Snoring is the most frequent symptom in OSA occurring in up to 94% of patients with this syndrome (Martin et al., 1985). In contrast, Bearpark and co-workers (1995) found that although sleep apnea is associated with snoring, snoring for much of the night does not necessarily predict the presence of sleep apnea.

An association between snoring and vascular disease has been described. More specifically, some authors have identified snoring as a risk factor for systemic hypertension, cerebral infarction and ischemic heart disease (Lugaresi et al., 1980; Koskenvuo et al., 1985 & 1987; Norton et al., 1985; Gislason et al., 1987; Partinen et al., 1985). However, this association may be influenced by inclusion of patients with OSA and the poor quantification of snoring, mainly from questionnaires (Waller and Bhopal, 1989). The key studies examining this association are detailed below.

1.7.1. Positive Epidemiological Studies

In the earliest study by Lugaresi and colleagues in 1980 examining the association between snoring and obesity, 5,713 men and women answered a questionnaire on their sleep
habits. The snoring history was correlated with one BP measurement made during the
daytime. Systemic hypertension was associated with age, obesity and snoring. In obese
patients (i.e. over 15% ideal weight), the association between BP and snoring disappeared
once weight was considered. However, in non-obese patients, a positive association between
snoring and BP was evident.

Similarly, Kuskenvuo and colleagues (1985) surveyed over 7,000 men and women by
mail and correlated the prevalence of self-reported snoring and blood pressure. They
reported a risk ratio of 2.68 for hypertension for snorers versus non-snorers. Once age and
weight were accounted for, this ratio fell to 1.94. Similarly, a risk ratio of 2.01 was observed
for the association between snoring and angina, after adjusting for hypertension and obesity.
However, these authors did not find a significant correlation in women.

In another survey by Norton and Dunn (1985) of 2001 men and women, snoring
correlated with several medical conditions including hypertension, obesity, smoking, chest
disease, depression, alcoholism, rheumatism and allergies. The risk ratio for hypertension
among snorers versus non-snorers was approximately 2, after correction for age but not
obesity. From another survey by Gislason and colleagues (1987) of 4064 men, snoring was
associated with age and obesity. In the overall group, snoring was not a risk factor for
hypertension once obesity was accounted for; however, in the subgroup of 40-49 year old
men, snoring was a significant risk factor for hypertension.

Several studies have examined the association between snoring and cardiovascular
disease or associated risk factors. In a case-control study, Partinen and Palomaki (1985)
examined the snoring history of 50 men with cerebral infarction and 100 men with other
disorders. The risk ratio for cerebral infarction in habitual snores versus non-snorers was 10.
However, the risk factors for hypertension and ischemic heart disease were not different between snorers and non-snorers. Although the groups were matched for weight, alcohol consumption and smoking were not considered.

Similarly, Koskenvuo and co-workers (1987) surveyed the snoring history of 4388 men. Over the next three years, the prevalence of admission or death from ischemic heart disease or stroke were monitored by reviewing hospital records and death certificates. A relative risk of 2.08 for ischemic heart disease and stroke was found in snorers after adjustments for age, weight, history of hypertension, smoking and alcohol use.

Finally, in a study of 804 elderly group of snorers (i.e. 70 year old), elevated plasma nor-epinephrine and abnormal glucose tolerance were found in self-reported snorers compared to non-snorers (Jenum et al., 1993). Snorers also had a higher blood pressure than non-snorers. This relationship between snoring and diastolic BP became non-significant when body mass index, physical activity, alcohol and smoking history were adjusted. However, systolic BP still showed an association with snoring.

The association between snoring and daytime hypertension may exist because of the nocturnal changes in BP in snorers (Lugaresi et al., 1975; 1978). The specific stimuli that contribute to the surges in nocturnal BP have not been defined. Lugaresi and colleagues (1975, 1978) have suggested a role for the more negative intrathoracic pressure that snorers produce during sleep. Alternately, Hoffstein and co-workers (1988) have argued that nocturnal hypoxemia that may accompany snoring contributes to the nocturnal increases in BP. However, when the relationship between snoring and nocturnal BP is examined, only snoring frequency and not oxygen desaturation correlate with changes in mean nocturnal BP
(Mateika et al., 1992). The exact stimuli and their interaction responsible for the changes in BP during snoring remain to be determined.

The positive studies reviewed above do not provide convincing evidence of an independent association between snoring and cardiovascular disease due to a number of limitations (Waller and Bhopal, 1989). First, there is a lack of standard definition and method of detection of snoring. Second, the use of questionnaires may provide a poor quantification of snoring. For example, in one study, the rate of habitual snoring in the population doubled if the bed partner was present at the interview (Stradling and Crosby, 1990). Third, confounding variables were not always adequately considered such as anti-hypertensive medications. Finally, snoring may be a marker of other conditions such as OSA that may be an independent risk factor for cardiovascular disease.

1.7.2. Negative Epidemiological Studies

Telakavi and colleagues (1988) studied fifty-men with both a questionnaire and full-night polysomnography. In contrast to other investigators (Stradling and Crosby, 1990; Hoffstein et al., 1995), these authors found self-reported snoring was a valid tool in estimating the actual prevalence of snoring. The diastolic BP level was related to obesity, but not to hypoxic events or snoring history. In a larger study, Hoffstein and co-workers (1988) estimated the relationship between systemic hypertension and snoring with and without the presence of OSA. Using overnight sleep studies, they measured the frequency of snoring and the apnea/hypopnea index in 372 snorers. A positive association existed between blood pressure and age, obesity, mean nocturnal desaturation and apnea/hypopnea index. However, no association was found between blood pressure and snoring, indicating that snoring was not an independent risk factor for hypertension.
Similar negative findings have been reported in larger scale studies. Schimdt-Nowara and co-workers (1990) studied a population-based sample of 1,222 adults. They reported 27.8% prevalence of snoring in men and 15.3% in women. No effect of snoring on hypertension was found once confounding factors were controlled. Similarly, in a study of 1,504 individuals (Jemnum and Sjol, 1993), self-reported snorers showed higher systolic and diastolic BP and total cholesterol and lower high density lipoprotein. These relationships became non-significant when age, sex, body mass index, alcohol and tobacco consumption were taken into account. Finally, Stradling and Crosby (1990) studied 836 men from a general practice and found that the number of episodes of desaturation at night correlated with daytime BP. However, once confounding variables were controlled (i.e., age and weight), this correlation disappeared. Only 5% of the sample was overweight and the rate of desaturation was only 12 per hour. They concluded that an association between snoring and cardiovascular disease is not proven and may be a function of confounding variables such as obesity, smoking and alcohol consumption. However, these authors did not specifically examine the occurrence of snoring as they did not measure sound or respiration.

The most convincing evidence of lack of an association between snoring and hypertension is the comprehensive study by Hoffstein (1994). A total of 1,415 patients was studied with nocturnal polysomnography including objective measurement of snoring, and BP was measured in the morning. When confounding variables were considered (i.e., sleep apnea, hypoxemia, and obesity), snoring was not a significant determinant of morning BP.

In summary, the balance of evidence suggests that snoring per se is not a direct risk factor for hypertension. The relations between snoring, sleep apnea and obesity may explain
the findings of some epidemiological studies of an association between snoring and hypertension.

1.8. Potential Cardiac Consequences of OSA

Along with the association between OSA and systemic hypertension, OSA has been considered a risk factor for other cardiovascular diseases. This section highlights the evidence implicating OSA with cardiac arrhythmia, pulmonary hypertension, right and left ventricular dysfunction, pulmonary hypertension, myocardial and cerebral infarction and cardiovascular mortality.

1.8.1. Arrhythmia

Evidence of rhythm disturbances has been observed in patients with OSA (see section 1.4.3), with sinus brady-tachyarrhythmia being the most common (Shepard, 1992). Treatment of OSA with tracheostomy effectively reduces cardiac arrhythmias that are related to OSA, specifically sinus bradychardia, sinus pauses and atrioventricular blocks (Coccagna et al., 1972b; Guilleminault et al., 1981; Tilkian et al., 1977; Conway et al., 1981). The prevalence of atrial fibrillation and flutter is also decreased after tracheostomy possibly due to reduction in ventricular afterload and atrial distention (Coccagna et al., 1972b). Ventricular ectopic beats, which are associated with desaturation below 60% and ventricular tachycardia are less common after tracheostomy (Shepard et al., 1985; Guilleminault et al., 1981; 1983; Tilkian et al., 1978; Conway et al., 1981). In addition, the application of nasal CPAP results in a reduction in the frequency of atrial and ventricular arrhythmias and the disappearance of nocturnal sinus pauses (Wagner and Pollack, 1988; Becker et al., 1989). These findings of considerable reduction of cardiac arrhythmias with successful treatment of OSA implicate OSA in the pathogenesis of these arrhythmias.
1.8.2. Myocardial Infarction

The highest prevalence of myocardial infarction occurs between 6 a.m. and noon (Muller et al., 1985; Rocco et al., 1987; Tofler et al., 1987). The typically prolonged periods of early morning REM sleep (with its associated BP variability) and the surge in BP associated with arousal from sleep may contribute to this increased risk of myocardial infarction. The underlying mechanisms may include rupture of an atherosclerotic plaque and/or increase platelet aggregability (Tofler et al., 1987; Willich et al., 1987). In addition, the hemodynamic mechanisms that accompany obstructive apneas (see section 1.4) such as hypoxemia, arousal from sleep, surges in BP and HR, and afterload effects from the negative intrapleural pressure have the potential to precipitate myocardial ischemia in patients with coronary artery disease (Hung et al., 1990).

Several studies have suggested a link between sleep-related complaints and myocardial infarction. Appels and colleagues (1987) studied 3,877 men between the ages of 39 and 65 years over a 4 year period. Complaints related to sleep, especially difficulties initiating sleep, non-refreshing sleep and chronic fatigue were correlated with increased risk of myocardial infarction. Similarly, in a study of 566 men, insomnia, short sleep duration and frequent napping were associated with increased risk of coronary heart disease (van Diest et al., 1990). Habitual snoring has also been related to increased risk of angina and ischemic heart disease, after adjustment for confounding variables such as hypertension, age and obesity (Koskenvuo et al., 1985 and 1987). The mechanisms underlying this association between sleep disturbance or snoring and myocardial infarction have not been defined.

More direct evidence of an association between OSA and myocardial infarction has been described by Hung and colleagues (1990). They studied 101 men that had suffered
myocardial infarction and 63 age-matched control subjects and found that an apnea index of greater than 5 carried a relative risk of acute myocardial infarction of 23.3, after controlling for hypertension, smoking, body mass, and cholesterol. Similarly, a short report by Hillman (1993) reported that 15.8% of a sample of patients with recent myocardial infarction had an apnea index of 10 or greater compared to 1.9% in the control group (without myocardial infarction). However, after adjustment for age, body mass index, hypertension, smoking and cholesterol levels, the relative risk factor for myocardial infarction in patients with sleep apnea was significant only for those patients with severe sleep apnea. These authors did not specify the type of sleep apnea observed (i.e., central or obstructive). Finally, in three case reports, the initial manifestations of OSA simulated angina or mimicked heart failure (Loui et al., 1994). Although Hung and colleagues (1990) and Hillman (1993) suggest that OSA was a risk factor for myocardial infarction, it is also possible that myocardial infarction may predispose patients to sleep disordered breathing (Shepard, 1992). Research is needed to establish a cause and effect relationship between myocardial infarction and OSA.

1.8.3. Left Ventricular Dysfunction

Congestive heart failure is often associated with periodic breathing known as Cheyne Stokes respiration. This pattern of ventilation includes episodes of central apneas. Although the association between congestive heart failure and central apnea is well established (Bradley, 1992), the association between OSA and left ventricular dysfunction has received less attention. Hedner and colleagues (1990) found a thicker left ventricular wall in normotensive patients with OSA as compared to normal subjects. However, other authors have reported no differences in echocardiographic measures of left ventricular diameter, wall
thickness and calculated mass in patients with OSA as compared to controls (Hanly et al., 1992; Davies et al., 1994).

The remainder of the evidence suggesting a correlation between OSA and left ventricular dysfunction is from studies showing improvements in left ventricular ejection fraction following management of OSA (Malone et al., 1991; Krieger et al., 1991c; Tal et al., 1988). In 8 patients with congestive heart failure due to idiopathic dilated cardiomyopathy and OSA, correction of OSA with CPAP resulted in a significant improvement in left ventricular function, both in terms of ejection fraction and clinical status (Malone et al., 1991). Furthermore, withdrawal of nasal CPAP for one week caused a deterioration in left ventricular ejection fraction (Malone et al., 1991). Krieger and co-workers (1991c) also reported small but significant improvements in left ventricular ejection fraction in 29 patients with OSA who had normal left ventricular function at baseline, after 1 year of nasal CPAP treatment. In children, surgical management of OSA resulted in a greater than 10% improvement in left ventricular ejection fraction (Tal et al., 1988). These findings indicate that OSA may have a detrimental effect on left ventricular function that is reversible with treatment of the disorder.

Although mechanisms underlying the association between OSA and left ventricular dysfunction have not been defined, there is evidence to implicate hypoxia. In a rat model of episodic hypoxia that mimics the hypoxia in OSA, exposure for 35 days results in an increase in left ventricle-to-body weight ratio and left ventricular size (Fletcher et al., 1992a). Another stimulus that may contribute to left ventricular impairment is the repeated exposure to negative intrapleural pressure, which increases left ventricular afterload and may promote hypertrophy and dysfunction (Bradley, 1992). There are two case reports in the literature in
patients with OSA who developed pulmonary edema as a manifestation of OSA (Chaudhary et al., 1982 and 1984). Systemic hypertension or the frequent surges in night-time BP with OSA may also contribute to left ventricular dysfunction (Bradley, 1992).

1.8.4. Cerebral Infarction

The rate of occurrence of non-hemorrhagic fatal strokes is highest during sleep (Marshall, 1977; Shepard, 1992). Several studies have provided data to link snoring with increased risk of stroke (Partinen and Palomaki, 1985; Koskenvuo et al., 1987; Palomaki et al., 1989). Koskenvuo and colleagues (1987) found an increased prevalence of stroke in habitual snorers and 35% of these patients were likely to have OSA. Partinen and Palomaki (1985) showed an increased risk ratio for cerebral infarction in snorers, after controlling for age and body mass index. Finally, Palomaki and co-workers (1989) studied 177 male patients with cerebral infarction and found that 35% of the patients had the strokes during sleep. Snoring was associated with an increased risk ratio for strokes occurring during sleep. Although these data suggest that snoring may influence the prevalence and timing of stroke, confounding variables and the method of detection of snoring may have influenced the high relative risk ratios observed (Waller and Bhopal, 1989).

Even though a definite association between OSA and stroke has not been established, some of the pathophysiological changes that accompany OSA could possibly predispose to cerebral infarction. First, cyclical increases in intracranial pressure occur in association with apneas (Jenum and Borgeson, 1990). Second, the reduction in cardiac output that accompanies apnea termination (Garpestad et al., 1992a) may decrease cerebral perfusion (Balfors and Franklin, 1994). Third, systemic hypertension may also increase the risk of cerebral infarct. Conversely, McGinty and co-workers (1988) described profound nocturnal
hypotension in elderly patients with OSA and suggested a defect in their sympathetic vasomotor response to hypoxia. Such a defect would allow the primary vasodilatation effects of hypoxia to occur and thereby compromise cerebral perfusion and lead to ischemia (McGinty et al., 1988).

1.8.5. Pulmonary Hypertension and Right Ventricular Dysfunction

Pulmonary artery pressure increases during apneas but returns to normal at termination of apnea and on arousal from sleep (Coccagna et al., 1972b; Schroeder et al., 1978). Schroeder and co-workers (1978) described mild pulmonary hypertension in over 50% of patients with severe OSA. The vast majority of these patients had an awake \( P_{O_2} \) of greater than 65 mm Hg. However, Bradley and colleagues (1985a) reported data on 50 patients with OSA and found right heart failure to be present in 12% of these patients. The presence of right heart failure did not correlate with the severity of OSA but correlated with diurnal hypoxemia and hypercapnea attributed to the co-existence of chronic lung disease. These findings were confirmed by Weitzenblum and co-workers (1988) who performed right heart catheterization in 49 patients with OSA. In their sample, 20% of patients presented with pulmonary hypertension at rest and these patients had daytime hypoxemia and hypercapnea from chronic lung disease. The presence of pulmonary hypertension did not correlate with the apnea/hypopnea index or lowest nocturnal oxygen saturation but correlated with daytime \( P_{O_2}, P_{CO_2} \) and forced expiratory volume in one second. A recent study by Laks and colleagues (1995) found a 42% prevalence of pulmonary hypertension in 100 consecutive patients with obstructive sleep apnea. Again, patients with pulmonary hypertension had evidence of coexisting airflow limitation with or without daytime hypertension. In agreement with these findings, treatment of OSA with nasal CPAP does not result in a change in
pulmonary artery pressure; in 54 patients with OSA treated with CPAP for one year, no significant reduction in daytime awake pulmonary artery pressure was detected (Sforza et al., 1990). Thus, the available evidence suggests that OSA produces pulmonary hypertension only in the presence of a co-existing respiratory condition associated with hypoxia and/or hypercapnea.

In contrast to the studies refuting a direct association between right ventricular dysfunction and OSA in the absence of other pulmonary disease, a study in children with OSA found reduced right ventricular ejection fraction in 37% of the 27 patients (Tal et al., 1988). Evidence of right ventricular dysfunction was not detected on clinical examination and was only found using radionuclide ventriculography. Following adenotonsillectomy, right ventricular ejection fraction returned to normal. This finding is in disagreement with the studies outlined above and may be the result of differences in the physiological responses of children and adults to the stress of OSA. Nevertheless, in adults, evidence of right ventricular hypertrophy on echocardiography has been reported in 41 to 80% of patients with OSA (Berman et al., 1991; Noda et al., 1995). This finding does not necessarily equate with the presence of pulmonary hypertension and may be the result of confounding variables. However, using Doppler echocardiography to measure pulmonary artery pressure in patients without chronic lung disease, Sajkov and co-workers demonstrated that 41% of patients with OSA had mild degree of pulmonary hypertension. The small sample size used (i.e., n=27) and the indirect method for measuring pulmonary artery pressure may have influenced the high rate observed in this study.

Although pulmonary hypertension in patients with OSA has not been established, exercise may induce a considerable degree of pulmonary hypertension in some patients with
OSA (Podszus, 1986; Weitzenblum et al., 1988). Pulmonary capillary wedge pressure increases during exercise in patients with OSA which may be due to impairment in left ventricular function (Weitzenblum et al., 1988).

1.8.6. Mortality

The mortality rate is highest between 2 and 8 a.m. (Mitler et al., 1987). In patients with OSA, it has been postulated that apneas at that time of the night may be longer, and therefore associated with greater degree of hypoxemia and possibly mortality (Parish and Shepard, 1990). The five-year mortality rate in patients with untreated OSA has been reported at between 11 and 13% (Partinen et al., 1988; He et al., 1988). In the study by Partinen and co-workers (1988), 57% of the deaths were the result of cardiovascular disease. The incidence of death was correlated with severity of the disease. Untreated patients with an apnea index greater than 20 had an eight year cumulative mortality of 37%, compared to 4% in those with an apnea index of less than 20 (He et al., 1988). Although the time of death was not specified, Guilleminault and co-workers (1983) found that death occurs unexpectedly during sleep in patients with OSA. Sleep apnea may also affect mortality indirectly, possibly through the increased risk for hypertension (Lavie et al., 1995). With effective treatment of OSA with nasal CPAP or tracheostomy, there were no deaths during a nine year follow-up period (He et al., 1988; Partinen et al., 1988).

In a retrospective review of 198 patients with OSA, Partinen and Guilleminault (1990) reported lower cardiovascular morbidity and mortality in those patients who underwent definitive treatment as compared to those who were simply encouraged to lose weight. However, 56% of the patients presented with evidence of cardiovascular disease at the start of the study. A similar retrospective review of cardiovascular mortality in an elderly
population revealed a mortality ratio of 2.7 in apneic versus non-apneic individuals (Bliwise et al., 1988). Finally, a study by Gonzalez-Rothi and colleagues (1988) found no increased incidence of mortality in OSA. However, the sample size (i.e., n=91) was small, resulting in low statistical power.

In summary, there are epidemiological and clinical studies that show an association between OSA and cardiovascular disease and mortality. However, this association may be secondary to the increased risk of hypertension in patients with OSA. There is a need for experimental data to determine whether OSA per se produces cardiovascular morbidity and mortality.

1.9 Animal Models of Sleep Apnea

The use of animal models in biomedical research has enhanced considerably the understanding of numerous diseases. Animal models provide powerful tools to examine the natural history, pathogenesis and pathophysiological consequences of a disorder. Studies of these different aspects of the disease often require invasive measures that are not feasible in patients. Moreover, animal models may be used in the development of novel methods of treatment, such as new surgical techniques.

Models of human diseases may occur spontaneously in animals. This type of model is useful in investigating the natural history and the primary underlying cause of the condition. Animal models may also be induced by experimentally creating a state akin to the disease observed in humans. Clearly, induced models cannot be used to study the cause of the condition, but they provide an effective method for the study of the consequences of the disease. Furthermore, induction of the condition may allow for manipulation of the presentation of the severity and duration of the disorder.
Both natural and induced animal models of OSA have been reported. Spontaneous sleep disordered breathing has been described in brachycephalic canine breeds such as the English bulldog (Amis et al., 1986; Hendricks et al., 1987). Several investigators have also produced short-term (≤ 48 hours) models of OSA in rats, new-born lambs, pigs and dogs (Cragg and Phillips, 1984; Fewell et al., 1988; Pinto et al., 1993; Scharf et al, 1992; O’Donnell et al., 1994). There is, however, only one report in the literature in conscious animals on the application of an induced model of OSA for more than 48 hours; Kimoff et al. (1994b) produced OSA in the dog for 5 consecutive days. Although an animal model of repetitive upper airway occlusions during sleep has not been applied long-term (i.e. more than one week), episodic hypoxia (without upper airway occlusions) has been induced experimentally in the rat for 20-35 days (Fletcher et al., 1992).

In this section, the natural models of OSA and the various short-term induced models in different species will be described. The model of intermittent repetitive hypoxia in the rat, without upper airway occlusions, will also be presented.

1.9.1. Natural Models of Sleep Apnea

The recognition of OSA in animals may be difficult since some of the clinical features of the disease (i.e., excessive daytime sleepiness, restless sleep, impaired function) may not be apparent. However, snoring during sleep is one clinical feature that may indicate the presence of OSA in animals. Given the companionship between the human and canine species, it is perhaps not surprising that the only natural model of OSA described to-date has been among dogs.

Upper airway obstruction often develops in brachycephalic dogs (Amis et al., 1985). These animals (e.g., English bulldogs and boxers) have wide and relatively flat faces, with
crowding in the pharyngeal area. Upper airway obstruction in these dogs is associated usually with a combination of abnormalities such as narrow nares, elongated or enlarged soft palate, collapse of the larynx and hypoplastic trachea (Amis et al., 1986). Of the various brachycephalic dogs, the English bulldog is the most severely affected with recurrent upper airway obstructions, and has been studied as a natural animal model of sleep apnea (Hendricks et al., 1987).

Brachycephalic dogs have a flattened flow-volume loop during breathing, consistent with the presence of increased upper airway resistance (Amis et al., 1984). During the daytime, the English bulldog shows evidence of hypersomnolence; although these bulldogs snore and demonstrate signs of increased upper airway resistance, they generally have no apneic events during n-REM sleep (Hendricks et al., 1987). A potential reason for the lack of apneic events during this stage of sleep may be the increased activation of upper airway dilator muscles in these dogs compared to the activity in control dogs (Hendricks et al., 1993). The compensatory hyperactivity of upper airway dilator muscles may be a response to the anatomically compromised airway and may prevent sleep-disordered breathing during n-REM sleep. However, the persistent load on the upper airway dilators leads to myopathic changes in these muscles that, over time, may diminish the ability of these muscles to maintain patency (Petrof et al., 1994).

Conversely, REM sleep in the English bulldog is characterized by distinct respiratory events ranging from 15 to 114 apneas/hour of sleep, and large dips in oxygen saturation with an average $S_\text{a}O_2$ nadir of 73% (Hendricks et al., 1987). The animals demonstrate episodes not only of obstructive apneas, but also of apparent central apneas. These respiratory events during REM sleep are associated with a consistent suppression, fractionation and asynchrony of
respiratory muscle activity, and a significant decrease in drive to the upper airway muscles (Hendricks et al., 1991).

This spontaneous model of OSA in the bulldogs has important advantages. It can be used to investigate the primary causes of apnea and the neural mechanisms controlling the upper airway dilator muscles during sleep (Pack, 1994). However, apneas in the bulldogs predominate in REM sleep, whereas, in OSA in human, apneas occur in both n-REM and REM sleep. Furthermore, the English bulldogs tend to have sleep-disordered breathing by the time they are acquired, making it difficult to obtain measures from ‘pre-disease’ state, and to distinguish between primary defects and secondary consequences of the disorder (Kimoff et al., 1994b).

1.9.2. Induced Models of OSA

*Lamb Model*

The first induced model of repeated episodes of upper airway occlusions in conscious animals was described by Fewell and colleagues (1988). Lambs, 8-14 days of age, were instrumented for recordings of EEG, electrooculogram, nuchal and diaphragmatic EMG, and measurements of BP and oxygen saturation. The lambs breathed through a fenestrated tracheostomy tube placed in the trachea. A balloon-tipped catheter was inserted into the tracheostomy tube so that airflow could be obstructed by inflation of the balloon. The animals were monitored with a closed-circuit video system and occlusions were initiated by the investigators whenever the animals went to sleep. Once the animal aroused from sleep, the obstruction was released by manual deflation of the balloon. The model was induced for 17 to 30 hours in 5 lambs and resulted in a longer time to arousal from active sleep and a greater decrease in arterial oxygen saturation in response to airway occlusions. Although this model
mimics closely the human syndrome of OSA, it requires continuous human intervention and physical attachments between the animals and the recording apparatus, thus restricting its long-term application.

Porcine Model

A similar model of repeated episodes of upper airway occlusions has been described in pigs (Pinto et al., 1993). Ten pigs were instrumented for recordings of EEG, electrooculogram and nuchal EMG and measurements of cardiovascular variables (i.e., coronary blood flow, arterial BP and heart rate). A modified tracheostomy tube was placed in the trachea to obstruct the upper airway during sleep. The pigs were kept in fiberglass open-topped boxes during the experiments and monitored by the investigators from an adjacent room. Changes were measured in arterial blood pressure, and in heart rate and coronary blood flow during wakefulness, n-REM and REM sleep, and in response to airway occlusion. Similar to the lamb model described above, the long-term application of this model is restricted since it requires continuous monitoring by the investigators, involves restriction of the activities of the animal, and introduces the potential for exit site infection from the exteriorized leads.

Canine Models

Three different induced canine models of repeated episodes of airway occlusions have been described, the first in conscious dogs for a 12 hour period (O’Donnell et al., 1994), the second in anaesthetized dogs (Scharf et al., 1992), and the third in conscious dogs for a 5 day period (Kimoff et al., 1994b). In the first model, four dogs were instrumented for recordings of electrooculogram, EEG and nuchal EMG and for the measurement of BP. The leads were exteriorized and attached to the recording apparatus in the adjacent room. The animals were monitored with a short-wave closed-circuit television for 12 hours. The upper airway was
obstructed by the investigators through the inflation of the luminal endotrachael tube whenever sleep was exhibited, and the airway obstruction was relieved with the subsequent arousal from sleep. The investigators repeated the experiment after each dog was sleep deprived for 24 hours to determine the effect of sleep deprivation on the responses to airway occlusions. This model in the dog is similar to those described above in the lamb and the pig (Fewell et al., 1988; Pinto et al., 1984). These short-term models involve the recording of electrophysiological variables through exteriorized leads and the induction of airway occlusion through a custom-designed tracheostomy whenever sleep is detected. Thus, these models in the pig, lamb and dog successfully mimic the human syndrome of OSA and provide insight into the short-term consequences of this disorder.

The second model of induced OSA used ten anaesthetized dogs that were instrumented for the measurement of cardiac output, heart rate, arterial BP, left ventricular end-diastolic and end-systolic transmural pressure, coronary blood flow and myocardial contractility. While spontaneously breathing from 100% oxygen, airway occlusions were produced by completely clamping the endotracheal tube for 60 seconds at functional residual capacity then unclamping for 60 sec. The authors quantified the changes in the cardiovascular variables for 7 consecutive cycles of occlusions. Although the use of anaesthetized dogs provided accurate and stable measurements of cardiovascular variables, the level of anesthesia can influence cardiovascular function (Chelly et al., 1986; Chiueh et al., 1978; Seyde et al., 1985). Furthermore, the time over which measurements can be made in anaesthetized animals is limited. Therefore, the anaesthetized preparation is not suitable for long-term studies of the consequences of OSA.

Finally, Kimoff and colleagues (1994b) described a novel canine model of OSA. The animal was tracheostomized, and a special custom-designed valve was connected to the
endotracheal tube through which the dog breathed. Sleep state was automatically monitored online by a computer that used telemetered signals from implanted EEG and nuchal EMG electrodes to make a judgment about sleep state. The valve was controlled by radiofrequency signals and programmed to close when the dog fell asleep and to open when the animal aroused. Kimoff et al. (1994b) applied a modified version of this induced-model of OSA in two dogs, for a 5 day period. It is important to note, however, that these 5 day experiments were performed before the telemetry unit and remote-controlled occlusion valve were operational. Thus, Kimoff and colleagues (1994b) manually operated the system and employed exteriorized EEG and EMG electrodes due to technical difficulties. The animals were connected to the standard laboratory amplifiers to record EEG during the occlusions, and the valve position was controlled with a standard valve controller. Nevertheless, during the short term application by Kimoff, the dogs demonstrated behavioral and objective evidence of somnolence. As will be described in chapter 2, the model of OSA on which this thesis is based is a modified version of the initial model described by Kimoff et al. (1994b).

Rat Model

There are two reports in the literature of rat models of sleep apnea, one in the anaesthetized rat, and the second in conscious rats (Cragg and Phillips, 1984; Fletcher et al., 1992). In the first model, the effects of repeated apneic episodes on brain function during intoxicated sleep were investigated. Rats were anaesthetized with alcohol and subjected to repeated episodes of asphyxia for 1-2 hours on 5 successive days. At the end of this period, none of the rats showed evidence of brain damage. This animal model mimics the short-term episodes of apneas that may be experienced after consumption of alcohol rather than the long-term, recurrent obstructions seen in OSA.
In the second model of OSA in the rat, only one aspect of the OSA disorder, precisely intermittent hypoxia, was induced. Fletcher and colleagues (1992) produced repetitive intermittent hypoxia in the rat that was patterned after the hypoxia in sleep apnea. Using a 12 second infusion of nitrogen into daytime chambers, rats were subjected to intermittent hypoxia (3-5% nadir ambient oxygen) every 30 seconds, 7 hours per day for up to 35 days. Periodic measurements of daytime BP were made. It is important to note that the rats in this model were not subjected to airway occlusions and the associated changes in intrapleural pressure. Furthermore, the hypoxic events were not synchronized to sleep state and may not have been associated with the arousal responses that accompany OSA. Therefore, the application of this model provide insight into the effects of only one aspect (i.e., hypoxia) caused by the syndrome of OSA.

In Conclusion

A natural model of sleep disordered breathing, the English bulldog, and models simulating repetitive airway obstructions have been described. The natural model can be used to study the pathophysiology and natural history of the disease, and to test new treatment approaches (e.g., stimulation of upper airway muscles). However, it is difficult in the natural model to obtain measures from 'pre-disease' state, and to distinguish between primary defects and secondary consequences of the disorder.

There are a number of limitations to the induced animal models of OSA. First, these models cannot be used to investigate the mechanisms that initiate apnea. Second, in the porcine and canine models described above, the occlusion of the airway occurred at the tracheal level, in contrast to clinical OSA in which occlusion is at the pharyngeal level. It is possible that the lack of input from reflexes in the upper airway may alter the responses observed in the animals.
compared to humans. Third, OSA in these animals is artificially produced and progressed and may not reflect the development of the syndrome in humans. Finally, to date, application of the induced models of repetitive upper airway occlusions during sleep have provided insight into only the short-term consequences of recurrent apneic events.
PART II: DEVELOPMENT OF METHODS

Animal models of a disease or a disorder are valuable for studying the natural history and promoting the understanding of a disease process. As outlined earlier (Chapter 1), both naturally occurring and induced animal models of OSA have been described. This thesis is based on the findings from an induced model of OSA in which the dog was chronically tracheotomized and the airway was occluded mechanically with a valve. Since this model bypasses the upper airway, it does not reveal any insights into the underlying pathogenesis of OSA, but provides a powerful tool to investigate the physiological consequences of this disorder.

The main feature of the model is that it functions independently and requires minimal human intervention except for routine monitoring. In addition, there is no physical attachment between the dog and the monitoring apparatus and the animal is free to move in its environment. These important features are accomplished with the use of biotelemetry and radio-frequency signals. Part II of the thesis describes the development and validation of OSA and sleep fragmentation model. In addition, the validation of the telemetry system for long-term cardiovascular measurements and the properties of the apparatus used in the experiments outlined in chapters 6-8 are presented.
Chapter 2: Development and Validation of an Animal Model of Obstructive Sleep Apnea

2.1. Introduction

This induced model of OSA was initially developed by Kimoff et al. (1994b). These authors described a biotelemetry system to monitor EEG and EMG, a computer algorithm for sleep detection and a remote-controlled occlusion valve to occlude the airway. However, Kimoff and colleagues did not apply this system but rather produced OSA in two dogs for a 5 day period, using standard methods that require continuous human intervention. The experiments were performed before the telemetry unit and remote-controlled occlusion valve were operational. Instead, exteriorized rather than implanted EEG and EMG electrodes were employed primarily because of technical difficulties with the surgery. The animals were connected to the standard laboratory amplifiers to record EEG during the occlusion, and the valve position was manually controlled with a standard valve controller. Nevertheless, during the short term application by Kimoff, the dogs demonstrated behavioral and objective evidence of hypersomnolence.

The purpose of this chapter is to describe the validation of an enhanced version of this induced model of OSA. This model was functional in the absence of human intervention. Although the model is based on the work by Kimoff et al. (1994b), substantial changes were made to allow for its long-term application. The main changes to the model are described:

1. The telemetry unit was rendered functional in vivo, allowing monitoring of the animal without restricting its activity. Furthermore, none of the components of the telemetry
unit (i.e. electrodes, wires and body of transmitter) were exteriorized, which eliminated the risk of exit site infection.

2. The method of implantation of EEG and EMG electrodes was improved to ensure that the leads remained adequately in place for the length of the experiment. Previously, the leads were dislodged within 2 weeks of implantation. With the improvements in the surgical technique, the EEG and EMG electrodes have remained in place up to 2 years from implantation.

3. The remote-controlled occlusion valve was functional so that no attachments to the animal were required. Furthermore, considerable changes were made to the valve to decrease its power requirements, minimize the incidence of malfunction, and decrease its weight.

4. The algorithm for sleep detection was significantly modified to improve the accuracy of detection of the state of the animal, especially when the dog was ambulatory in its pen.

5. A low pressure endotracheal tube was used to decrease the incidence of tracheal irritation, particularly when used nightly for 3-4 months. In an earlier pilot study, the use of a high pressure cuff for two consecutive nights in one dog resulted in acute tracheal irritation and discomfort to the animal.

In short, the induced model of OSA described in this chapter functioned entirely independently and required minimal human monitoring except for routine maintenance. Furthermore, there were no lead attachments to the animal so it was allowed it to move freely in its pen. Finally and most significantly, the system described in this chapter was operational on
a nightly basis for several months in each of the dogs, allowing us to examine the long-term consequences of OSA.

This chapter will describe in detail the surgical preparation of the animal, the model hardware and the sleep analysis software for the OSA model. Validation data for each component of the system will also be presented.

2.2. Methods

2.2.1. Animal Preparation

Studies were performed on a total of three adult female dogs and one male dog (weight, 23-31 kg) trained to sleep in the laboratory. All surgical and experimental procedures were approved by the Animal Care Committee of the University of Toronto.

The dogs underwent two surgeries, the first to create a tracheostomy that bypasses the upper airway, and the second to implant a telemetry unit (TLM11M3D70-CCP, Data Sciences, St Paul, MN). The telemetry unit consisted of three channels to record arterial blood pressure, EEG and EMG. These surgical procedures (i.e. tracheostomy and implantation of the telemetry unit) were performed in separate operations at least one month before the start of the studies. Surgery was performed aseptically under general anesthesia. Prior to the operative procedures, the dog was pre-medicated with atropine (0.02-0.05 mg/kg i.m.). Induction of anesthesia was achieved with a short-acting barbiturate (thiamyl sodium, 10-20 mg/kg i.v.) and maintained either with halothane (titrated to effect typically 0.5-2%) or a long-acting barbiturate (pentobarbitone, IV, titrated to effect, typically 30 mg/kg). Halothane was used for the surgery involving the implantation of the telemetry unit; pentobarbitone was used for the surgery involving the creation of the chronic tracheostomy. The permanent tracheostomy allowed
intubation of the dog with an endotracheal tube to which the occlusion valve was attached. Long-acting penicillin (15,000-20,000 units/kg, I.M.) and suitable analgesics (buprenorphine, 0.01-0.02 mg/kg I.M.) were administered after the surgery (Canadian Council on Animal Care, 1984).

2.2.1a. Placement of EEG electrodes

The bipolar EEG electrodes were implanted through a midline scalp incision. The temporalis fascia, muscles and the periosteum were dissected over the frontoparietal region to expose the skull (Miller et al, 1964). The two EEG electrodes were attached to the skull, on each side of the midline, using washers and stainless steel screws. Washers were used to minimize fraying of the wire and to increase the surface area of contact between the wire and the skull. Resin cement was placed on top of the screws to minimize signal contamination by activity of the overlying layer of muscles. The common ground electrode was attached to the skull using the same hardware (i.e. washer and screw) and was placed anteriorly, remote from the pair of EEG electrodes.

2.2.1b. Placements of EMG Electrode

Two incisions were made in the neck on each side of the midline. The first muscle layer, the rhomboids, was dissected to reveal the splenius muscles (Miller et al, 1964). The EMG electrodes were looped, sutured to the splenius muscle layer and covered with resin cement to prevent unlooping of the wire. The rationale for the placement of the electrodes on either side of the midline was to allow detection of muscle activity in any posture; for example, if one side of the neck was stretched and the other was relaxed, activity would still be detected between the EMG electrodes.
2.2.1c. Transmitter body and BP catheter

The EEG and EMG electrode wires (length, 75 cm) were tunneled subcutaneously to a pouch created in the lower abdomen where the body of the transmitter was placed. We used silk sutures to secure the transmitter body (diameter, 56 mm; thickness, 14 mm; weight, 40g) and prevent rotation in the abdominal pouch. The fluid-filled arterial pressure catheter (diameter, 1.2 mm; length, 35 mm), connected to the sensor in the body of the transmitter, was tunneled subcutaneously from the abdominal pouch to the area of the femoral triangle. The catheter was inserted into the deep femoral artery and advanced into the external iliac artery to the level of bifurcation of the aorta (see chapter 3 for more details on the blood pressure catheter). The lower abdominal pouch was irrigated with an antibiotic solution (ampicillin sodium, 125 mg/ml) and then closed.

2.2.2. Model Hardware

OSA was produced in the dog using the model illustrated in figure 1. Briefly, sleep-wake state was continuously monitored overnight by a computer that received telemetered EEG and EMG signals from the chronically implanted electrodes. Whenever the computer detected sleep, it sent a signal to close an occlusion valve connected to an endotracheal tube through which the dog breathed; when the dog awoke from sleep, the occlusion was released. Another computer received and stored cardiovascular parameters (i.e. blood pressure and heart rate).

The following sections will describe the hardware components of this model of induced OSA and present relevant validation data. Information regarding the hardware used for cardiovascular monitoring is presented in Chapter 3.
2.2.2a. Signal Processing: from the dog to the computer

A schema of the transmission and processing of the EEG and EMG signals is shown in figure 2. For continuous monitoring of EEG and EMG signals, the radio-frequency signals emitted by the telemetry unit were detected by three water resistant receivers (RL2000, Data Sciences, St. Paul, MN) located around the pen in which the dog was housed. The signals from the receivers were processed by a multiplexer (RMX10, Data Sciences, St. Paul, MN) which selected the signals from the receiver in closest proximity to the dog, based on the strength of the signal. The signals were then converted from digital to analog and amplified (ratio of 1:100) (DL10, Data Sciences, St. Paul, MN). The signals were then further amplified (ratio of 1:4) and filtered (1-50 Hz for EEG, 10-100 Hz for EMG) (see validation of amplifier and filter in the next section). The signals were then relayed from the amplifier and filter to the analog-to-digital board (Lab Master DMA; amplification of 1:10) of a personal computer (IBM compatible 386 SX/16 MHz with math co-processor) and sampled at 300 Hz. The EEG and EMG signals could also be displayed on chart or oscilloscope and stored on tape.

Nyquist's sampling theory states that the sampling frequency for a signal must be at least twice the highest frequency component of interest in the signal (Brook and Wyne, 1988). For EEG and EMG analysis, the highest significant components in the signal were 30 and 100 Hz respectively. Thus, a sampling rate of 300 Hz met the criterion from the Nyquist sampling theory and thus prevented aliasing. Low pass filter of 50 Hz (for EEG) and 100 Hz (for EMG) were used to avoid contamination from higher frequencies.
Figure 1: A schematic of the obstructive sleep apnea model in the dog. Each dog had an implanted telemetry system with three channels (EEG, EMG and BP). Signals were sent by telemetry to two computers, one for the detection of sleep-wake state, and the other for analysis of hemodynamic data. Dashed lines represent telemetered connections.
Figure 2: Schematic diagram of transmission of EEG and EMG signals from the dog to the computer and valve control. Dotted lines indicate telemetered connections.
Prior to implantation, an EEG calibrator (100 µV, 10 Hz) was connected to the EEG and EMG leads in order to test the fidelity of signal transmission from the telemetry unit to the computer. The amplifier-telemetry units were found to transmit these calibration signals accurately. After implantation, the transmission of signals was further tested by visually comparing the EEG signal from the implanted electrodes to those obtained by subdermal needle electrodes (Beckman bioelectric). Similarly, the EMG signals from the implanted electrodes were compared to those recorded with subdermal needle electrodes and standard EMG amplifiers (Grass P511K). Visual comparison revealed that the EEG and EMG signals obtained by telemetry were similar to those obtained by standard laboratory equipment, during both wakefulness and sleep.

**Function of Amplifier and Filters**

As mentioned above, an amplifier was used to amplify the analog signals of EEG and EMG (amplification ratio of 1:4) before they were processed by the computer. Amplification was required to ensure that the signals were sufficiently large to allow proper detection by the computer program. The function of the amplifier was validated using an input signal (5 Volt square wave, 0.05Hz) from a function generator (Wavetek) and recording the output voltage on an oscilloscope. As shown in figure 3, at frequencies of 10, 100 and 500 Hz, and input voltages from 2-2000 mV, the relationship between input and output voltage was linear and the ratio of the two signals was consistently four.

Similarly, the low pass and high pass filters were validated using a known input signal and recording the output voltage on the oscilloscope. The function of the filters was to filter out extraneous noise from the EEG and EMG signals. Thus, the filters used were 1-50 Hz for EEG,
Figure 3: Relationship between input voltage and output voltage from the amplifier (ratio 1:4). The top graph represents the function of the amplifier for input signals of 50-2000 mV peak to peak. The bottom graph represents the function of the amplifier for input signals of 1-50 mV peak to peak. The relationship between input and output voltage was reproducible for input signals of 10, 100 and 500 Hz.
Figure 4: Relationship between input frequency and the ratio of output to input voltage. The top graph represents the function of the high pass filter and the bottom graph represents the function of the low pass filter. The amplitude of the input signal was 20mV. The EEG signal was filtered using 1 Hz high pass filter and 50 Hz low pass filter. The EMG signal was filtered using 10Hz high pass filter and 100 Hz low pass filter.
10-100 Hz for EMG. An input signal of 20 mV typical of voltages in an EMG and EEG signals after amplification, was used. The ratio of output to input voltage was plotted as a function of input frequency. Figure 4 shows that the filters allowed the desired signal to pass and filtered out unwanted frequencies.

2.2.2b. Signal Processing: from the computer to the dog

After amplification and filtering, the EEG and EMG signals were processed by the computer which utilized the frequencies in the EEG and the magnitude of the EMG to make a judgment of sleep-wake state, using the algorithm described below. Once a period of sleep of pre-determined length was detected, the computer sent a signal (4.8 V, square signal) via a digital-to-analog converter to an FM transmitter; this signal was then transmitted to the battery-powered receiver-controller placed in the dog jacket, which closed the valve attached to the endotracheal tube through which the dog was breathing. Thus, an obstructive apnea was produced. When arousal occurred, the signal from the computer to the FM transmitter terminated (returned to 0 volts), resulting in release of the occlusion.

The silent occlusion valve (figure 5) was attached to the outer end of the endotracheal tube. The valve consisted of a Plexiglas block with a hole (9 mm inner diameter) that could be occluded by a rotating shutter. The shutter position was controlled by an electric motor placed on top of the shutter. A battery-powered receiver-controller unit for the motor was placed in a pocket of the jacket worn by the dog (figure 6). The receiver-controller detected remote signals from the FM transmitter and provided power to the motor which controlled the position of the shutter. Prior to their use in the dogs, the functioning of the valve, valve motor, receiver-controller unit and FM transmitter were tested for continuous use with an input signal (5 Volt
Figure 5: Photograph of the occlusion valve and the battery-powered receiver-controller. The controller detected remote signals from the FM transmitter and provided power to the motor which controlled the position of the valve.
Figure 6: Photograph of the endotracheal tube, occlusion valve and dog jacket. The battery-powered receiver-controller was placed in the pocket of the jacket.
square wave, 0.05Hz) for extended periods of time, to ensure that the valve would not fail overnight.

**Valve Resistance**

The magnitude of the resistance of the valve was determined both in the open and closed position. The rationale for measuring the resistance of the valve in the open position was to ensure that the animal was not subjected to an increased breathing load when attached to the valve. The rationale for measuring the resistance of the valve in the closed position was to ensure that there was no significant leakage of air through the shutter and that the animal was truly being subjected to airway occlusions.

The valve resistance was determined by measuring the pressures on either side of the valve at a given flow rate. A rotameter was used to generate a pre-determined flow. A schematic of the set-up is shown in figure 7. The resistance was calculated using the equation (Strong, 1970):

\[
\text{Resistance} = \frac{\text{Pressure gradient across the valve}}{\text{Flow}} = \frac{P_1 - P_2}{\text{Flow}}
\]

Since \(P_2\) was assumed equal to 0 (atmospheric pressure) for convenience, then:

\[
\text{Resistance} = \frac{P_1}{\text{Flow}}
\]

In the open position, the resistance was 3.1-4.9 cm H\(_2\)O/L/sec for flows of 0.6-2.7 L/sec which represent the flows during quiet and labored breathing in a dog (figure 8). Thus, even at a high flow rates, such as during the hyperpnea that follows the release of an occlusion, the valve resistance was low. This resistance was comparable to the upper airway resistance which was bypassed in the dogs by tracheostomy and endotracheal tube.
Figure 7: Schematic of set-up for measuring valve resistance in the open and closed position.
Figure 8: Relationship between flow and valve resistance. The top graph represents valve resistance in the open position. The bottom graph represents the valve resistance in the closed position.
In the closed position, the resistance of the valve was 300-325 cm H$_2$O/L/sec at flows of 0.4-1.2 L/sec (figure 8). From the studies of the responses of the dogs to airway occlusions (see chapter 6), the dogs generated peak tracheal pressures against the occluded valve of 37 cm of H$_2$O in nREM sleep, and 50 cm of H$_2$O in REM sleep. At these peak pressures and assuming a linear relationship, the maximum flows that could be generated through the closed valve were 0.12-0.17 L/sec (Flow=Pressure/Resistance; assume resistance of 300 cm H$_2$O/L/sec). Since these peak pressures were maintained for only a fraction of a second and since the relationship was unlikely to be linear, the tidal volume generated during the occluded respiratory efforts were substantially less than the anatomical deadspace of the dog and therefore, did not result in any effective alveolar ventilation. These small leaks were inherent to the design of the valve and were a function of the gap required for rotation of the shutter between the open and closed position.

**Endotracheal Tube**

The dogs were intubated with a cuffed low-pressure endotracheal tube (outer diameter=12.3 mm; inner diameter=9.0 mm; Aire-Cuf; Bivona, Indiana). In preliminary experiments, we attempted to use a cuffed latex rubber high pressure endotracheal tube (Rusch no.42); however, following two nights of use, the dog developed severe tracheal irritation, characterized by erythema of the tracheal mucosa and occasional blood streaking of tracheal secretions. With the use of the low-pressure endotracheal tube and careful selection of cuff volume, no further problems of tracheal irritation were encountered.

The volume of the cuff needed to properly seal the trachea but, at the same, minimize damage to the airway, was individually determined in each dog as follows. The dog was
mechanically hyperventilated, using a volume-cycled ventilator, to a steady state hypocapnic pCO₂ of approximately 30 mm Hg, which was sufficient to abolish spontaneous breathing movements as judged by smooth and reproducible pressure profiles. The volume of the ventilator was set at 700 mL, and the rate was adjusted to achieve the desired pCO₂. Peak inspiratory tracheal pressures were recorded (as described in Chapter 4) for each unit of cuff volume between 0 and 20 cc. With a poor seal of the trachea, air leakage around the cuff resulted in low peak tracheal pressures; when a seal of the airway was produced with a sufficient cuff volume, a further increase in cuff volume resulted in no further change in peak tracheal pressures (figure 9). The cuff volume used in each dog was selected as 1 cc above the minimum volume required to seal the airway. The cuff volume ranged from 8 to 12 cc in the four dogs.

Preliminary experiments revealed that the cuff membrane of the endotracheal tube was semi-permeable to air as it deflated overnight, but impermeable to fluid. Thus, in order to prevent deflation of the cuff in overnight intubation, the cuff was inflated with physiological saline rather than air.

2.2.2c. Summary

With the hardware technology described in this section, specifically the use of telemetry and a remote-controlled valve, the dog was free of any attachments during monitoring, allowing the animal to move without any constraints. The lack of exteriorized leads or connections also eliminated the risk of their damage by the animal. In all four dogs that were subjected to OSA, the EEG and EMG electrodes remained in place for up to two years from the date of implantation. The fidelity of the signals was also maintained over this time period.
Figure 9: Relationship between cuff volume and peak inspiratory tracheal pressure. Cuff volumes of less than 6 cc resulted in poor seal of the trachea and therefore a low peak pressure. A seal of the airway was produced at 6 cc since a further increase in cuff volume resulted in no further change in peak tracheal pressure.
2.2.3. Sleep Analysis Software

In this section, the method for determining the sleep-wake state will be detailed. The method for EEG and EMG analysis, the algorithm for sleep detection and the validation of the algorithm in each of the dogs will be described. Finally, the accuracy of the algorithm at the start and the end of OSA will be outlined.

The analysis of sleep-wake state in humans is usually carried out according to the criteria set out by Rechtschaffen and Kales in 1968. Determination of sleep stage is based on the combined data from EEG, electrooculogram (EOG) and EMG. Relaxed wakefulness is generally characterized by high muscle tone and irregular eye movement (Mendelson, 1987). With the eyes closed during wakefulness, the EEG predominantly shows alpha waves (7.5-11.5 Hz) intermixed with lower amplitude irregular beta waves (13.5-30 Hz) (Moorcroft, 1993). The change from waking to drowsy (Stage 1 sleep) is characterized by a decrease in the amount of alpha wave, moderation of muscle activity, the appearance of a mixture of EEG frequencies of relatively low voltage (delta 1 and theta, 2-7.5 Hz) and the emergence of slow eye movements (Moorcroft, 1993). In stage 2 sleep, EEG shows theta activity (4-7.5 Hz), mixed frequency activity (with less than 20% delta activity) and the presence of K-complexes (a high amplitude negative wave followed by a positive wave), and sleep spindles (burst of 12-14 Hz waves) (Moorcroft, 1993; Rechtschaffen and Kales, 1968). Stages 3 and 4 (slow-wave sleep) are characterized by high amplitude, slow waves (delta 1 and 2, 0.5-4 Hz). Stage 3 is scored when delta activity is between 20-50% of the record. In stage 4, delta activity makes up more than 50% of the record (Rechtschaffen and Kales, 1968; Mendelson, 1987). During REM sleep, the EEG shows mixed frequency pattern without any sleep spindles or K complexes, the EMG
drops to a very low amplitude and the EOG shows bursts of sharp waves reflecting rapid eye movements (Mendelson, 1987). In dogs, the EEG, EMG and EOG during sleep and wakefulness show similar patterns as in humans, although the different stages of nREM sleep are often not differentiated (Phillipson et al., 1976). In the program used for the OSA model, only EEG and EMG are used to differentiate sleep state.

2.2.3a. EEG analysis

The computer program utilized the frequencies in the EEG signal to determine the sleep-wake state of the dog. In order to determine these frequencies, the computer program used a modification of the interval histogram method described by Kuwahara and colleagues (1988) to analyze the signal. In short, the amplitude of the EEG signal was divided into 32 equally spaced horizontal voltage lines. A period was then designated as the time interval between the two points at which the same slice line crossed consecutive positive-going slopes of the EEG signal. An illustration of the modified interval histogram method is shown in Appendix 1. For each 5 seconds, a histogram was made of all these time intervals and converted to percent distribution for frequencies. The frequency ranges are presented in Table 8. This approach for analysis of the frequencies in the EEG signal has been validated in humans and animals (Kuwahara et al., 1988; Van Gelder et al., 1991).

Table 8: The frequency ranges and corresponding bands used in the computer program for sleep detection

<table>
<thead>
<tr>
<th>Frequency bands</th>
<th>Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ₁</td>
<td>0.5-2</td>
</tr>
<tr>
<td>δ₂</td>
<td>2-4</td>
</tr>
<tr>
<td>θ</td>
<td>4-7.5</td>
</tr>
<tr>
<td>α</td>
<td>7.5-11.5</td>
</tr>
<tr>
<td>β₁</td>
<td>13.5-20</td>
</tr>
<tr>
<td>β₂</td>
<td>20-30</td>
</tr>
</tbody>
</table>
The accuracy of the computer software in detecting the frequencies in the EEG signal was examined using signals from a wave generator (Wavetek). Sine waves equivalent to an EEG input of 25-100 μV and frequencies of 0.5 to 30 Hz were used, typical of frequencies and amplitude of the canine EEG. Signal detection was 100% accurate for each of the frequency bands tested (δ2 to β2). This rate of accuracy was maintained for all sizes of signals equivalent to an EEG input of 25 to 100 μV. Thus, these tests showed that the program provided excellent detection of the frequency of the signals in the canine EEG, both in wakefulness and sleep.

2.2.3b. EMG Analysis

The computer program also utilized the amplitude of the EMG signals in determining the sleep-wake state of the dog. A moving-time average of the EMG signal was performed and the amplitude of the averaged signal was compared to pre-determined thresholds.

The accuracy of the computer software in distinguishing changes in the amplitude of the EMG signal was examined using signals from a wave generator (Wavetek). Sine waves equivalent to an EMG input of 2.5-70 μV were used, typical to the amplitude of canine EMG. The magnitude of the detected EMG signal was linearly related to the magnitude of the input signals. The frequency of the input signal (10-100 Hz) did not affect this linear relationship. Thus, these tests showed that the EMG was adequately magnified to allow accurate detection of changes in the EMG amplitude, both in wakefulness and sleep.

2.2.3c. Program for Sleep Detection

Every 6 seconds, the EEG and EMG signals from the dog were sampled for 5 seconds and processed for 1 second, during which the judgment of wakefulness, nREM or REM was made. The detection of sleep-wake state was based on the algorithm illustrated in figure 10.
The sleep detection program was developed using Quick Basic (Microsoft Corp., 1987) and the parameters are described below. The computer program is listed in Appendix 2.

**Ratio of %β2/%δ1**

After examination and analysis of EEG signals in several dogs, the ratio of %β2/%δ1 was determined to best represent the EEG changes associated with the different sleep-wake states (Kimoff et al., 1994). The changes in the ratio were less variable than the changes in percentage within a bandwidth. The β2 band included the high frequency waves, whereas the δ1 band represented the low frequency waves. Thus, a low ratio indicated the presence of low frequency activity in the EEG, whereas a high ratio indicated high frequency activity in the EEG. A cut-off or threshold value for this ratio that provided the best discrimination was individually determined in each dog.

**%β2/%δ1 below threshold**

A low ratio was observed in two conditions:

1) during nREM sleep which was associated with an increase in slow-frequency activity (figure 11, panels C and D; figure 12); and

2) during activity, such as grooming and drinking, where a low frequency movement artifact was introduced on the EEG (figure 11 panel A; figure 12).

We therefore used the averaged EMG, which was low in nREM sleep but high during active movement, to distinguish between these two conditions (figures 11 and 12).

**%β2/%δ1 above threshold**

A high ratio was observed in two conditions which were associated with an increase in high frequency activity:
1) during wakefulness without movement artifact (figure 11, panel B; figure 12); and

2) during REM sleep (figure 11, panel E).

We again used the averaged EMG, which was low in REM but high in wakefulness, to distinguish between these two conditions (figure 11).

**Apnea Index**

The apnea index was defined as the number of occlusions in each hour of sleep. The apnea index could be altered though the sleep detection program by changing the number of consecutive epochs of sleep required to generate the signal to close the occlusion valve. For example, if we mandated that there be a long period of sleep before the computer sent the signal to occlude the valve, the frequency of occlusion would be low, resulting an a low apnea index. The minimum number of epochs of sleep required to generate the signal was three (i.e. 18 seconds). This requirement ensured that the sleep was established before an occlusion was initiated and thus minimized the incidence of occlusion during wakefulness.

In each of the dogs, the number of epochs of sleep required before initiation of occlusion was empirically set to produce 10-15 apneas/hour of sleep for the first 7 nights. This interval was shortened to produce 50-60 apneas/hour of sleep by 14 nights of OSA. This progressive increase in apnea index provided the dog with time to adjust to overnight intubation and airway occlusions. To ensure consistency, we reviewed, on a daily basis, the computer records from the previous night [from 1930 hr (lights off) until 0730 hr (lights on)] and calculated the apnea index, mean apnea duration, total hours of sleep and total number of occlusions. The apnea index in each dog during the OSA period is shown in figure 13.
2.2.3d. Establishing thresholds

Three thresholds were individually determined in each of the dogs: the threshold for \%\text{\beta}2/%\delta 1; the EMG threshold 1, to distinguish nREM from movement artifact; and the EMG threshold 2, to distinguish REM from wakefulness. The cut-off values for \%\text{\beta}2/%\delta 1 and for the EMG thresholds were chosen to minimize errors in sleep detection. The most important error to minimize was the misinterpretation of wakefulness as sleep since such an error would result in an occlusion during wakefulness and distress the animal. The following will outline the guidelines used to select these thresholds.

In preliminary studies, the ratio of \%\text{\beta}2/%\delta 1 and the amplitude of the averaged EMG signal were calculated in each dog for over 3000 epochs of awake, nREM and REM, as determined by human judgment. The data were collected on different days and the human scoring was performed according to standard canine EEG criteria by an experienced technician who was blinded to the computer generated values (Phillipson et al., 1976). The mean and standard deviation for \%\text{\beta}2/%\delta 1 and for the amplitude of the averaged EMG were calculated for each state. A box plot was used to represent the full distribution of the data (mean, median and percentile points). We used the following as guideline for choosing the appropriate thresholds:

1) The \%\text{\beta}2/%\delta 1 threshold was set at the mean \%\text{\beta}2/%\delta 1 in nREM + either 1 or 2SD.

2) The EMG threshold 1 (to distinguish movement from nREM) was set at the mean EMG in nREM (+2SD).

3) The EMG threshold 2 (to distinguish awake from REM) was set at: mean EMG in REM (+2SD).
Figure 10: The algorithm for sleep detection.
<table>
<thead>
<tr>
<th>State</th>
<th>EEG</th>
<th>EMG</th>
<th>%(\beta_2/\delta_1)</th>
<th>EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alert Awake</td>
<td>50µV</td>
<td>50µV</td>
<td>0.9</td>
<td>64.2</td>
</tr>
<tr>
<td>Quiet Awake</td>
<td></td>
<td></td>
<td>3.9</td>
<td>12.2</td>
</tr>
<tr>
<td>'Light' n-REM</td>
<td></td>
<td></td>
<td>1.4</td>
<td>11.6</td>
</tr>
<tr>
<td>'Deep' n-REM</td>
<td></td>
<td></td>
<td>0.7</td>
<td>8.6</td>
</tr>
<tr>
<td>REM</td>
<td></td>
<td></td>
<td>5.4</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Figure 11: Recorder tracing of EEG and EMG signals and corresponding computer values for %\(\beta_2/\delta_1\) and EMG thresholds in W, nREM and REM.
Figure 12: Recorder tracing of EEG and EMG signals, state as detected by the computer and valve position. The corresponding computer values for \%\(\beta_2/\delta_1\) and EMG thresholds are also presented. The arrow indicates the point of arousal from sleep. Note that there was minimal lag time between arousal and release of the occlusion.
Figure 13: Changes with time in the apnea indices in the 4 dogs. Note that dog 4 required a slower rate of increase in apnea index due to tracheal irritation.
Although the above were used as guidelines, the accuracy for different combinations of threshold values was determined. The threshold values that minimized errors in detection were chosen.

2.2.3e. Accuracy of Detection

The box plots representing the distribution of the %\(\beta_2/\%\delta_1\) and EMG values in wakefulness, and nREM and REM sleep in each dog are shown in Figures 14 to 17. Based on the mean values, several algorithms with different values of threshold were examined for accuracy of detection. The best discrimination of sleep-wake state was found with %\(\beta_2/\%\delta_1\) thresholds ranging from 1.8 to 4.4, EMG thresholds (for separation of W versus nREM sleep) ranging from 24 to 58, and EMG thresholds (for separation of REM versus W) ranging from 12.6 to 32 (table 9).

The accuracy of detection of wakefulness for individual epochs ranged from 90 to 97% in the 4 dogs (table 9). Although between 2-10% of the individual awake epochs were detected by the computer as sleep, none of these epochs occurred consecutively. Since three consecutive epochs of sleep were require before initiation of an occlusion, the risk of an occlusion during wakefulness was eliminated.

The accuracy of detection of nREM sleep ranged from 84 to 96% in the 4 dogs. However, the accuracy of detection of nREM as sleep (i.e., either nREM or REM) ranged from 89 to 97%. Since misinterpreting nREM sleep as REM sleep would still result in an occlusion, this type of error is irrelevant for the purposes of this model. The lower rate of accuracy for the detection of nREM sleep in dog 2 (i.e., 84%) was due to the many periods of transitional sleep
which were associated with body twitches and were misinterpreted by the computer as W or REM.

We encountered the lowest accuracy in the detection of REM sleep (range of 39-72%). However, the accuracy of detection of REM as sleep (i.e., either nREM or REM) ranged from 56 to 93%. As noted earlier, misinterpreting REM sleep as nREM sleep would still result in an occlusion. In some cases (7-44%), REM sleep was wrongly detected as awake by the computer; this error has been reported for other sleep programs and is the result of twitches and body movements that are inherent to REM sleep (VanGelder et al., 1991).

2.2.3f. Long-term Accuracy of Detection

The long-term accuracy of the program during OSA period (2-4 months) was monitored in each of the dogs on a weekly basis. During a consistent 2 hour period each week, we recorded all signals on paper to validate the functioning of the model. The computer judgment was then compared to human judgment. The ratio of %β2/%δ1 remained consistent throughout the OSA period but slight adjustments to the EMG threshold 2 (for separation of REM and W) were required over time to maximize accuracy. The accuracy at the end of the OSA phase is presented to demonstrate long-term accuracy of sleep detection.

The threshold values and the accuracy rates at the end of the OSA phase are presented in table 10 for the 4 dogs. The rates of accuracy were comparable to those seen at the start of the OSA period. The accuracy rate for the detection of wakefulness ranged from 93 to 100%. Although between 0 and 7% of the individual awake epochs were detected by the computer as sleep, none of these epochs occurred consecutively, eliminating the risk of an occlusion during wakefulness.
Figure 14: Box plots of %β2/δ1 and EMG during wakefulness, nREM and REM sleep in dog 1. Data represent measurements during periods of stable wakefulness, nREM, and REM sleep based on human judgment using standard criteria. The boxes represent the median and the upper and lower quartiles, and the circles represent the 5th and 95th percentile points.
Figure 15: Box plots of $\% \beta_2/\% \delta_1$ and EMG during wakefulness, nREM and REM sleep in dog 2. Format is the same as in Figure 14.
Figure 16: Box plots of $\% \beta_2/\% \delta_1$ and EMG during wakefulness, nREM and REM sleep in dog 3. Format is the same as in Figure 14.
Figure 17: Box plots of $\%\beta_2/\%\delta_1$ and EMG during wakefulness, nREM and REM sleep in dog 4. Format is the same as in Figure 14.
Table 9: Rate of Accuracy for detection of wakefulness, nREM and REM at the beginning of the OSA period

<table>
<thead>
<tr>
<th>Computer Judgment</th>
<th>Human Judgment (Reference)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Awake</td>
<td>nREM</td>
<td>REM</td>
</tr>
<tr>
<td><strong>Dog 1</strong></td>
<td>%β2/δ/δ1 = 3.2</td>
<td>EMG (nREM-W) = 40</td>
<td>EMG (REM-W) = 20</td>
</tr>
<tr>
<td>Awake</td>
<td>95%</td>
<td>4%</td>
<td>37%</td>
</tr>
<tr>
<td>nREM</td>
<td>2%</td>
<td>96%</td>
<td>24%</td>
</tr>
<tr>
<td>REM</td>
<td>3%</td>
<td>0%</td>
<td>39%</td>
</tr>
<tr>
<td><strong>Dog 2</strong></td>
<td>%β2/δ/δ1 = 4.4</td>
<td>EMG (nREM-W) = 51</td>
<td>EMG (REM-W) = 25</td>
</tr>
<tr>
<td>Awake</td>
<td>90%</td>
<td>11%</td>
<td>44%</td>
</tr>
<tr>
<td>nREM</td>
<td>7%</td>
<td>84%</td>
<td>16%</td>
</tr>
<tr>
<td>REM</td>
<td>3%</td>
<td>5%</td>
<td>40%</td>
</tr>
<tr>
<td><strong>Dog 3</strong></td>
<td>%β2/δ/δ1 = 2.2</td>
<td>EMG (nREM-W) = 58</td>
<td>EMG (REM-W) = 32</td>
</tr>
<tr>
<td>Awake</td>
<td>97%</td>
<td>9%</td>
<td>7%</td>
</tr>
<tr>
<td>nREM</td>
<td>1%</td>
<td>89%</td>
<td>21%</td>
</tr>
<tr>
<td>REM</td>
<td>2%</td>
<td>2%</td>
<td>72%</td>
</tr>
<tr>
<td><strong>Dog 4</strong></td>
<td>%β2/δ/δ1 = 1.8</td>
<td>EMG (nREM-W) = 24</td>
<td>EMG (REM-W) = 12.6</td>
</tr>
<tr>
<td>Awake</td>
<td>98%</td>
<td>3%</td>
<td>9%</td>
</tr>
<tr>
<td>nREM</td>
<td>2%</td>
<td>96%</td>
<td>19%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>1%</td>
<td>72%</td>
</tr>
</tbody>
</table>

*the human judgment of sleep-wake state was used as a reference or standard. The computer judgment was compared to the human judgment.*

The accuracy of detection of nREM sleep ranged from 89 to 98%, which is similar to the rates observed at the beginning of the OSA period. The accuracy of REM sleep improved at the end of the OSA phase compared to the beginning (range, 83-100%). This variability in the detection of REM is due to the inconsistent nature of this stage of sleep.
Table 10: Rate of Accuracy for detection of wakefulness, nREM and REM at the end of the OSA period

<table>
<thead>
<tr>
<th>Human Judgment</th>
<th>Awake</th>
<th>nREM</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dog 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>100%</td>
<td>2%</td>
<td>17%</td>
</tr>
<tr>
<td>nREM</td>
<td>0%</td>
<td>98%</td>
<td>31%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>0%</td>
<td>52%</td>
</tr>
<tr>
<td><strong>Dog 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>93%</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>nREM</td>
<td>7%</td>
<td>94%</td>
<td>44%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>4%</td>
<td>56%</td>
</tr>
<tr>
<td><strong>Dog 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>99.5%</td>
<td>11%</td>
<td>0%</td>
</tr>
<tr>
<td>nREM</td>
<td>0.5%</td>
<td>61%</td>
<td>12%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>28%</td>
<td>88%</td>
</tr>
<tr>
<td><strong>Dog 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>98%</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>nREM</td>
<td>0%</td>
<td>95%</td>
<td>27%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>1%</td>
<td>67%</td>
</tr>
</tbody>
</table>

2.2.3g. Summary

In summary, the computer program for sleep detection was able to detect sleep from wakefulness consistently over the period of OSA. There were minimal changes in the threshold values over the sleep apnea period. Thus, the algorithm for sleep detection was adequate for producing sleep apneas in the dogs. Specific validation in each dog was required to maximize the accuracy of detection.
2.3. Concluding Remarks

This chapter has described the model hardware and software used to produce the animal model of OSA. This model mimicked the clinical syndrome of OSA by producing repeated episodes of airway occlusions and arousal from sleep. With the biotelemetry and computer software, the animal was freely ambulatory and the system required no human intervention except for routine monitoring. Most importantly, the OSA model functioned independently on a nightly basis for up to 136 days period, and could have functioned longer if required by the protocol, within the limits of the battery life.
Chapter 3: Validation of a Telemetry System for Long-term Measurement of Blood Pressure

3.1. Introduction

Experimental protocols frequently require continuous and accurate measurements of cardiovascular variables, such as blood pressure (BP) and heart rate (HR). In anesthetized animals, the conventional method for direct measurement of BP usually involves the insertion of an exteriorized arterial catheter into a carotid or femoral artery. Although this method provides accurate and stable measurements, the level and type of anesthesia can influence cardiovascular function (Seyde et al., 1985; Chelly et al., 1986; Rogers et al., 1986; Brown et al., 1989). Furthermore, the length of time over which measurements can be made in anesthetized animals is limited. Therefore, the anesthetized preparation is not suitable for long-term studies.

Exteriorized arterial catheters have also been used frequently in studies that require chronic monitoring of BP in conscious laboratory animals. Such a system requires a physical connection between the animal, the pressure sensor, and the recording apparatus. This arrangement introduces the potential for exit site infection, restricts the animal's activity, and limits the duration of unattended monitoring to relatively brief periods of time. In addition, some degree of restraint may be needed during the measurement period, which can introduce stress-related changes in BP and HR (Adams et al., 1988; Chiueh et al., 1978; Kvetnansky et al., 1978). Furthermore, repeated flushing of the catheter with an antithrombogenic solution may be required to maintain catheter patency, which further increases the risk of infection. Although intermittent arterial catheterization avoids many of the problems inherent in long-term
exteriorized catheters, repeated insertions of a catheter pose logistical difficulties (e.g. allowing adequate time between studies for wound healing). Furthermore, intermittent catherization does not allow for continuous uninterrupted measurement of BP over 24 hour periods.

Non-invasive cuff measurements of BP circumvent some of the problems encountered with exteriorized arterial catheters, particularly the risk of exit site infection and the need for repeated arterial punctures. However, the cuff method has a number of limitations in conscious animals (Hassler et al., 1979). Although such measurements correlate with direct measurements obtained from arterial catheters, significant and inconsistent differences between the two methods have been reported (Pettersen et al., 1988). Furthermore, the cuff method requires physical restraint of the animal, and is sensitive to movement (Hassler et al., 1979), thus reducing its value in long-term monitoring. Moreover, the act of inflating the cuff to record BP may be sensed by the animal and affect the measurement (Davies et al., 1994). Obtaining measurements during sleep is particularly difficult with this approach because inflation of the cuff can cause arousal from sleep (Davies et al., 1994). In addition, continuous measurement of BP is not possible with the cuff method; thus, dynamic beat-to-beat changes in BP cannot be recorded.

In contrast to the problems encountered with the preceding techniques, radiotelemetry of biological signals potentially allows for long-term continuous monitoring in freely behaving animals, during wakefulness and sleep, 24 hours a day. In particular, with telemetry of hemodynamic signals, the need for exteriorized arterial catheters and for a physical connection between the sensor and the animal is eliminated. Furthermore, telemetry is free of movement artifact, and restriction of the animal's activity or behavior is not required. Since no part of the
unit is exteriorized, the risk of exit site infection is avoided. Thus, a telemetry system allows measurement of hemodynamic variables over extended periods of time without the need for anesthesia and with minimal disturbance of the animal.

In order to investigate the long-term cardiovascular consequences of this experimentally-induced sleep apnea, continuous and accurate measurements of BP are required over a period of several months. Given the preceding considerations, we used an implanted telemetry system for this purpose. This chapter describes the application and validation of the radiotelemetry system which is designed for continuous, long-term measurements of BP and HR. Although the use of telemetry for cardiovascular measurements has been reported previously (Appleby et al., 1990; Armentano et al., 1990; Brockway et al., 1991; DePasquale et al., 1994; Guiol et al., 1992; Krulan et al., 1992; Sadoff et al., 1992; Schnell and Wood, 1993), there have been no critical evaluations of this technique over prolonged periods of time.

3.2. Methods

3.2.1. Animal Preparation

The validations reported in this chapter are from three adult female dogs (23-31 kg). The dogs initially underwent surgery to implant a three channel telemetry unit (TLM11M3D70-CCP, Data Sciences, St Paul, MN) for monitoring of arterial blood pressure, EEG and EMG from the dorsal neck muscles. Surgery was performed under general anesthesia and aseptic conditions as described in chapter 2. Before surgery, the dogs were pre-medicated with atropine (0.02-0.05 mg/kg I.M.) and anesthesia was induced with a short-acting barbiturate (thiamyl sodium, 10-20 mg/kg I.V.). Anesthesia was maintained with halothane (titrated to effect, typically 0.5-2%). The EEG and EMG electrodes were implanted as described in chapter 2, and
the electrode wires were tunneled to a subcutaneous pouch located in the lower abdomen, where the body of the transmitter was placed. The deep femoral artery was exposed, and the arterial catheter, connected to the pressure sensor in the body of the transmitter, was inserted into this artery and advanced to the external iliac artery (Miller et al., 1964). The tip of the catheter was positioned at the bifurcation of the aorta. Silk ligatures secured the catheter in place in the proximal artery and were used to tie off the distal deep femoral artery. Other remaining collateral vessels provided blood supply to the leg. The site was irrigated with an antibiotic solution (ampicillin sodium, 125mg/ml) before closure in two layers. Penicillin (15,000-20,000 units/kg, I.M.) and buprenorphine (0.01-0.02 mg/kg I.M.) were administered post-operatively.

3.2.2. Overview of the telemetry system

The electronics of the telemetry unit for the measurement of BP and of the data acquisition system have been described previously (Guiol et al., 1992). A schematic of the system for transmission of signals in our study is shown in Figure 18. For the measurement of BP, the radio-frequency signals emitted by the telemetry unit were detected by receivers, processed by a multiplexer, sent to a computer (IBM compatible 486/33MHz, 16MB RAM) and sampled by a data acquisition system (Dataquest IV, Data Sciences, St. Paul, MN) operating in an OS/2 (IBM) environment. Since the pressure implant sensed absolute pressure (i.e. relative to vacuum), an electronic barometer (C11PR, Data Sciences, St.Paul, MN) was incorporated into the system to correct any changes in barometric pressure. Analog signals of BP for display on chart, oscilloscope and tape recorder were also obtained following conversion of the digital signals (DL10, Data Sciences, St. Paul, MN).
Figure 18: Schematic of system for the measurement of arterial blood pressure by telemetry. Solid lines indicate direct connections; dotted lines indicate telemetered connections.
With the use of this telemetry system, the animal was freely ambulatory. An antithrombogenic substance was applied by the manufacturers to the exterior surface of the fluid-filled catheter. Because of the antithrombogenic properties of the catheter tip, the dog did not require anticoagulation. The telemetry unit could be turned on and off using a magnetically activated switch. The nominal battery life of the implants was 3.5 months of continuous use. This battery life was extended to as long as 17 months in our study by switching the transmitter off when not in use.

3.2.3. System Validation

Validation of the telemetry system prior to implantation was not possible because the tip of the arterial pressure catheter was covered by a protective plastic cap (which was removed at surgery) to preserve its antithrombogenic properties. However, the system was pre-calibrated by the manufacturer. We did not sacrifice any telemetry units to verify the calibrations by the manufacturers because we were specifically examining in-vivo validation, and the cost of the implanted catheters was prohibitive ($1300 US). Nevertheless, prior to implantation, we verified that the measured pressure was correctly registered as 0 mm Hg, relative to atmosphere. Testing of the EEG and EMG channels was performed with an EEG calibrator, as described in chapter 2.

The implanted arterial catheter was tested for accuracy and stability of BP recordings in each dog beginning 4 weeks following implantation. Once the animal had fully recovered from the surgical procedure, we arbitrarily chose to validate the implanted system on a monthly basis, with less frequent validations in the later part of the study. For this purpose, the telemetered BP
signal was compared to a signal recorded simultaneously from an acutely inserted manometer-tipped arterial catheter (Model MPC-500, Millar Instruments, Houston, Texas). This exteriorized catheter was calibrated with a mercury manometer and was inserted into the contralateral femoral artery via an introducer (Model HIA-338, Ingeion Corporation, Minneapolis, MN), with the dog under very light halothane anesthesia. The catheter tip was advanced the same distance into the external iliac artery as the tip of the implanted catheter. The position of the dog was adjusted so that both catheters were at the level of the heart. The dog was then subjected to a series of maneuvers to alter BP, including intravenous injections of phenylephrine (20-120 mg) and sodium nitroprusside (25-125 mg), airway occlusion, and mechanical lung inflation. As a result of these interventions, a range of BP values was obtained from 40 to 200 mm Hg. The data acquisition software was configured to sample the BP signal at 355 Hz. Values of BP from the implanted system were adjusted for barometric pressure. Signal detection and feature extraction could be performed on-line. However, the data were also stored on the hard drive for later retrieval and detailed analysis.

For validation of the telemetry system, the beat-by-beat systolic and diastolic pressures were measured simultaneously through the exteriorized and the chronically implanted catheters, and were recorded on computer and chart recorder. Over 200 values of BP obtained from the two systems were compared on each experimental day. In addition, values of mean systolic, mean diastolic and mean arterial BP and of HR from the implanted system were calculated by hand and compared to the computer-derived values.
3.2.4. **Data Analysis**

The beat-by-beat data obtained from each catheter during periods of stable BP and during transient changes in BP were compared for each experimental day by least squares linear regression analysis. The slopes of the regression lines were compared to the line of identity to examine the agreement between the two methods of BP measurement.

To determine the offset in the values of BP measured by the implanted catheter, the mean ± standard error (S.E.) of all the differences between the values of BP obtained with the two systems (BPexteriorized - BPimplanted) were calculated for each experimental day and were compared to zero by t-test. The differences were also normalized [(BPexteriorized - BPimplanted)/BPexteriorized]. The drift (mm Hg per week of implantation) was calculated by dividing the absolute mean difference in BP values by the number of weeks between the two consecutive measurements.

The accuracy of the computer-derived values of BP and HR was determined by paired t-test analysis, comparing the computer-derived with the hand-calculated values from the chart record. Two comparisons were performed in each dog, one during a stable period and one during transient changes in BP, to ensure that the computer-derived values were accurate under both steady state and dynamic conditions.

3.3. **Results**

Figure 19 shows a representative tracing of the BP signals in one dog recorded simultaneously with the chronically implanted catheter and the acutely-inserted exteriorized catheter. Similar tracings were obtained in the other dogs. The waveform of the BP signal obtained from the implanted catheter at 5 and 75 weeks after implantation was virtually
identical to the signal obtained from the exteriorized catheter. In addition, BP recordings from the two catheters showed very similar responses to dynamic changes in BP. However, although the absolute values of BP obtained by the two systems were similar at 5 weeks following implantation, a significant offset in BP values obtained from the implanted catheter was evident at 75 weeks.

Linear regression analyses of BP values recorded in the three dogs with the chronically implanted catheter and the exteriorized catheter are shown in Figure 20. Each regression line was derived from at least 200 values of BP recorded simultaneously by the two systems. A very high correlation was found between the two measurements (all p values ≤0.0001, all r ≥ 0.966, table 11). For most of the regression analyses, the slope of the line was statistically different from one (table 11), although many of the differences between the lines of regression and the line of identity were very small and of little biological relevance.

The offset in the BP values obtained by telemetry was determined by calculating the differences in BP between the values obtained by the two systems (table 11). The mean differences were all different from zero (p<0.001) and were typically positive, indicating that the implanted catheter underestimated the true value of BP. Figure 21 shows the normalized differences as a function of time following implantation of the telemetry system. Each data point represents measurements during periods of stable BP readings. As demonstrated, the normalized differences of blood pressure measured by the two systems were not consistent over time. The absolute weekly drift measured by telemetry varied from 0.08 to 3.31 mm Hg/week (table 12).
Figure 19: Recorder tracings in one dog of blood pressure (BP) obtained simultaneously by the implanted telemetry system and through an acutely-inserted exteriorized catheter at 5 weeks (panel A) and 75 weeks (panel B) after implantation of the telemetry system. Blood pressure was decreased transiently by lung inflation.
Figure 20: Relationship between blood pressure (BP) values measured with the exteriorized and implanted systems. Each solid line represents the linear regression of over 200 values of BP recorded on a single experimental day. The dotted line is the line of identity.
Figure 21: Mean (± S.E.) of the normalized differences [(BP_{exteriorized} - BP_{implanted})/BP_{exteriorized}] between the values of blood pressure (BP) obtained via the implanted and exteriorized catheters as a function of time following implantation of the telemetry system. Data represents measurements during periods of stable BP. The dotted line indicates zero percent normalized difference between the two measurements. The standard error bars are hidden within the symbols.
Figure 22: Comparison of the computer-derived and hand-calculated values of mean systolic, mean diastolic, and mean arterial BPs (MABP) and of heart rate obtained via telemetry system. Each graph contains 2 values from each dog, one during steady-state conditions (solid symbols) and one during dynamic changes in BP (open symbols.)
TABLE 11. Relationship between arterial blood pressure measure by the implanted telemetry system and through an acutely-inserted exteriorized arterial catheter.

<table>
<thead>
<tr>
<th>Dog 1</th>
<th></th>
<th>Dog 2</th>
<th></th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (wks)</td>
<td>Slope (S.E.)</td>
<td>r</td>
<td>Mean difference (S.E.) (mm Hg)</td>
<td>Time (wks)</td>
</tr>
<tr>
<td>5</td>
<td>1.05 (0.0019)</td>
<td>0.999</td>
<td>+0.8 (0.10)</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>0.88 (0.0072)</td>
<td>0.982</td>
<td>+5.5 (0.34)</td>
<td>12</td>
</tr>
<tr>
<td>17</td>
<td>1.01* (0.0058)</td>
<td>0.995</td>
<td>+9.2 (0.21)</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>1.15 (0.0039)</td>
<td>0.999</td>
<td>-1.5 (0.25)</td>
<td>19</td>
</tr>
<tr>
<td>25</td>
<td>1.01 (0.0028)</td>
<td>0.999</td>
<td>+3.3 (0.11)</td>
<td>23</td>
</tr>
<tr>
<td>31</td>
<td>1.01 (0.0035)</td>
<td>0.999</td>
<td>+14.5 (0.12)</td>
<td>27</td>
</tr>
<tr>
<td>75</td>
<td>0.98 (0.0031)</td>
<td>0.998</td>
<td>+22.4 (0.09)</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43</td>
</tr>
</tbody>
</table>

Time = time from implantation of the telemetry system; Slope = regression coefficient; r = correlation coefficient; Mean difference = $P_{\text{exteriorized}} - P_{\text{implanted}}$

Values were derived from over 200 measurements on each experimental day. Slope values were statistically different than 1 at a p value ≤ 0.005, except for * (p=0.087), ** (p=0.109), *** (p=0.175).
TABLE 12. Average weekly drift in blood pressure measured by telemetry.

<table>
<thead>
<tr>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong> (weeks)</td>
<td><strong>Drift</strong> (mm Hg/week)</td>
<td><strong>Time</strong> (weeks)</td>
</tr>
<tr>
<td>5 - 12</td>
<td>0.68</td>
<td>8 - 12</td>
</tr>
<tr>
<td>12 - 17</td>
<td>0.74</td>
<td>12 - 15</td>
</tr>
<tr>
<td>17 - 21</td>
<td>2.70</td>
<td>15 - 19</td>
</tr>
<tr>
<td>21 - 25</td>
<td>1.20</td>
<td>19 - 23</td>
</tr>
<tr>
<td>25 - 31</td>
<td>1.90</td>
<td>23 - 27</td>
</tr>
<tr>
<td>31 - 75</td>
<td>0.18</td>
<td>27 - 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31 - 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43 - 47</td>
</tr>
</tbody>
</table>

*Time = time from implantation of the telemetry system. Drift = absolute weekly change in baseline blood pressure between successive validation studies.*

Figure 22 shows the values of mean systolic, mean diastolic and mean arterial BP and of HR, derived by the telemetry data-acquisition program compared to the values calculated from the analog tracing of BP obtained from the implanted catheter. There were no differences between the calculated and computer-derived values (all p values ≥ 0.3).

**3.4. Discussion**

This chapter described the *in-vivo* application and validation of a telemetry system for measurement of blood pressure over several months of implantation. The advantages of this telemetry system are that it provides direct and continuous measurements of BP with minimal disturbance to the animal, without the risk of percutaneous infection or the need for equipment maintenance.
Measurements for a period of 28 to 75 weeks after implantation showed a consistently high correlation between the BP values recorded by telemetry and those obtained by a conventional exteriorized manometer-tipped catheter. The BP profile obtained with the implanted catheter for up to 75 weeks after implantation was virtually identical to the waveform recorded with the exteriorized catheter. The three implants provided reliable blood pressure signals for the duration of the study period (75, 47 and 28 weeks in dogs 1, 2 and 3 respectively). The differences in study duration among the three dogs were not due to failure of the system, but rather related to the dogs being involved in other ongoing studies. The finding of a reliable pressure waveform suggests that no thrombi formed on the tip of the implanted catheter. However, there was a significant and variable offset in the measurement of BP by the implanted catheter, the magnitude of which was dependent on the absolute level of prevailing pressure (Figure 20). Furthermore, the drift in the value of BP from the implanted catheter was not consistent over time, necessitating calibration in order to minimize the magnitude of error of the measurement.

Because the BP values from the implanted catheter were consistently highly correlated with the values obtained by the exteriorized catheter, the telemetry system could be used to accurately measure acute changes in BP. However, for the measurement of long-term changes in BP in response to a physiological stimulus (or in our application, induction of obstructive sleep apnea), the major disadvantage of the telemetry system is the need to determine when a drift in the measurement has occurred (Brockway et al., 1991). The small sample size used in this study and the variation in offset among the three systems tested does not allow prediction of the offset at a given point in time following implantation. The sample size in this study was
limited by the cost of the implants and dogs and the long-term nature of the study. Nevertheless, the magnitude and variation in the offset observed among the three implants indicates that the system requires calibration at a frequency that is appropriate to the particular study, with the interval between calibrations being dependent on the magnitude of any expected changes in BP over time.

Several previous studies have validated telemetry systems similar to that used in our investigation (Appleby et al., 1990; Armentano et al., 1990; Brockway et al., 1991; DePasquale et al., 1994; Guiol et al., 1992; Krulan et al., 1992; Sadoff et al., 1992; Schnell and Wood, 1993), but none has reported in vivo validation for the length of time described in this study. A number of studies that described the accuracy of the telemetry system shortly after implantation (less than 3 weeks) reported offsets in BP ranging from -5 mm to 2 mm Hg (Appleby et al., 1990; Armentano et al., 1990; DePasquale et al., 1994; Krulan et al., 1992). Similarly, the manufacturer specifies a maximum offset drift of 4 mm Hg in the first two weeks of implantation, and 5 mm Hg per month thereafter. We did not validate the telemetry unit intraoperatively, nor within the first 3 weeks of implantation since we were interested in long-term validation in conscious animals after full recovery from the surgical procedure. However, the first validation in each dog (at 4-8 weeks) showed offsets that were similar to those noted above (0.75, 3.8 and 5.1 mm Hg in dogs 1, 2 and 3 respectively). An earlier validation in our study may have shown a smaller difference between the BP values measured by the two catheters, in agreement with the findings of the short-term studies and the manufacturer's specifications.
In terms of long-term validation, two studies have examined the accuracy of the telemetry system in rats up to 12 weeks following implantation (Brockway et al., 1991; Guiol et al., 1992). In one study, the accuracy was found to be ±5 mm Hg in 85% of the rats at 3 and 8 weeks following implantation, and in 78% of the rats at 12 weeks (Brockway et al., 1991). In contrast, in our study, the implanted system offset was greater than 5 mm Hg in all three dogs at 12 weeks after implantation. However, the study in rats did not document the variability of the offset in BP nor the range of BP values over which the measurements were made, which may account for the differences between our findings and those of the earlier studies.

In another study in unrestrained rats, Guiol and colleagues (1992) compared BP responses to pharmacological stimuli measured by telemetry at 9 weeks after implantation to the responses obtained by direct arterial catheterization. They found a strong linear correlation between the two measurements. The mean difference between the two techniques ranged from -2.1 to +6.9%, and the slope of the regression was 1.12 (r=0.973), similar to the findings of our study. However, it should be noted that these authors did not simultaneously record BP by telemetry and conventional methods in the same animals, but rather used two different groups of rats for comparison.

We are aware of only one study that examined the offset of values measured by the implanted catheter for a period greater than 6 months. In marmosets, Schnell and Wood (1993) surgically removed the telemetry units from their animals following 6 to 11 months of implantation, and found that 13 of the 50 sensors had an offset of >5 mm Hg. Despite the drift, the BP measured by telemetry was highly correlated with the pressure measured by manometer ex-vivo (r=0.999). It is difficult to compare the results of these ex-vivo single point validations
to our *in-vivo* findings since we have not removed the units from our dogs because of their involvement in ongoing studies. Furthermore, the relationship between *ex-vivo* static measurements and *in-vivo* dynamic measurements at body temperature has not been established.

We conclude that this telemetry system for hemodynamic monitoring is particularly useful for studies requiring unattended, continuous measurement of blood pressure, and in studies in which the results could be affected by stress artifacts associated with restraint or handling of the animal. The results of the present study demonstrate that BP measured with this telemetry system for a period of 28 to 75 weeks following implantation is highly correlated with BP measured with a conventional exteriorized catheter. Hence the telemetry system can detect acute changes in BP with a high degree of accuracy. The system can also measure blood pressure over extended periods of time (weeks to months) without the need for antithrombogenic therapy or maintenance. However, there is a drift over time in BP values obtained with this device, necessitating validation of the measurements at intervals appropriate for the particular study. Finally, validation should be performed over the range of pressure values expected to be encountered by the system given that the offset is dependent on the absolute level of BP.
Chapter 4: Development and Validation of the Model of Sleep Fragmentation without Airway Occlusion

4.1. Introduction

Patients with OSA can have as many as 400 to 500 brief arousals each night, resulting in marked changes in overall sleep architecture. This fragmentation of sleep is believed to play an important role in causing the excessive daytime sleepiness associated with OSA and in contributing to the increased risk of motor-vehicle accidents in this patient population (Findley et al, 1988). Sleep fragmentation may also lead to adverse psychosocial consequences (Kales et al. 1985). Furthermore, recurrent arousals from sleep may contribute to the development of systemic hypertension in OSA since graded arousals from sleep cause acute increases in BP that are dependent on the degree of arousal (Davies et al, 1988).

The overall purpose of this research project was to examine the effect of OSA on BP in a canine model of this disorder. A number of stimuli, including acute asphyxia, repeated respiratory efforts against a closed airway, and arousal from sleep may play a role in the pathogenesis of hypertension in OSA. To determine the effect of this latter stimulus, we produced a model of sleep fragmentation, without airway occlusion. We postulated that sleep fragmentation did not account for all the increase in BP associated with OSA. At least 6 months after the completion of the OSA protocol, all four dogs were re-studied using the model of sleep fragmentation. For this purpose, we again used the telemetry system and computer algorithm to detect sleep from the implanted EEG and EMG electrodes. Once a period of sleep of pre-determined length was identified by the computer, it sent a signal to activate an acoustic alarm. When the dog aroused from sleep, the alarm ceased. Thus, the repeated episodes of
arousal from sleep produced fragmentation of sleep without the changes in blood gases and intrathoracic pressure associated with obstructive apneas.

Similar to OSA, the induced model of sleep fragmentation functioned entirely independently and required minimal human monitoring except for routine maintenance. Furthermore, there were no lead attachments to the animal allowing it to move freely in the pen. Finally, the system for sleep fragmentation was operational on a nightly basis for up to 52 days, allowing us to examine the long-term consequences of sleep fragmentation and to compare them to the long-term consequences of OSA.

The purpose of this chapter is to describe this induced model of acoustic sleep fragmentation, without airway occlusion. The components of the system that differed from the OSA model will be highlighted. The details of the surgical preparation of the animal, the model hardware and the validation of the sleep analysis software will be described.

4.2. Methods

4.2.1. Animal Preparation

Studies were performed on the same 4 dogs (3 female, 1 male) that were used in the OSA protocol. All surgical and experimental procedures were approved by the Animal Care Committee of the University of Toronto. All dogs had been prepared with a permanent side-hole tracheostomy and an implanted telemetry unit (TLM11M3D70-CCP, Data Sciences, St Paul, MN). The telemetry unit consisted of three channels to record arterial blood pressure, EEG and EMG. The nominal life of the implant was 3.5 months of continuous use. The telemetry unit could be turned on and off using a magnetically activated switch to extend its life. At the completion of the OSA protocol, three of the dogs had functional telemetry systems.
However, in one dog, the life of the battery had expired and surgery was required to replace the telemetry implant.

4.2.1.a. Surgery for Replacement of Telemetry Implant

This surgery was performed aseptically under general anesthesia two months before the start of the studies. Prior to surgery, the dog was pre-medicated with atropine (0.02-0.05 mg/kg i.m.). Induction of anesthesia was achieved with a short-acting barbiturate (thiamyl sodium, 10-20 mg/kg i.v.) and maintained with halothane (titrated to effect; typically 0.5-2%).

The subcutaneous pouch that housed the ‘old’ implant was localized. The ‘old’ implant was exposed and a length of the wires was cleared. The 5 wires (2 EEG, 2 EMG and 1 ground) were cut and crimped to the corresponding 5 wires of the new telemetry unit. The new implant was stabilized in the pouch with silk sutures.

Since the BP catheter was gel-filled, it was not possible to connect the new BP catheter to the ‘old’ catheter. For this reason, the ‘old’ catheter had to be removed from the artery and replaced by the new one. We therefore dissected and exposed clearly the vessel in which the ‘old’ catheter had been inserted. The ‘old’ catheter was removed and the new catheter was slipped into the same artery. Silk sutures were placed around the artery and catheter to secure it.

Post-operatively, long-acting penicillin (15,000-20,000 units/kg, i.m.), suitable analgesics (buprenorphine, 0.01-0.02 mg/kg, i.m.) and anti-coagulant therapy (Heparin, 2,000 USP, i.v.) were administered (Canadian Council on Animal Care, 1984).

4.2.2. Model Hardware

Sleep fragmentation without airway occlusion was produced in the dog using the model illustrated in figure 23. Briefly, sleep-wake state was continuously monitored overnight by a
Figure 23: Schematic diagram of the sleep fragmentation model in the dog. Each dog had an implanted telemetry system with three channels (EEG, EMG and BP). Signals were sent by telemetry to two computers, one for the detection of sleep-wake state, and the other for analysis of hemodynamic data. Dashed lines represent telemetered connections.
computer that received telemetered EEG and EMG signals from the chronically implanted electrodes. Whenever the computer detected sleep, it sent a signal to activate an alarm that generated a sound. The frequency of the sound increased progressively until the dog aroused from sleep, when the alarm ceased. Another computer received and stored cardiovascular variables (BP and HR) from the dog.

The signals from the dog were detected by the receivers, transmitted through the multiplexer, amplifiers and filters to the analog-to-digital board of a personal computer as described in chapter 2. The EEG and EMG signals were processed by the computer which utilized the frequencies in the EEG and the magnitude of the EMG to make a judgment of sleep-wake state, using the algorithm described earlier. Once a period of sleep of predetermined length was detected, the computer sent a signal (4.8 V, square wave) via a digital-to-analog converter to the alarm, generating a sound. When arousal occurred, the signal from the computer to the alarm terminated (i.e. returned to 0 volts), resulting in cessation of the sound. In order to maintain the experimental conditions the same as OSA, the dogs wore a jacket and breathed through an endotracheal tube at similar cuff pressure as in OSA.

*Alarm Properties*

The alarm was custom-built to produce sounds at frequencies ranging from 17 to 36 kHz, using a power amplifier and a microphone (see attached detailed schematic in Appendix 3). Once the alarm received the 5 Volt signal from computer, a sound was produced at a frequency of 17 kHz which is inaudible for most humans but within the canine hearing range. If the dog did not arouse from sleep immediately, the alarm frequency (but not intensity) progressively increased to 36 kHz at a variable ramp of between 10 to 30 seconds. A variable
ramp was used to minimize acclimatization by the dog. The alarm remained at 36 kHz until the dog aroused from sleep, or for a maximum of 18 epochs (i.e. 108 seconds).

Prior to initiation of the protocol, the dogs were assessed to ensure that they were able to hear the frequencies of the sound generated by the alarm. For this purpose, the dogs were connected to the standard laboratory equipment. The data were collected on several days and human scoring of sleep-wake state was performed according to the standard EEG criteria by an experienced technician (Phillipson et al., 1976). Once sleep was established, the alarm was triggered at a frequency of 17 kHz and progressively increased. In 3 out of the 4 dogs, we confirmed that the animals consistently and repeatedly aroused from sleep at sound frequencies between 17 and 36 kHz. A representative trace of the responses is shown in figure 24.

In contrast, dog 4 did not arouse from sleep at sound frequencies in the above range. Prior to testing, we had suspected hearing impairment in this dog, based on her behavior. We therefore modified the alarm to produce sounds ranging from 1-20 kHz to test this animal’s hearing range. On different occasions, once sleep was established, the alarm was triggered at frequencies within this range. We established that this animal could only hear frequencies of less than 12 kHz. Therefore, to produce sleep fragmentation in this dog, we used a modified alarm that generated frequencies between 4 and 12 kHz.

To ensure that the animals continued to arouse to the sound produced by the alarm, we reviewed, on a daily basis, the computer records from the previous night, from 1930 hrs (lights off) until 0730 hrs (lights on), and calculated the arousal index, time to arousal, total hours of sleep and total number of arousals overnight. The arousal indices in each dog during the sleep fragmentation period are shown in figure 25.
Figure 24: Representative recorder tracing in one dog demonstrating the response to the acoustic alarm. Activation of the alarm resulted in an immediate arousal from sleep which was associated with an increase in heart rate (HR) and blood pressure (BP).
Figure 25: Changes with time in the apnea and arousal indexes. The filled squares represent the apnea index during OSA, and the open circles represent the arousal index during sleep fragmentation. Due to the limited battery life of the implant, the sleep fragmentation protocol was shorter than the OSA protocol.
4.2.3. Sleep Analysis Software

The method for EEG and EMG analysis, the algorithm for sleep detection, and the method for establishing the thresholds and validating the algorithm were the same as for the OSA model (see chapter 2). The accuracy of the algorithm for sleep detection over the period of sleep fragmentation model is outlined below.

4.2.3a. Arousal Index

The arousal index was defined as the number of arousals in each hour of sleep. The arousal index could be altered in the sleep detection program by changing the number of consecutive epochs of sleep required to generate the signal to activate the alarm. For example, if a long period of sleep was required before the computer sent the signal to activate the alarm, the frequency of the arousals would be low, resulting in a low arousal index. The minimum number of epochs of sleep required to generate the signal was three (i.e. 18 seconds) to ensure that sleep was established, before activation of the alarm. The arousal index was increased progressively from 10-30 events per hour of sleep on nights 1-7, to 50-60 events per hour of sleep after 14 nights. In each of the dogs studied, the arousal index was similar during sleep fragmentation to the apnea index during the OSA period (figure 25).

4.2.3b. Accuracy of Detection

Three thresholds were individually determined in each of the dogs: threshold for $\%\beta_2/\%\delta_1$; EMG threshold 1 to distinguish nREM from movement artifact; and EMG threshold 2 to distinguish REM from wakefulness. Once the animal was surgically prepared, the ratio of $\%\beta_2$ to $\%\delta_1$ and the amplitude of the averaged EMG signal were determined for over 3000 epochs of wakefulness, nREM and REM sleep based on human judgment of sleep-wake state.
The data were collected on several days and the human scoring was performed according to the standard EEG criteria (Phillipson et al., 1976). The mean and standard deviation for %β2/δ1 and the amplitude of the averaged EMG were calculated for each state. A box plot was used to represent the distribution of the data. The same guidelines as described in chapter 2 were used to choose the appropriate thresholds.

The thresholds for %β2/δ1 and EMG values during sleep fragmentation were generally higher than the threshold values during OSA, possibly due to a lower signal-to-noise ratio. We postulate that over time, the resin cement that had been placed on top of the EEG electrodes and screws to minimize contamination of the signal by the overlying layer of muscles, may have degraded, resulting in increased noise on the EEG signal and a higher %β2/δ1 threshold. Scar tissue formation may have caused the change in the EMG threshold. Nevertheless, with proper selection of thresholds for %β2/δ1 and EMG, the accuracy of detection of wakefulness versus sleep generally exceeded 85%.

The box plots representing the distribution of the %β2/δ1 and EMG values in wakefulness, nREM and REM sleep in each dog are shown in Figures 26 to 29. The best discrimination of sleep-wake state was found with %β2/δ1 threshold ranging from 2.8 to 5.4, EMG thresholds (for separation of W versus nREM sleep) ranging from 44 to 60, and EMG threshold (for separation of REM versus W) ranging from 30 to 55 (table 13).

The accuracy of detection of wakefulness for individual epochs ranged from 85 to 89% in the 4 dogs (table 13). Although 11-15% of the individual awake epochs were detected by the computer as sleep, none of these epochs occurred consecutively. Since three consecutive
epochs of sleep were required to activate the alarm, the possibility of alarm activation during wakefulness was eliminated.

The accuracy of detection of nREM sleep ranged from 72 to 93% in the 4 dogs. However, the accuracy of detection of nREM as sleep (i.e., either nREM or REM) ranged from 90 to 95%. Since misinterpreting nREM sleep as REM sleep would still result in activation of the alarm, this type of error is irrelevant for the purposes of this model.

Table 13: Accuracy rates for the detection of wakefulness, nREM and REM sleep at the beginning of the sleep fragmentation period

<table>
<thead>
<tr>
<th>Human Judgment</th>
<th>Awake</th>
<th>nREM</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dog 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>87%</td>
<td>10%</td>
<td>14%</td>
</tr>
<tr>
<td>nREM</td>
<td>8%</td>
<td>72%</td>
<td>43%</td>
</tr>
<tr>
<td>REM</td>
<td>5%</td>
<td>18%</td>
<td>43%</td>
</tr>
<tr>
<td><strong>Dog 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>85%</td>
<td>5%</td>
<td>35%</td>
</tr>
<tr>
<td>nREM</td>
<td>8%</td>
<td>90%</td>
<td>20%</td>
</tr>
<tr>
<td>REM</td>
<td>7%</td>
<td>5%</td>
<td>45%</td>
</tr>
<tr>
<td><strong>Dog 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>85%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>nREM</td>
<td>8%</td>
<td>72%</td>
<td>0%</td>
</tr>
<tr>
<td>REM</td>
<td>7%</td>
<td>21%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Dog 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>89%</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>nREM</td>
<td>11%</td>
<td>93%</td>
<td>42%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>1%</td>
<td>58%</td>
</tr>
</tbody>
</table>
Figure 26: Box plots of \( \%\beta / \%\delta \) and EMG during wakefulness, nREM and REM sleep in dog 1. Format is the same as in Figure 14.
Figure 27: Box plots of $\%\beta_2/\%\delta_1$ and EMG during wakefulness, nREM and REM sleep in dog 2. Format is the same as in Figure 14.
Figure 28: Box plots of $\%\beta_2/\%\delta_1$ and EMG during wakefulness, nREM and REM sleep in dog 3. Format is the same as in Figure 14.
Figure 29: Box plots of %\(\beta_2/\%\delta_1\) and EMG during wakefulness, nREM and REM sleep in dog 4. Format is the same as in Figure 14.
The accuracy of detection of REM sleep was variable among dogs and ranged from 43 to 100%. However, the accuracy of detection of REM as sleep (i.e., either nREM or REM) ranged from 65 to 100%. As noted earlier, misinterpreting REM sleep as nREM sleep would still result in activation of the alarm. In some cases (0-35%), REM sleep was wrongly detected as awake by the computer due to body twitches and movements that are inherent to REM sleep.

4.2.3c. Long-term Accuracy of Detection

The accuracy of the sleep detection program during the period of sleep fragmentation was monitored in each of the dogs, on a weekly basis. During a consistent 2 hour period each week, we recorded all signals on paper to validate the functioning of the sleep detection program. Human judgment was compared to computer judgment. Throughout the sleep fragmentation period, the threshold forREM/REM remained consistent but slight adjustments to the EMG threshold were required over time to maximize accuracy. To demonstrate long-term accuracy of sleep detection, the rates of accuracy at the end of the period of sleep fragmentation are presented in table 14.

The threshold values and the accuracy rates at the end of the sleep fragmentation phase are presented in table 14 for the 4 dogs. The rates of accuracy were comparable to those seen at the start of the sleep fragmentation period. The accuracy rate for the detection of wakefulness ranged from 85 to 98%. Although between 2 and 15% of the individual awake epochs were detected by the computer as sleep, none of these epochs occurred consecutively, eliminating the risk of triggering the alarm during wakefulness.

The accuracy of detection of nREM sleep ranged from 75 to 99%, which is similar to the rates observed at the beginning of the sleep fragmentation period. The accuracy of detection
of REM sleep deteriorated at the end of the sleep fragmentation period (range, 0–69%). However, the rate of detection of REM as sleep ranged from 60 to 100%. This variability in the detection of REM is due to the inconsistent nature of this stage of sleep.

Table 14: Accuracy rates for the detection of wakefulness, nREM and REM sleep at the end of the sleep fragmentation period

<table>
<thead>
<tr>
<th>Human Judgment</th>
<th>Awake</th>
<th>nREM</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dog 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>85%</td>
<td>21%</td>
<td>22%</td>
</tr>
<tr>
<td>nREM</td>
<td>13%</td>
<td>75%</td>
<td>23%</td>
</tr>
<tr>
<td>REM</td>
<td>2%</td>
<td>4%</td>
<td>56%</td>
</tr>
<tr>
<td><strong>Dog 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>98%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>nREM</td>
<td>2%</td>
<td>86%</td>
<td>31%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>9%</td>
<td>69%</td>
</tr>
<tr>
<td><strong>Dog 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>87%</td>
<td>1%</td>
<td>40%</td>
</tr>
<tr>
<td>nREM</td>
<td>13%</td>
<td>99%</td>
<td>60%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Dog 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>87%</td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>nREM</td>
<td>13%</td>
<td>83%</td>
<td>40%</td>
</tr>
<tr>
<td>REM</td>
<td>0%</td>
<td>16%</td>
<td>57%</td>
</tr>
</tbody>
</table>

4.2.3d. Summary

In summary, the computer program for sleep detection was consistently able to distinguish sleep and wakefulness over the period of the sleep fragmentation protocol with
minimal changes in the threshold values. Thus, the algorithm for sleep detection was adequate for producing sleep fragmentation in the dogs. Specific validation in each dog was required to maximize the accuracy of detection.

4.3. Concluding Remarks

This chapter has described the hardware and software used to produce the animal model of sleep fragmentation without airway obstruction. Each dog was studied using this model during a control period before (for 30-60 days), during (for 44-52 days) and after cessation (for 14-40 days) of sleep fragmentation. To allow comparison of the results with those obtained during OSA, the experimental conditions remained the same as during the OSA protocol. The diet and daily routine of the dogs remained constant during all phases of the study, and there was no change in their weight during either protocol (p=0.6). Thus, the model of sleep fragmentation without airway obstruction allowed us to determine the role of recurrent arousal from sleep in the pathogenesis of hypertension.
Chapter 5: Apparatus and Methods Common to Chapters 6-8

5.1. Introduction

The studies described in Chapters 6-8 rely on measurements of respiratory and cardiovascular variables. The different apparatuses used to make these physiological measurements during the different phases of the study (i.e. control, OSA, sleep fragmentation and recovery periods) were validated. Other methods that were specifically applied in one or more experiments are described in the particular chapter describing these experiments, under the Methods section. Properties of the apparatus used to produce OSA and sleep fragmentation are described in chapters 2-4.

Measurements of respiratory and cardiovascular variables were made at standardized laboratory temperature (20-24 °C), while the animal lay in the lateral position, either during wakefulness or sleep.

5.2. Methods

5.2.1. Respiratory Monitoring

During studies of the responses to airway occlusion (Chapter 6), and to progressive hypoxia and hypercapnea (Chapter 8), as well as during measurements of daytime BP (Chapter 7), several respiratory variables were recorded on paper on an eight channel recorder (Beckman R612) at a speed of 5 mm/sec. These variables included: rate of airflow, tidal volume, airway pressure, arterial oxygen saturation, and airway CO₂ concentrations. All signals were also recorded on magnetic tape (3968A tape recorder, Hewlett Packard) for later playback and analysis. Measurements were made during quiet wakefulness, non-REM and REM sleep as judged from behavioral and EEG criteria (Phillipson et al., 1976).
All dogs had a permanent side-hole tracheostomy and, during the studies they breathed through a cuffed endotracheal tube (10 mm internal diameter) inserted through the tracheostomy. Respiratory flow was derived from a pneumotachograph (Fleish No. 2) with a differential pressure transducer (Validyne, MP45-871). This signal was integrated electronically (Beckman, 9873B resetting integrator coupler) to obtain a volume signal. The airflow signal was also fed to a microcomputer via a 12 bit analog-to-digital converter (sampling interval of 10 msec). The computer calculated inspiratory time ($T_i$) and expiratory time ($T_e$) of each breath by searching for a zero-crossing of the airflow signal, and tidal volume by integrating the airflow signal during inspiration. Tracheal gas was sampled continuously through a vapor permeable line to an infrared CO$_2$ analyzer (LB-2, Beckman Instrument Inc.) for measurement of airway CO$_2$ concentration. This instrument was calibrated daily with several precision gases. For studies of the acute responses of the dogs to airway occlusion, airway pressures were recorded as a measure of the magnitude of inspiratory effort made by the dogs (Statham P23Db transducer, Gould Inc.). Arterial O$_2$ saturation was monitored with an ear oximeter (Model 47201A, Hewlett-Packard) that was calibrated with internal standards.

Since all data were displayed on the chart recorder (Beckman, R612), the ability of this system to record the various signals was examined. Three properties were considered: the voltage linearity, the frequency response of the pen, and the response time. The results of these validations are presented in Appendix 4.

When measurement of airflow was required, the end of the endotracheal tube was attached to a pneumotachograph (Fleish No. 2), and a differential pressure transducer (Validyne MP45-871) was used to measure airflow. The pneumotachograph consisted of a
hydraulic resistance head with a fine mesh screen. The flow through the mesh was laminar and behaved according to Poiseuille's law, which states that the pressure differential across the mesh is proportional to the mean flow velocity (Strong, 1970). A small heating coil that surrounded the pneumotachograph prevented precipitation of water vapor on the mesh. A very low range differential pressure transducer was used to measure the pressure across the mesh in the pneumotachograph. The frequency response, linearity and resonant frequency of this differential pressure transducer allowed accurate measurement of airflow (see Appendix 4).

We detected the degree of hypoxemia during airway occlusion and hypoxic rebreathing by continuous measurement of \( S_aO_2 \) using an ear oximeter (Hewlett Packard 47201A). The ear oximeter was accurate in measuring \( S_aO_2 \) in 3 out of the 4 dogs. In one of the dogs, we were unable to obtain an adequate and stable reading of \( S_aO_2 \) possibly due to the dark color of her skin. The accuracy of the oximeter measurements in the dog has been validated previously against arterial blood samples (Phillipson et al., 1978). These authors found that over the range of 70-98%, the correlation between oximeter and blood \( S_aO_2 \) was 0.98 and the mean difference between the two values was \( 1.1 \pm 0.2\% \) (mean \( \pm \) SE). We repeated this validation in one dog and showed similar findings (Appendix 4).

The changes in airway pressure were measured with a strain gauge pressure transducer (Statham P23Db transducer, Gould Inc.). The properties of this system allowed accurate measurement of airway pressure (Appendix 4).

5.2.2. Cardiovascular Monitoring

During the experiments measuring daytime BP, an electrocardiogram (ECG) was recorded via two platinum coated subdermal needle electrodes (Type E2, Grass Instrument Co.)
placed in the chest wall. In addition to the raw ECG signal, the instantaneous heart rate was also derived (Cardiotachometer Coupler, Beckman, type 9857). This system accurately recorded the instantaneous heart rate as shown in Appendix 4.

Finally, during the studies to validate the implanted blood pressure measurement system, the telemetered values of blood pressure were compared to those obtained from a manometer-tipped catheter (Model MPC-500, Millar Instruments) that was inserted acutely into the femoral artery via an introducer (Model HI6-338, Ingeion Corporation), with the dog under light halothane anesthesia. Since the validity of the implanted system was determined based on values obtained simultaneously from the manometer-tipped catheter (chapter 3), it was important to establish the linearity and response time of the manometer-tipped catheter. The properties of this manometer-tipped catheter were adequate for accurate measurements of physiological BP signals, and for validation of the implanted system, as shown in Appendix 4.
PART III: STUDIES

The induced model of OSA described in chapter 2 can be used to investigate the consequences of OSA. The sleep fragmentation model described in chapter 4 can be applied to determine the effects of sleep disruption, without airway occlusion, on OSA. The focus of this thesis is on the cardiovascular consequences of OSA. Part III details the results of the studies performed using these two canine models.

Although the acute physiologic responses to apnea in patients with OSA have been well documented, the changes in these responses over the course of the disease have not been investigated. Therefore, the purpose of the studies in Chapter 6 was to use a canine model of OSA to examine the long-term effects of sleep apnea on the acute responses to airway occlusion during sleep. The sleep fragmentation model was used to determine the impact of sleep disruption without OSA on the acute responses to airway occlusion.

Although several epidemiological studies have identified OSA as a risk factor for systemic hypertension (chapter 1), a direct etiologic link between the two disorders has not been established definitively. Furthermore, the specific physiological mechanisms underlying the association between OSA and systemic hypertension have not been identified. The purpose of the studies in Chapter 7 was to examine systematically the effects of OSA on daytime and night-time blood pressure (BP). As stated in Part 1, sustained hypertension, in the context of this research project, is defined as elevation in blood pressure that persists during periods of uninterrupted breathing, i.e., in the absence of acute airway occlusions.
Chapter 6: Effect Of Obstructive Sleep Apnea Versus Sleep Fragmentation

On Acute Responses To Airway Occlusion In The Dog

6.1. Introduction

OSA is characterized by repeated episodes of airway occlusion during sleep that are associated with progressive hypoxia, hypercapnea and increasing respiratory effort, resulting in transient arousal from sleep and restoration of upper airway patency (Phillipson, 1988). The recurrent arousals from sleep produced by OSA result in sleep fragmentation and lead to excessive daytime sleepiness (Kribbs et al., 1993; Levine et al., 1987). The obstructive apneas are also associated with transient surges in blood pressure (BP) that may lead to nocturnal hypertension (Ringler et al., 1994).

Although the acute respiratory and cardiovascular responses to airway occlusion during sleep have been described (O'Donnell et al., 1994a; Scharf et al., 1992), it is neither clear whether the acute responses change with time, nor whether such changes are of clinical importance. The lack of relevant long-term data is largely due to the fact that patients with OSA do not come to medical attention until their disorder has been present for a number of years; and to date the only studies of animal models of OSA have been limited to short applications of less than one week (O'Donnell et al., 1994a; Kimoff et al., 1994b) except in the English bulldog (Hendricks et al., 1987) where control (i.e. pre-OSA) data are also difficult to obtain. There is evidence, however, that the sleep disruption associated with OSA affects the responses to acute airway occlusion and may aggravate the disease process. For example, Leiter and colleagues (1985) reported that a single night of sleep deprivation decreases upper airway dilator muscle activity during hypercapnic rebreathing, thereby predisposing to upper airway collapse. Short-
term sleep deprivation for 24 hours has also been shown to decrease ventilatory responses to hypercapnea and hypoxia (Cooper and Phillips, 1982; White et al., 1983), and thereby alter the responses to airway obstruction. In addition, 24 hours of sleeplessness in the dog results in increased apnea duration in response to airway occlusion, and in greater arterial hemoglobin desaturation, independent of upper airway function (O’Donnell et al., 1994a). However, these studies all involved complete sleep deprivation, which may not be applicable to human OSA.

In contrast to sleep deprivation in the dog, short-term sleep fragmentation with acoustic stimuli results in impairment of arousal responses to respiratory stimuli, but no change in hypercapnic or hypoxic ventilatory responses (Bowes et al., 1983). In normal subjects, two consecutive nights of sleep fragmentation with an auditory stimulus also produces no change in the hypercapnic ventilatory response (Espinoza et al., 1991). However, there are no reports in the literature on the effects of long-term sleep fragmentation on the responses to acute airway occlusion.

Thus, in the present study, we utilized the canine model of OSA to characterize the long-term effects of sleep apnea on the acute responses to airway occlusion during sleep, independent of any change in upper airway function. More specifically, we examined the effects of OSA on apnea duration and on the associated degree of arterial hemoglobin desaturation, inspiratory effort, and arterial hypertension. We hypothesized that long-term OSA, independent of any changes in airway collapsibility, would result in progressively decreased arousability and in altered physiological responses to airway occlusion during sleep. To determine whether these changes were primarily the result of the sleep fragmentation
associated with OSA, we also examined in the same dogs, the effects of sleep fragmentation without airway occlusion on the acute responses to airway occlusion.

6.2. Methods

6.2.1. Animal Preparation

All surgical and experimental procedures were approved by the Animal Care Committee of the University of Toronto. Studies were performed on four adult dogs (3 female, 1 male; weight, 23-31 kg; age, 5-12 years) trained to lie quietly and to sleep in the laboratory. The dogs underwent two surgical procedures at least two months before initiation of the study, the first to create a permanent side-hole tracheostomy, and the second to implant a three channel telemetry unit as described in chapters 2 and 3. The EEG and EMG electrodes and BP catheter were implanted as described previously (see chapters 2 and 3).

6.2.2. Induction of OSA

The description and validation of the hardware and software systems for producing OSA have been described (chapter 2).

OSA was produced in each dog over a period of 83-133 days. The severity of the disorder, defined by the number of apneas per hour of sleep (i.e., apnea index), was adjusted by changing the number of consecutive epochs of sleep required to generate the signal to close the occlusion valve. By decreasing the number of epochs, the apnea index was allowed to increase during the initial two weeks, reaching a plateau of 50-60 events per hour of sleep after 14 nights.
6.2.3. Sleep Fragmentation without Airway Occlusion

In a separate protocol, the same four dogs were re-studied at least 6 months after completion of the OSA protocol to determine the effects of sleep fragmentation without airway occlusion on the acute responses to airway occlusion during sleep (chapter 3). For this purpose, we again used the telemetry system and computer algorithm to detect sleep from the implanted EEG and EMG electrodes. Once a period of sleep of pre-determined length was identified by the computer, it sent a signal to activate an acoustic alarm. The frequency of the sound increased progressively along a variable ramp over 10-30 sec, to minimize acclimatization by the animal. When the dog aroused from sleep, the alarm ceased. The repeated episodes of arousal from sleep produced fragmentation of sleep without the changes in blood gases and intrathoracic pressure associated with obstructive apneas. The arousal index (i.e., number of arousals per hour of sleep) was controlled by changing the number of consecutive epochs of sleep required to generate the signal to activate the alarm and was allowed to increase progressively, as was the case for the apnea index in OSA.

6.2.3. Experimental Protocol

Each animal was studied during a control period before induction of OSA (for 30-60 days), during OSA (for 83-133 days) and after cessation of OSA (for 14-40 days). Before and after the period of OSA the dogs were allowed to sleep without airway obstructions. During the period of OSA, the dogs were kept awake during the day with human companionship and were connected to the monitoring-occlusion apparatus at night or whenever they displayed pre-sleep behavior. In each phase of the study (control, OSA and recovery) we systematically examined, every 7-14 days, the acute responses of the dogs to airway occlusion during sleep by measuring
the time to arousal from sleep, and the associated changes in oxygen saturation and BP. These
studies were performed at the same time of day (1000-1200 hr.), at a stable laboratory
temperature.

After completion of the OSA protocol, the dogs were re-studied on the sleep
fragmentation protocol. Each dog was studied before (for 30-60 days), during (for 38-52 days)
and after cessation (for 14 days) of sleep fragmentation. We again examined systematically the
acute responses to airway occlusions during sleep every 7-14 days. The experimental conditions
remained the same as during the OSA protocol. Throughout both protocols the diet and daily
routine of the dogs remained constant, and there was no change in their weight. The studies of
the responses to acute airway occlusion were performed at the same time of day (1000-1200
hr.), at a stable laboratory temperature.

6.2.4. Responses to Acute Airway Occlusion

The daytime studies of the acute responses to airway occlusion were performed in the
laboratory, with the dogs sleeping in the same position for all experiments. Sleep-wake state of
the dog was determined according to EEG and behavioral criteria, as described previously
(Phillipson et al., 1976).

During the experiments, the dogs breathed through a cuffed endotracheal tube (internal
diameter, 10 mm) inserted through the chronic tracheostomy. The endotracheal tube was
attached to a breathing circuit that consisted of a pneumotachograph (Fleish No. 2) and a
manually operated three-way valve. Airflow was measured with the pneumotachograph and
differential pressure transducer (Validyne MP-45). The airflow signal was integrated (Beckman
9873B resetting integrator coupler) to provide tidal volume. Airway pressure was measured
with a transducer (Gould Statham P23Db transducer) that was calibrated against a water manometer. Arterial O$_2$ saturation (S$_a$O$_2$) was measured with an ear oximeter (Hewlett-Packard, no. 47201A) that was calibrated with internal standards and validated in the laboratory using arterial blood gases (Phillipson et al., 1978 and Appendix 4).

Arterial BP was measured with the implanted catheter and telemetry system that we have validated (see details in Chapter 3). The implanted catheter was calibrated periodically (generally every 4 weeks) by comparing the BP values from the telemetry system to those measured with a manometer-tipped catheter inserted acutely into the contralateral femoral artery (Chapter 3). Thus, the BP values recorded with the implanted catheter were corrected for the amount of drift observed on the nearest calibration date to the day of measurement.

During the studies, the dogs breathed room air. When sleep was detected, the airway was occluded at end-expiration by manual closure of the three-way valve attached to the breathing circuit. When the dog aroused from sleep, the occlusion was released. Airway occlusions were repeated once S$_a$O$_2$ and ventilation had returned to baseline values and the dog had returned to sleep. On each study day, 12-24 airway occlusions were produced during nREM sleep and 6-10 during REM sleep.

6.2.5 Data Analysis

For each airway occlusion, we measured the time from the start of the occlusion to the point of arousal from sleep, and the peak airway pressure at arousal, which reflects the respiratory effort generated by the dog against the closed valve. The change in S$_a$O$_2$ in response to the airway occlusion was determined as the difference between the control S$_a$O$_2$ (measured during 20 seconds prior to the occlusion), and the lowest level of S$_a$O$_2$ associated with the
occlusion. Maximum systolic and diastolic BP were measured during 20 sec periods of stable sleep, immediately before initiation of airway occlusion, and for 30 sec periods beginning with arousal of the dog and release of the occlusion. The change in BP in response to airway occlusion was quantified as the difference between the maximum systolic and diastolic BP values measured before the occlusion and immediately after termination of the apnea.

Three separate questions were asked: (i) did long-term OSA result in a change in the acute responses to airway occlusion during sleep, as measured by time to arousal, degree of hemoglobin desaturation, magnitude of respiratory effort, and change in BP? (ii) did sleep fragmentation result in changes in the responses to acute airway occlusion during sleep? (iii) were the maximum changes in the responses to acute airway occlusion resulting from OSA larger than the maximum changes associated with sleep fragmentation? The first two questions were addressed using a one-way analysis of variance (ANOVA) with repeated measures (Sigmastat, Jandel Scientific, San Rafael, CA), and post-hoc analysis was performed using the Student-Newman-Keuls method. Since we were only interested in whether the changes in late OSA were different from the changes in late sleep fragmentation, the third question was addressed with a paired t-test (rather than a two-way analysis of variance). The analyses for the 3 questions were performed using one mean value for each dog, for the different time periods of the two protocols (i.e., the sample size was 4 for each of the analyses). Thus, we ensured that no one dog was over-represented in the analysis. For all tests, differences were considered statistically significant if the null hypothesis was rejected at a level of P<0.05 using a two-tailed test. Data are presented as mean ± standard error (S.E.).
Finally, to determine the role of hypoxia in the BP surges associated with airway occlusion, we examined the correlation between the absolute value for the degree of arterial oxygen desaturation during airway occlusion and the surges in BP associated with arousal from sleep, using the Pearson correlation coefficient. Correlations were performed for measurements made during the control period (before initiation of OSA or sleep fragmentation) and during the later stages of OSA or sleep fragmentation.

6.3. Results

Each of the four dogs completed both the OSA and the sleep fragmentation protocols. In each dog the arousal index during sleep fragmentation was similar to the apnea index during the OSA protocol (see Figure 25). Statistical analysis revealed no differences in the apnea and arousal indices between the two protocols (paired t-test, p=0.172).

Figure 30 shows a representative tracing of the acute response to airway occlusion in one dog during nREM sleep. Airway occlusion was associated with progressive arterial oxygen desaturation, a progressive increase in inspiratory effort, and a transient increase in arterial BP and heart rate.

The time to arousal during airway occlusion (i.e. length of apnea) is shown in Figure 31. Both OSA and sleep fragmentation resulted in an increase in length of apnea compared to control values (for OSA, p<0.001 for nREM, p=0.019 for REM; for sleep fragmentation, p=0.007 for nREM, p=0.0123 for REM). There were no differences between the changes observed during OSA and sleep fragmentation (p=0.366 for nREM; p=0.161 for REM).

Figure 32 shows the changes in \(S_4O_2\) associated with airway occlusion. There was no change in baseline \(S_4O_2\) during either the OSA or sleep fragmentation protocols. \(S_4O_2\) could
be reliably measured in only 3 of the 4 dogs, due to skin pigmentation in one dog; in the fourth dog with dark skin pigmentation, we were unable to obtain a stable accurate measurement of $S_4O_2$, on room air. Both OSA and sleep fragmentation resulted in greater desaturation in response to airway occlusion during nREM sleep, compared to control values ($p=0.039$ for OSA; $p=0.041$ for sleep fragmentation). The changes associated with sleep fragmentation were not different from those associated with OSA ($p=0.963$). During REM sleep there was also a trend to greater desaturation during both OSA and sleep fragmentation, but the results did not reach statistical significance ($p=0.08$ for OSA; $p=0.10$ for sleep fragmentation).

The changes in peak tracheal pressure during airway occlusion, reflecting inspiratory effort, are shown in Figure 33. Both OSA and sleep fragmentation resulted in an increase in absolute peak tracheal pressure compared to control values (for OSA, $p=0.002$ for nREM, $p=0.042$ for REM; for sleep fragmentation, $p=0.002$ for nREM and $p=0.002$ for REM). There were no differences between the changes observed during OSA and sleep fragmentation ($p=0.452$ for nREM; $p=0.502$ for REM).

The changes in systolic BP associated with airway occlusion are shown in Figure 34. Both OSA and sleep fragmentation were associated with greater surges in maximum systolic BP compared to control values (for OSA, $p<0.001$ for nREM, $p=0.003$ for REM; for sleep fragmentation, $p=0.007$ for nREM, $p<0.001$ for REM). There were no differences between the changes observed during OSA and sleep fragmentation ($p=0.558$ for nREM; $p=0.481$ for REM).
The changes in diastolic BP associated with airway occlusion are also shown in Figure 34. Both OSA and sleep fragmentation were associated with a greater surge in maximum diastolic BP during nREM sleep, compared to control values (for OSA p=0.003; for sleep fragmentation, p<0.001). During REM sleep, despite a trend to greater surges in maximum diastolic BP, the results did not reach statistical significance during OSA (p=0.213), possibly due to the large variability in responses in REM sleep; but were significant during sleep fragmentation (p<0.03). There were no differences between the changes observed during OSA and sleep fragmentation (p=0.249 for nREM; p=0.757 for REM).

For all ANOVA tests that showed a significant effect of OSA or sleep fragmentation on either length of apnea, peak tracheal pressure, change in $S_2O_2$, or change in systolic or diastolic BP, post-hoc analysis with the Student-Newman-Keuls test consistently revealed significant differences between the values during late OSA or sleep fragmentation (i.e., > 4 weeks) and both the control and the final recovery values, but no differences between the control values and the final recovery values.

We examined the relationship between the degree of arterial oxygen desaturation during airway occlusion and the surges in BP associated with arousal from sleep, using the Pearson correlation coefficient. During nREM sleep (in both the OSA and the sleep fragmentation protocols), r values ranged from 0.05 to 0.55 between the absolute change in $S_2O_2$ and the absolute change in maximum systolic or diastolic BP (all p>0.05, Table 15). The correlation coefficients for airway occlusions during REM sleep ranged from 0.14-0.77, and occasionally reached statistical significance (Table 16). Data are presented for dogs 2-4 only, due to poor oximetry recordings in dog 1.
Figure 30: Recorder tracings in one dog of the acute responses to airway occlusion. Airway occlusion (indicated by the cessation of airflow) resulted in a progressive increase in tracheal pressure ($P_{tr}$), a decrease in oxygen saturation ($S_aO_2$) and a transient increase in blood pressure (BP) and heart rate (HR). The arrow indicates the point of arousal from sleep, resulting in restoration of ventilation. Note the latency in the response of $S_aO_2$ to airway occlusion due to circulatory and electronic (which is less than 1 sec) delay.
Figure 31: Changes in length of apnea in response to acute airway occlusion during OSA (filled squares) and sleep fragmentation (open circles), in nREM (left) and REM sleep (right). Data points represent the mean ± S.E.. Data to the left of the first dotted line represent the control period; data between the dotted lines represent the period of OSA or sleep fragmentation; and data to the right of the second dotted line represent the recovery period. Data points are joined for ease of interpretation; note, however, that the time periods between points on the x axis are unequal. In addition, the apnea index was progressively increased over the first 2 weeks (starting at 10 apneas/hour of sleep), but remained constant after 14 nights (range of 50-60 apneas/hour of sleep). * denotes statistically significant difference (p<0.05) from baseline values (pre-OSA) on post hoc analysis. Al reflects apnea index for OSA and arousal index for sleep fragmentation.
Figure 32: Change in arterial oxygen saturation ($S_aO_2$) in response to acute airway occlusion during OSA (filled squares) and sleep fragmentation (open circles), in nREM (left) and REM sleep (right). The format of the data points is the same as in Figure 31.
Figure 33: Change in absolute peak tracheal pressure in response to acute airway occlusion during OSA (filled squares) and sleep fragmentation (open circles), in nREM (left) and REM sleep (right). The format of the data points is the same as in Figure 31.
Figure 34: Change in minimum systolic and diastolic BP in response to acute airway occlusion during OSA (filled squares) and sleep fragmentation (open circles), in nREM (left) and REM sleep (right). The format of the data points is the same as in Figure 31.
6.4. Discussion

We have utilized a canine model to examine the long-term effects of OSA and of sleep fragmentation on physiological responses to upper airway occlusion during sleep. We have demonstrated that OSA, independent of any changes in upper airway function, alters responses to airway occlusion in several respects, including prolongation of the time to arousal, greater arterial hemoglobin desaturation, increased peak inspiratory effort, and greater surges in BP. In addition, we have demonstrated that long-term sleep fragmentation, without airway obstruction, results in similar alterations in the acute responses to airway occlusion. This latter finding indicates that the changes in response to airway occlusion with OSA can be attributed primarily to the associated sleep fragmentation, rather than to other stimuli, such as recurrent asphyxia.

Kimoff and colleagues (1994a) have previously reported preliminary findings of the effects of short-term application of the canine model of OSA on measures of apnea severity. In two dogs, 5 days of OSA resulted in a significant increase in apnea duration, in the number of respiratory efforts during each apnea, and in the tracheal pressure of the last obstructed breath before arousal, and in a decrease in arousal \( S_2O_2 \) (Kimoff et al., 1994a). The present study extends these findings to the long-term application of the model of OSA and provides insight into the effects of sleep fragmentation (without airway occlusion) on the responses to airway occlusion.

Several investigators have produced short-term models of OSA in new-born lambs, pigs, and dogs (O'Donnell et al., 1994a; Fewell et al., 1988; Pinto et al., 1993). However, the studies utilizing these models were generally limited to less than 40 hours. Two of the studies examined the effects of sleep fragmentation on the arousal response to upper airway obstruction (Fewell et al., 1988; O'Donnell et al., 1994a). Fewell and colleagues (1988) subjected 6 new-
Table 15: Correlation between absolute changes in BP and absolute change in oxygen saturation during nREM sleep

<table>
<thead>
<tr>
<th></th>
<th>Changes in $S_aO_2$ vs. changes in systolic BP</th>
<th>Changes in $S_aO_2$ vs. changes in diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ values $^{**}$</td>
<td>$r$ values $^{**}$</td>
</tr>
<tr>
<td>Dog 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0498</td>
<td>0.206</td>
</tr>
<tr>
<td>OSA</td>
<td>0.215</td>
<td>0.110</td>
</tr>
<tr>
<td>Fragment. $^*$</td>
<td>0.085</td>
<td>0.340</td>
</tr>
<tr>
<td>Dog 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.200</td>
<td>0.119</td>
</tr>
<tr>
<td>OSA</td>
<td>0.395</td>
<td>0.376</td>
</tr>
<tr>
<td>Fragment. $^*$</td>
<td>0.435</td>
<td>0.441</td>
</tr>
<tr>
<td>Dog 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.233</td>
<td>0.449</td>
</tr>
<tr>
<td>OSA</td>
<td>0.336</td>
<td>0.255</td>
</tr>
<tr>
<td>Fragment. $^*$</td>
<td>0.374</td>
<td>0.550</td>
</tr>
</tbody>
</table>

$^*$'Fragment.' : sleep fragmentation  
$^{**}$ none of these regressions reached statistical significance
Table 16: Correlation between absolute changes in BP and absolute change in oxygen saturation during REM sleep

<table>
<thead>
<tr>
<th></th>
<th>Changes in $S_aO_2$ vs. changes in systolic BP r values</th>
<th>Changes in $S_aO_2$ vs. changes in diastolic BP r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 2</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.433</td>
<td>0.464</td>
</tr>
<tr>
<td>OSA</td>
<td>0.497</td>
<td>0.364</td>
</tr>
<tr>
<td>Fragment.*</td>
<td>0.462</td>
<td>0.689**</td>
</tr>
<tr>
<td>Dog 3</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.138</td>
<td>0.235</td>
</tr>
<tr>
<td>OSA</td>
<td>0.514</td>
<td>0.661</td>
</tr>
<tr>
<td>Fragment.*</td>
<td>0.375</td>
<td>0.771**</td>
</tr>
<tr>
<td>Dog 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.621</td>
<td>0.344</td>
</tr>
<tr>
<td>OSA</td>
<td>0.673**</td>
<td>0.422</td>
</tr>
<tr>
<td>Fragment.*</td>
<td>n/a***</td>
<td>n/a***</td>
</tr>
</tbody>
</table>

*Fragment.*: sleep fragmentation
** $p$ values less than 0.05
*** n/a: insufficient data for calculation of correlation coefficient
born lambs to occlusion of the upper airway whenever they fell asleep, before and after a 36-42 hour period of sleep fragmentation with an acoustic stimulus. Following sleep fragmentation, the time to arousal and the degree of arterial hemoglobin desaturation at arousal, in response to upper airway occlusion, were increased during quiet sleep, but not active sleep. However, the changes observed were small, being of the same magnitude that we observed after 1-2 weeks of OSA or sleep fragmentation. In a similar model in dogs, O’Donnell and colleagues (1994a) found that 24 hours of sleep deprivation resulted in increased apnea duration, a greater degree of hemoglobin desaturation, and an increased peak negative inspiratory effort at arousal. Our findings extend the results of these short-term studies by demonstrating that over a period of weeks, sleep fragmentation results in delayed arousal responses to airway obstruction.

In addition to animal studies, several studies have examined the effects of sleep fragmentation or deprivation on arousal responses to cardiorespiratory stimuli in normal subjects and in patients with OSA. In normal subjects two nights of sleep fragmentation did not change the slope or position of the ventilatory response curve to hypercapnea (Espinoza et al., 1991). However, the short duration of sleep fragmentation in these studies is not comparable to the sleep disruption of months or years associated with OSA (Espinoza et al., 1991). In 4 patients with OSA, Guilleminault and Rosekind (1981) reported that 38-63 hours of sleep fragmentation increased the time to arousal from apnea and the degree of associated arterial hemoglobin desaturation. Several other studies have also reported changes in hypoxic and hypercapnic ventilatory responses with sleep loss (Cooper and Phillips, 1982; White et al., 1983; Cooper and Phillips, 1982; Schiffman et al., 1983); however, sleep deprivation may have different physiological effects than sleep fragmentation.
**Methodological Considerations**

In our model of sleep disruption, we were able to produce sleep fragmentation without sleep deprivation since the acoustic alarm was not activated until a period of sleep had been established (at least 18 sec). We had to consider the possibility of habituation of the dogs to the acoustic stimuli since repeated noise has been shown to result in decrements in sensitivity to the acoustic stimulus and in failure to arouse from sleep (Roehrs et al., 1994). In addition, sleep deprivation increases the arousal threshold to auditory stimuli in both animals and humans (Frederickson and Rechtschaffen, 1978; Williams et al., 1964). Therefore in our study, if the dog failed to arouse when the alarm was activated, the frequency of the sound increased progressively along a variable ramp until the dog aroused from sleep. If, however, the dog still failed to arouse, the sound ceased after 18 epochs of sleep (108 sec). The ramping of the frequency minimized acclimatization by the animal. Furthermore, we reviewed the sleep records on a daily basis, and if more than 10% of acoustic stimuli failed to induce arousal, the amplitude of the sound was increased on the subsequent night. Thus, the design of the sound profile and the change in amplitude of the sound minimized habituation of the response. As a result, throughout the sleep fragmentation period, the dogs aroused to more than 85% of the acoustic stimuli on a nightly basis.

We strove to create a similar degree of sleep disruption during the OSA and sleep fragmentation protocols. In each dog, the arousal index during sleep fragmentation was similar to the apnea index during OSA (Figure 25). In addition, the total sleep time and the degree of increase in night-time BP were comparable in each dog during OSA and sleep fragmentation (see chapter 7). It will be noted that the duration of the sleep fragmentation
protocol was less than that of the OSA protocol. However, since the changes in the responses to acute airway occlusion were maximum after 4 weeks of OSA, we did not consider it necessary to study the dogs on the sleep fragmentation protocol beyond a 5-7 week period, when the changes in the responses to acute airway occlusion had reached a plateau. In addition, from a practical standpoint, the duration of the protocols had to be restricted because of the limited life of the battery in the telemetry unit.

**BP Response During Airway Occlusion**

In the present study, there were significant increases in maximum systolic and diastolic BP with arousal from sleep and termination of airway occlusion. This finding is in agreement with previous animal studies that demonstrated increases in mean arterial pressure of 5-18 mm Hg at arousal from apnea, mediated by the sympathetic nervous system (Pinto et al., 1993; O'Donnell et al., 1994b). Similarly, in patients with OSA, arterial systolic and diastolic BP may increase by 15-50 mm Hg in response to apneas, with the peak rise coinciding with arousal from sleep and resumption of ventilation (Tilkian et al., 1976; Shepard, 1985; Motta et al., 1978; Ringler et al., 1990). Following arousal, BP typically remains elevated for 10-15 sec before returning slowly to control levels (Shepard, 1985). This pattern of BP change is similar to the one we observed in our dogs (Figure 30).

Several stimuli may contribute to these BP changes. A number of studies have highlighted the importance of hypoxia. For example, in patients with OSA, Shepard (1985) found that the rise in BP before arousal correlated with the degree of associated oxygen desaturation. In addition, administration of supplemental oxygen has been reported to prevent the typical post-apneic increase in BP, in both humans and dogs (Iwase et al., 1992; Van Den
Aardweg et al., 1992). In contrast, Ringler and co-workers (1990) found that administration of supplemental O₂, sufficient to maintain SₐO₂ above 90% did not diminish the increase in BP during apneas in patients with OSA, and concluded that other stimuli (arousal from sleep, changes in intrathoracic pressure, or resumption of breathing) were responsible for the BP surge. However, O'Donnell and colleagues (1996) demonstrated in dogs that termination of airway occlusion before arousal still resulted in a BP surge. Resumption of breathing may also contribute to the surge in BP at the termination of apnea through the effects on cardiac volumes and function as described in chapter 1 (section 1.4.3). Taken together, these studies suggest that while hypoxia may play a role in the BP increase associated with obstructive apneas, other stimuli are also involved. Support for this conclusion can be derived from our observation of a lack of correlation between the changes in maximum systolic or diastolic BP and the fall in SₐO₂ during nREM sleep, but a somewhat stronger correlation (i.e., occasionally reaching statistical significance) between these variables in REM sleep. This difference may relate to the finding of O'Donnell and co-workers (1994b) who demonstrated a significant correlation between the change in mean arterial BP and the degree of hypoxia only for obstructions in which SₐO₂ fell below 80%. In our study, only airway occlusions in REM sleep resulted in SₐO₂ levels below 80%. Thus, in the absence of severe hypoxia, other stimuli associated with airway occlusion appear to play a more important role in causing acute increases in BP; whereas in the presence of severe hypoxia, the contribution of other stimuli may be proportionately less.
Responses to Airway Occlusion Over Time

Both sleep fragmentation and OSA were associated with progressively increased time-to-arousal from sleep in response to acute airway occlusion. Theoretically, this longer duration of apnea could have resulted in part from decreases in ventilatory responses to hypoxia and hypercapnea. However, in both dogs and humans, 2-3 days of sleep fragmentation does not change the ventilatory responses to hypoxia and hypercapnea, yet impair arousal responses to these stimuli (Bowes et al., 1980; Bowes et al., 1983; Espinoza et al., 1991). This later finding suggests that delayed arousal responses consequent to sleep fragmentation can be attributed directly to alterations in the arousal threshold of the central nervous system, rather than being secondary to changes in ventilatory chemoresponsiveness (Bowes et al., 1980).

Previous studies suggest that the primary stimulus producing arousal from sleep during an obstructive apnea is the degree of inspiratory effort produced by the patient (Gleeson et al., 1990; Kimoff et al., 1994b). Indeed, there appears to be a threshold of inspiratory effort at which arousal occurs, whether the effort is increased by chemical or mechanical stimuli (Gleeson et al., 1990). In our study, the peak inspiratory effort in response to acute airway occlusion increased with both sleep fragmentation and OSA, supporting the notion that the central threshold for arousal has been reset. Thus, it appears that under conditions of sleep disruption, the pressure to maintain sleep can override, within limits, the demands for effective ventilation.

Effect of Sleep State

Both OSA and sleep fragmentation resulted in a similar pattern of change in the acute responses to airway occlusion during nREM and REM sleep. However, the changes during
REM sleep occasionally failed to reach statistical significance, possibly due to the larger variability of responses and the fewer observations made during this stage of sleep. Similarly, short-term sleep deprivation in the dog resulted in longer duration of apneas in REM sleep, but no greater change in arterial hemoglobin desaturation or peak negative airway pressure during the apneas (O’Donnell et al., 1994a). Moreover, in sleep-fragmented newborn lambs, Fewell and co-workers (1987) failed to show changes in time to arousal and in oxygen desaturation during ‘active’ sleep, despite statistically significant changes during ‘quiet’ sleep. These authors therefore suggested that different mechanisms may mediate the arousal response in the two sleep states. Sleep stage may also influence the hemodynamic response to apnea, since Garpestad and co-workers (1995) found that apneas during REM sleep were associated with greater surges in BP than were apneas in nREM sleep, even when oxygen desaturation was similar. However, these inconsistent findings in REM sleep may simply be a function of the greater variability in responses associated with this stage of sleep.

In summary, we have compared the effects of long-term OSA and of sleep fragmentation on the acute physiological responses to upper airway occlusion during sleep, measured during the daytime. OSA altered the acute responses to airway occlusion, resulting in longer time to arousal from apnea, greater arterial hemoglobin desaturation, increased peak inspiratory effort, and greater surges in BP. These changes could be attributed to the fragmentation of sleep associated with OSA.
Chapter 7: Effects of OSA and Sleep Fragmentation on BP

7.1. Introduction

The National Commission on Sleep Disorders Research has identified disorders of sleep as a major public health burden affecting the lives of millions of Americans (National Commission on Sleep Disorders, 1993). One of the most common and serious of these disorders is OSA (Phillipson, 1993). As presented in Chapter 1, OSA is a prevalent disease; population studies have estimated that 4 percent of women and 9 percent of men between the ages of 30 and 60 years suffer from a clinically important degree of sleep apnea (Young et al., 1993).

Several epidemiological studies have identified OSA as an important risk factor for systemic hypertension, myocardial infarction, stroke and sudden death (Koskenvuo et al., 1987; He et al., 1989; Hla et al., 1994) (see Chapter 1). Given the high prevalence of sleep-disordered breathing, the potential importance of OSA in contributing to cardiovascular morbidity and mortality is considerable. The strongest association demonstrated to-date is between OSA and hypertension, but a direct etiologic link between the two disorders has not been established definitively. Furthermore, epidemiological and clinical studies are limited in this regard since by the time patients with OSA come to clinical attention, the disorder and its possible long-term sequelae have often been present for several years. In addition, patients with OSA typically present with other risk factors for hypertension, notably obesity.

Given these considerations, the aims of this study were to use a canine model of OSA to determine whether OSA per se leads to sustained systemic hypertension. Since each animal served as its own control, we were able to examine the specific effects of OSA on blood
pressure (BP) in the absence of other confounding variables. In addition, we investigated the effects on BP of recurrent arousals from sleep without airway occlusion.

7.2. Methods

7.2.1. Animal Preparation

OSA was produced in 4 dogs (3 female, 1 male; weight, 23-31 kg) using the model described in Chapter 2. All surgical and experimental procedures were approved by the Animal Care Committee of the University of Toronto. The dogs were trained to sleep in the laboratory, and then underwent two surgical procedures at least two months before initiation of studies; the first to create a permanent side-hole tracheostomy, and the second, to implant a three-channel telemetry unit for monitoring of arterial BP, the electroencephalogram (EEG) from implanted skull electrodes, and nuchal electromyogram (EMG). Details of these telemetry systems and surgical procedures are described in Chapters 2 and 3.

7.2.2. Induction of OSA

The details of the model are presented in Chapter 2. It is important to note that there were no physical attachments between the dog and the recording equipment with this model, allowing it to move about freely; and the system required no human intervention (except for routine monitoring and maintenance).

OSA was produced in each dog over a period of 1-3 months. The apnea index increased progressively from 10-30 events per hour of sleep on nights 1-7, to 50-60 events per hour of sleep after 14 nights.
7.2.3. Sleep Fragmentation without Airway Occlusion

In a separate protocol, the same four dogs were re-studied, at least 6 months after completion of the OSA protocol, to determine the effects on BP of sleep fragmentation without airway occlusion. The details of this model are presented in Chapter 4. The dogs were subjected to repeated episodes of arousal from sleep, but without the changes in blood gases and intrathoracic pressure associated with obstructive apneas. The arousal index (i.e., number of arousals per hour of sleep) was similar in each dog to the apnea index of the OSA model (p=0.2). Graphic presentation of apnea and arousal indices are shown in chapter 4.

7.2.4. Experimental Protocol

Each animal was studied during a control period of 1-2 months before induction of OSA, during 1-3 months of OSA, and during a recovery period of 1 month after cessation of OSA. During the period of OSA, the dogs were kept awake during the day with human companionship. However, whenever they displayed pre-sleep behavior (e.g., eyes closing, nodding of the head, stupor), the monitoring-occlusion system was activated. After completion of the OSA experiments, the dogs were re-studied on the sleep fragmentation protocol for 1-2 months before, 1-2 months during, and up to 1 month following cessation of sleep fragmentation. Throughout both protocols, the experimental conditions, diet, weight and daily routine of the dogs remained constant. However, from a practical standpoint, the duration of the protocols had to be restricted because of the limited life of the battery in the telemetry unit; thus, the exposure to OSA and sleep fragmentation varied from dog to dog.
7.2.5. Analysis of Night-time and Daytime BP

BP was measured using the telemetry system described in Chapter 3. The implanted BP system was calibrated periodically (generally every 4 weeks) using a manometer-tipped catheter inserted acutely into the contralateral femoral artery. The BP values obtained from the implanted system were corrected for the amount of drift from the closest calibration date.

Arterial BP was recorded continuously every night, and was analyzed for 12 hours (in a dark room from 1900-0730) by the computer on a minute-by-minute basis. Mean night-time arterial BP was calculated for 4 nights in the control period, 2 nights of early OSA or sleep fragmentation (apnea or arousal index<30 per hour of sleep), 4 nights in the later stages of OSA or sleep fragmentation (apnea or arousal index>45 per hour of sleep), and 1 night following cessation of OSA or sleep fragmentation. Four nights was arbitrarily chosen for the control period and later stages of OSA or sleep fragmentation. The nights were randomly chosen from each of the phases. Note that night-time BP was the mean arterial BP overnight regardless of the dog’s state or activity. Measurements of daytime arterial BP during wakefulness were made every 7 to 14 days during the control, OSA or sleep fragmentation, and recovery periods. Mean daytime arterial BP was calculated from over 6,000 cardiac cycles at a standardized time period during the day, with a stable laboratory temperature. For these measurements, the dogs lay recumbent and were in a state of relaxed wakefulness, according to standard EEG criteria (Phillipson et al., 1976). Measurements were also made during uninterrupted period of nREM and REM sleep at a standardized time in the daytime.
7.2.6. Data Analysis

The data were analyzed to answer three separate questions: (1) did OSA result in a change in night-time or daytime BP? (2) did sleep fragmentation result in a change in night-time or daytime BP? (3) Were the changes in BP with OSA the same as with sleep fragmentation? Questions 1 and 2 were addressed with a one-way analysis of variance (ANOVA) with repeated measures, using commercially available software (Sigmasat, Jandel Scientific, San Rafael, CA) and post-hoc analysis was performed using the Student-Newman-Keuls method. Question 3 was analyzed using a paired t-test, comparing in each dog the maximum changes in BP during OSA with that during sleep fragmentation. A paired t-test and not a two-way analysis of variance was used because only the values in the late stages of OSA and sleep fragmentation were compared. The analyses for the 3 questions were performed using one mean value for each dog, for the different time periods of the two protocols (i.e., the sample size was 4 for each of the analysis). Thus, we ensured that no one dog was over-represented in the analysis. For all tests, differences were considered statistically significant if the null hypothesis was rejected at a level of P<0.05, using a two-tailed test. The night-time and daytime arterial BP values during the different phases of the study and the changes from control were graphically represented. In addition, the change in BP from control the arterial night-time and daytime BP values were also normalized, with control value being 100%; for example, the normalized value for mean daytime BP during OSA would be \( \frac{BP_{OSA}}{BP_{control}} \times 100 \).
7.3. Results

Night-time BP

OSA resulted in increases in mean night-time BP (Figure 35) to a maximum of +13.0±2.0 mm Hg (mean ± S.E.; range +9.4-+18.5 mm Hg; one-way analysis of variance [ANOVA] with repeated measures, p=0.005). The normalized means for all 4 dogs during the different phases of the study are shown in Figure 36. Immediately following cessation of OSA, night-time arterial BP returned to control values. The immediate return of night-time arterial BP to the control value was probably related to the fact that on the first night following cessation of OSA, the sleep of the dogs was considerably more consolidated than in the pre-OSA control period (Homer et al., 1996).

Sleep fragmentation without airway occlusion resulted in an increase in mean night-time arterial BP (Figures 35 and 36) of +11.2±1.0 mm Hg (range +8.8-+13.7 mm Hg; ANOVA, p=0.04). There was no difference between the change in night-time arterial BP produced by sleep fragmentation and that produced by OSA (paired t-test, p=0.4). There were no changes in grouped mean night-time heart rate during either OSA or sleep fragmentation (ANOVA, p values >0.5, Figure 37).

Daytime Awake BP

OSA resulted in increases in mean daytime awake BP (Figure 38) to a maximum of +15.7±4.3 mm Hg (range +6.0-+26.8 mm Hg; ANOVA, p=0.004). The normalized means for all 4 dogs during the different phases of the study are shown in Figure 39. Following cessation of OSA, BP returned to control levels over a period of 1-3 weeks (Figures 38 and 39).
In contrast to the changes in night-time BP, sleep fragmentation produced only a small
elevation in mean daytime BP (Figures 38 and 39) of 4.0±4.0 mm Hg (range -4.5-14.3 mm
Hg; ANOVA, p=0.72). The change in daytime arterial BP during sleep fragmentation was
significantly less than the change during OSA (paired t-test, p<0.001). There were no changes
in daytime awake heart rate during either OSA or sleep fragmentation (Figure 40, ANOVA, all
p values>0.5) Daytime BP during nREM sleep

OSA resulted in increases in mean arterial BP during periods of uninterrupted nREM
sleep (Figure 41) to a maximum of +15.5±3.3mm Hg (ANOVA, p=0.002). The normalized
means for all 4 dogs during the different phases of the study are shown in Figure 42. Sleep
fragmentation produced only a small elevation in mean arterial BP in nREM sleep (Figures 41
and 42) of +4.4±2.5 mm Hg (ANOVA, p=0.28). The change in BP in nREM sleep during
sleep fragmentation was significantly less than the change during OSA (paired t-test, p<0.002).

Daytime BP during REM sleep

OSA resulted in increases in mean BP in REM sleep (Figure 43) to a maximum of
+16.8±3.6 mm Hg (ANOVA, p=0.002). The normalized means for all 4 dogs during the
different phases of the study are shown in Figure 44. Sleep fragmentation produced only a
small elevation in mean arterial BP in REM sleep (Figures 43 and 44) of +3.7±3.4 mm Hg
(ANOVA, p=0.60). The change in BP in REM sleep during sleep fragmentation was
significantly less than the change during OSA (paired t-test, p=0.04).
Figure 35: Night-time arterial blood pressure (MABP) during OSA (filled squares) and sleep fragmentation (open circles) in each of the 4 dogs. The dashed lines indicate the beginning and end respectively of the OSA or sleep fragmentation phase. The error bars, representing the 95% confidence intervals of the mean, are hidden in the symbols. Data points are joined for ease of interpretation; note, however, that the time periods between points on the x axis are unequal. In addition, the apnea index was progressively increased over the first 2 weeks (starting at 10 apneas/hour of sleep), but remained constant after 14 nights. AI represents apnea index in OSA and arousal index in sleep fragmentation.
Figure 36: Grouped mean night-time arterial blood pressure in 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The format is the same as in Figure 35 but the error bars represent the S.E. of the mean. * denotes statistically significant difference (p<0.05) from baseline (pre-OSA) value on post-hoc analysis. The left graph shows the percent change in BP; the right graph shows the actual change in mm Hg.
Night-time mean HR (bpm) control
1 - 2 weeks > 4 weeks first night control

Figure 37: Night-time heart rate in each of the 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The
Figure 38: Daytime awake arterial blood pressure (MABP) during OSA (filled squares) and sleep fragmentation (open circles) in each of the 4 dogs. The format is the same as in Figure 35.
Figure 39: Grouped mean daytime awake arterial blood pressure in 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The format is the same as in Figure 35 but the error bars represent the S.E. of the mean. * denotes statistically significant difference (p<0.05) from baseline (pre-OSA) on post-hoc analysis. The left graph shows the percent change in BP; the right graph shows the actual change in mm Hg.
Figure 40: Daytime awake heart rate in each of the 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The format is the same as in Figure 42.
Figure 4.1: Mean arterial blood pressure (MABP) in nREM sleep during wakefulness and sleep fragmentation (open circles) in each of the 4 dogs. The format is the same as in Figure 3.

- Dog 1
- Dog 2
- Dog 3
- Dog 4

- Control
- > 5 weeks
- > 2.5 weeks
- > 1.2 weeks
- > 1 week
- > 3 weeks
- > 5 weeks
- > 2.5 weeks
- > 1.2 weeks
- > 1 week
- > 3 weeks

MABP in nREM sleep (mm Hg)
Figure 42:  Grouped mean arterial blood pressure in nREM sleep in 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The format is the same as in Figure 35 but the error bars represent the S.E. of the mean. * denotes statistically significant difference (p<0.05) from baseline (pre-OSA) on post-hoc analysis.
Figure 43: Mean arterial blood pressure (MABP) in REM sleep during OSA (filled squares) and sleep fragmentation (open circles) in each of the 4 dogs. The format is the same as in Figure 35.
Figure 44: Grouped mean arterial blood pressure in REM sleep in 4 dogs during OSA (filled squares) and sleep fragmentation (open circles). The format is the same as in Figure 35 but the error bars represent the S.E. of the mean. * denotes statistically significant difference (p<0.05) from baseline (pre-OSA) on post-hoc analysis.
7.4. Discussion and Conclusions

There are two major findings from this study: first, that OSA \textit{per se} produces a sustained elevation in daytime BP; and second, that recurrent arousals from sleep alone cannot account for the daytime elevation in BP observed in OSA. These data provide the first direct evidence of a cause and effect relationship between OSA and the development of systemic hypertension.

Spontaneous sleep disordered breathing has been described in brachycephalic dogs such as the English bulldog (Hendricks et al., 1987). However, apneas in the bulldog occur only during rapid-eye-movement sleep, unlike the human disorder. Short-term (\( \leq 24 \) hours) models of OSA have been produced in adult pigs (Pinto et al., 1993) and dogs (O'Donnell et al., 1994), but these models are not suitable for the study of the long-term consequences of the disorder (see section 1.9 for more details on \textit{animal models of OSA}). The present study represents the first report of the long-term application of an induced model of repetitive upper airway occlusion during sleep. Our findings extend those of the short-term studies, which showed that periods of upper airway obstruction result in acute transient increases in arterial BP (Pinto et al., 1993; O'Donnell et al., 1994), by demonstrating that over a period of weeks, OSA results in a sustained elevation in daytime BP, even during relaxed wakefulness.

Several authors have reported a remarkably high prevalence of OSA in hypertensive compared to normotensive patients (Kales et al., 1984; Williams et al., 1985), although this finding has not been consistent (Hirshkowitz et al., 1989) (see Chapter 1). Other studies have demonstrated that the prevalence of hypertension among patients with OSA is higher than in the general population (Hla et al., 1994; Burack, 1984; Grunstein et al., 1993). A major problem
with this type of epidemiological evidence is the presence of confounding variables, particularly obesity, that predispose to both OSA and hypertension. Nevertheless, even in studies in which obesity, gender and age were statistically controlled, sleep apnea continued to be an independent risk factor for hypertension (Hla et al., 1984; Grunstein et al., 1993). A few clinical studies have described a decrease in blood pressure after effective treatment of OSA (Motta et al., 1993; Wilcox et al., 1993), but interpretation of these studies is complicated by concurrent changes in body mass, alcohol consumption, and antihypertensive medications, and by the direct effects of treatment, such as continuous positive airway pressure, on the cardiovascular system (Wilcox et al., 1993).

In contrast to these epidemiological and clinical studies, the present study in dogs has demonstrated a direct link between OSA and hypertension, in the absence of confounding variables. Several stimuli that characterize OSA may contribute to this relationship, including repetitive episodes of hypoxia and hypercapnea, disruption of sleep architecture, and fluctuations in intrathoracic pressure during the occluded respiratory efforts (Hla et al., 1994). However, the results of our study indicate that disruption of sleep architecture by recurrent arousals is not the underlying stimulus, suggesting that hypoxia and/or fluctuations in intrathoracic pressure are of critical importance. Support for the role of hypoxia can be derived from the observation that rats subjected to chronic intermittent hypoxia patterned after the hypoxia of sleep apnea develop sustained elevations of BP after 20 days of exposure (Fletcher et al., 1992a).
In contrast to daytime blood pressure, we found that recurrent arousals from sleep resulted in the same degree of night-time increase in BP as did OSA (Figures 35 and 36). This finding suggests that the transient BP increases associated with apneic events are primarily the result of arousal from sleep, and is in agreement with studies in humans demonstrating that supplemental oxygen or manipulations of ventilation and intrathoracic pressure have little effect on post-occlusion increases in BP (Ringler et al., 1994; Ringler et al., 1990). In contrast, graded arousals from sleep produce blood pressure increases that vary with the degree of arousal (Davies et al., 1993). Taken together, these studies suggest that BP elevations at the end of obstructive apneas can be largely attributed to arousal alone. However, as demonstrated in the present study, these night-time surges in BP do not necessarily translate into a daytime elevation in BP, in the absence of additional stimuli.

Caution must be used when extrapolating the results of this study to clinical applications because of potential species differences; however, the consequences and manifestations of experimentally induced OSA in the dog are similar to humans (Kimoff et al., 1994b). Another limitation of this model of OSA is that it bypasses the upper airway since the animals are tracheostomized. It is theoretically possible that bypassing the upper airway may alter the BP response to OSA. Finally, daytime BP returned to control values within 2 weeks of cessation of OSA in the dogs; it is conceivable that the recovery may have been considerably longer, if the duration of OSA had been greater than 1-3 months.

In conclusion, we have used a canine model of recurrent upper airway occlusion during sleep to demonstrate that OSA causes an elevation in daytime BP. The development of hypertension could not be attributed to the recurrent arousals from sleep that characterize the
OSA syndrome. Given the high prevalence of hypertension and of OSA in the general population, these findings suggest that the possibility of OSA should be considered in all patients with essential hypertension (i.e. by taking a sleep history from hypertensive patients).
PART IV: ADDITIONAL STUDIES AND INSIGHTS.

CONCLUSIONS AND FUTURE STUDIES

The aims of this research project were three-fold; first, to develop a canine model of OSA for long-term studies of the consequences of the disorder; second, to determine whether OSA per se causes sustained daytime hypertension; third, if OSA leads to daytime hypertension, to examine the role of sleep fragmentation in the pathogenesis of the daytime hypertension. The details of the development of the OSA model were outlined in Part 2 of this thesis. Part 3 of the thesis, specifically chapter 7, dealt with aims 2 and 3.

In addition to these objectives, we have also validated extensively a telemetry system for long-term continuous monitoring of cardiovascular variables (Chapter 3). The results of this validation (which have been published in the Journal of Applied Physiology) may be applied in many different research settings in the pharmacological and cardiovascular areas. In addition, we have examined the effects of OSA and sleep fragmentation, not only on BP, but also on the acute responses to airway obstruction (Chapter 6).

Part IV of this thesis includes two chapters. Chapter 8 outlines some preliminary results that provide further insight into the mechanisms contributing to the pathogenesis of daytime hypertension in the OSA canine model. These findings are presented as a background for the final chapter (Chapter 9), in which conclusions and recommendations for future research are made.
Chapter 8: Insight into Mechanisms Underlying Hypertension in OSA

This chapter will outline some preliminary results that provide further insight into the mechanisms that may be responsible for the pathogenesis of daytime hypertension. Two specific mechanisms were examined; changes in arterial baroreceptor reflex function and the effects of OSA on ventilatory responses to progressive hypoxia and hypercapnea.

8.1. Effects Of OSA on Arterial Baroreflexes

8.1.1. Introduction

The arterial baroreceptors play a major role in the reflex regulation of BP under normal and pathological conditions and maintain BP within certain limits over a relatively short time frame (Sved and Gordon, 1994). The reflex consists of different components, mainly the sensors with stretch receptors located in the cardiovascular and other tissues, the afferent input to the central nervous system and an efferent component consisting of the autonomic innervation of the vasculature and heart (Sved and Gordon, 1994). An inverse relationship exists between the sensitivity of the baroreflex and BP variability has been established (Floras et al., 1988).

In the human syndrome of OSA, it has been postulated that the paroxysmal sympathetic discharges and large BP variability associated with apneas during sleep may eventually lead to daytime hypertension (Levinson and Millman, 1991). There is evidence that patients with OSA have a higher level of sympathetic activity during wakefulness than normal subjects, as indicated by microneurography (Somers et al., 1995; Hedner et al., 1988). Sympathetic activity
is also increased during sleep in patients with OSA; and treatment of OSA with the application of CPAP decreases sympathetic activity (Somers et al., 1995).

These changes in sympathetic activity in patients with OSA may be due to changes in baroreflex function. Normally, an increase in BP results in an increase in baroreceptor firing (Scher et al., 1991). As a result, sympathetic activity decreases and vagal firing increases, causing a decrease in peripheral resistance, stroke volume and HR. Thus, a rise in BP initiates compensatory responses that eventually restore pressure to lower levels (Scher et al., 1991). However, recurrent exposure to elevated BP, as encountered in OSA, may lead to resetting of the baroreceptors to a higher pressure. Resetting can be defined as 'decreased pressure-sensitivity' of the baroreceptors, i.e., the baroreceptor discharge decreases for a given level of pressure (Chapleau et al., 1989). Resetting of the baroreceptors may in turn lead to changes in sympathetic nerve activity. Acute resetting that occurs within minutes and stabilizes within 5-15 minutes consists of decreased baroreceptor activity for the same pressure, without a change in gain or sensitivity (Chapleau et al., 1989). Thus, the sigmoid relationship between R-R interval and BP shifts to the right (on the pressure axis curve) with no change in its slope (for a definition of sensitivity of the arterial baroreflex, please refer to Chapter 1). With chronic resetting, baroreceptor activity is further reduced for a given pressure and there is a decrease in the gain of the relationship between baroreceptor activity and BP (Chapleau et al., 1989).

Given these considerations, the aims of this study were to use the canine model to determine whether OSA leads to changes in the arterial baroreceptor reflex control of HR. Since
each animal served as its control, we were able to examine the specific effects of OSA on the arterial baroreceptor reflex control of HR in the absence of other confounding variables.

8.1.2. Methods

Experimental Protocol

OSA was produced in 4 dogs as described earlier (Chapter 2). The arterial baroreflex control of HR was examined in 3 of the 4 dogs in each of the three phases of the study; before induction of OSA, during OSA, and after cessation of OSA. Control measurements were made 2-3 weeks before initiation of the model. OSA was then produced for 1-3 months and measurements of arterial baroreflex control of HR were completed during the last week of this phase. During the recovery phase, measurements of arterial baroreflex control of HR were made at least 4 weeks after cessation of OSA, when daytime BP had returned to control levels.

Methods of Measurements of Arterial Baroreflex Control of HR

During the daytime studies of arterial baroreflex control of HR, the dogs lay awake in the same position for each study. The electroencephalogram (EEG) was monitored and ECG was recorded via two needle electrodes (Type E2, Grass Instrument Co.) placed in the chest wall. Testing of baroreflexes was performed while the animal was awake, as determined by EEG and behavioral criteria (Phillipson et al., 1976).

During the experiments, the dogs breathed through a cuffed endotracheal tube (10 mm internal diameter) inserted through the chronic tracheostomy. The endotracheal tube was attached to a pneumotachograph (Fleish No. 2) and a differential pressure transducer (Validyne MP-45) to measure airflow. The airflow signal was integrated (Beckman 9873B resetting integrator coupler) to provide tidal volume. Airway pressure was measured with a transducer
(Gould Statham P23Db transducer) which was calibrated against a water manometer. In addition to the raw ECG signal, the instantaneous heart rate was also derived (Cardiotachometer Coupler, Beckman, type 9857). BP was measured with the implanted catheter and telemetry system, as described in Chapter 3.

For purposes of these studies, the dogs were mechanically hyperventilated using a volume-cycled ventilator (Model No. 613, Harvard Medical Apparatus Inc.) to abolish spontaneous respiratory rhythm and avoid the confounding influence of changes in spontaneous breathing pattern and blood gases on blood pressure. The dogs were hyperventilated with room air to a steady state hypocapnic PCO₂, 2 mm Hg below the PCO₂ measured during spontaneous breathing. This degree of hyperventilation was sufficient to abolish spontaneous breathing movements, as judged by the smooth and reproducible flow and pressure traces produced by the ventilator and as confirmed previously by silencing of respiratory muscle discharges (Horner et al., 1994a and b). The volume of the ventilator was set at 100 ml above the tidal volume observed during spontaneous breathing. After the desired PCO₂ had been reached, the level of mechanical ventilation was held constant; the rate of inspiratory time to expiratory time was 2:3.

Once a stable ventilatory pattern was established, the arterial baroreflex control of HR was examined by measuring heart rate responses to graded changes in blood pressure that were produced with intravenous infusions of the vasoactive agents phenylephrine (20-120 μg, i.v.) and sodium nitroprusside (25-125 μg, i.v.), administered over 30-60 seconds. To minimize the effects of respiratory sinus arrhythmias, steady state systolic BP and R-R intervals were measured over 45-60 seconds. Different dosages of the vasoactive agents were used to obtain a range of BP
changes (± 20 mm Hg) from control level. The different dosages and vasoactive agents (i.e. phenylephrine vs. nitroprusside) were administered in a random order.

**Data Analysis**

For each drug infusion trial, steady state systolic BP and R-R interval were measured over 45-60 seconds. Multiple drug infusions (n=10-14) were performed in each dog to obtain a representation of the arterial baroreflex control of HR over a range of pressures. The relationship between R-R interval and systolic BP was graphed in each dog for the 3 phases of the study.

In general, two questions were asked: (i) did OSA result in a shift of the systolic BP-R-R interval curve to higher pressures?, (ii) did OSA result in a change in gain (i.e. slope) of the systolic BP-R-R interval curve? A t-test was performed using a commercially available software (*Sigmastat*, Jandel Scientific, San Rafael, CA) to determine the change in intercept and slope during OSA compared to control values (pre-OSA). Differences were considered statistically significant if the null hypothesis was rejected at a level of P<0.05 using a two-tailed test.

**8.1.3. Results**

All 3 dogs developed sustained daytime hypertension during OSA compared to the control period (range of daytime BP increase: 6.0-26.8 mm Hg, see Chapter 7).

Figure 45 shows the relationship between the systolic BP and R-R interval in each of the three dogs, before, during and after cessation of OSA. To simplify the statistical analysis, the relationship between systolic BP and RR interval was assumed linear. OSA resulted in a rightward shift of the relationship on the pressure axis in each of the three dogs tested (t-test in
each dog on intercept, pre-OSA vs. post OSA, all p values<0.03), but no change in slope (t-test in each dog, pre-OSA vs. OSA, all p values >0.1). The lack of change in baroreceptor sensitivity (i.e., slope) is also demonstrated in Figure 46, where the change in R-R interval is plotted against the change in systolic BP. Measurements during OSA fall on the same curve as control values (pre and post OSA).

8.1.4. Discussion

The findings demonstrate that OSA in the dog produces sustained daytime hypertension, a rightward shift on the pressure axis of the R-R interval-systolic BP curve, but no change in slope of the baroreflex. These data suggest that OSA was associated with in an increase in the set-point of the baroreflex, without a change in sensitivity.

Our findings are in agreement with previous studies of baroreflex function in OSA. In a study by O'Donnell and co-workers (1994), sleep-deprived dogs subjected to 12 hours of airway obstructions developed a significant increase in mean arterial BP, but no decrease in heart rate. These findings led the authors to suggest that the night of repetitive airway obstruction ‘increased the set point of the cardiac baroreflex’, which may have contributed to maintaining an elevated BP after cessation of airway obstruction. However, their conclusions were based on only a single measurement on the baroreflex curve. In humans, Ziegler and co-workers (1995) reported that apneic patients (both hypertensive and normotensive) had an enhanced pressor response to phenylephrine infusion compared to non-apneic subjects. However, there was no statistically significant difference in baroreflex slope between apneics and non-apneics. There are no other reports in the literature on the effects of OSA on baroreflex function.
Figure 45: Relationship between systolic BP and R-R interval pre-OSA (filled circles), during OSA (open squares), and post-OSA (open triangles). Note that the OSA points, specially in dog 1, are shifted to the right compared to pre- and post-OSA points.
Figure 46: Relationship between changes in systolic BP and changes in R-R interval pre-OSA (filled circles), during OSA (open squares), and post-OSA (open triangles). The curves represent the best-fit for the control (pre-OSA) data.
The rightward shift of the baroreflex curve on the pressure axis without a change in slope (or gain) has been referred to as 'acute' resetting of the baroreflex (Chapleau et al., 1991), although there is no agreement in the literature on the precise meaning of this terminology. 'Acute' resetting consists of decreased baroreceptor activity for the same pressure, without a change in gain (Chapleau et al., 1989). In 'chronic' resetting, baroreceptor activity is further reduced for a given pressure and there is also a decrease in the gain of the relationship (Chapleau et al., 1989). Early hypertension in humans may be associated with 'acute' resetting (Eckberg and Sleight, 1992). In intermediate and late hypertension, the slope of the sigmoid relationship between R-R interval and BP (i.e., sensitivity or gain) is often reduced (Eckberg and Sleight, 1992).

In contrast, in this OSA model, we demonstrated evidence of 'acute' but not 'chronic' resetting of the arterial baroreflex control of heart rate; however, we were not able to determine with the resetting was the cause or consequence of hypertension. Could the rightward shift of the baroreflex curve on the pressure axis, without a change in slope, have contributed to maintaining the elevated blood pressures in the dogs with OSA? The parallel shift of the curve indicates that the same systolic BP was associated with a lower R-R interval (or higher HR) during OSA compared to control. This change would suggest that the baroreceptors were no longer counteracting the higher blood pressure (Chapleau et al., 1989) and may therefore be acting indirectly to maintain it (Sleight, 1991). However, our data do not allow us to establish whether the shift in the baroreflex curve has contributed to the elevated BP for two reasons. First, we have only examined the arterial baroreflex control of HR and not of
vascular resistance or stroke volume. Second, changes in the central nervous system may have contributed to the observed change in arterial baroreflex control of HR.

**Rationale for methods**

We chose to use vasoactive drugs to test arterial baroreflex control of HR because this method holds a number of advantages over other approaches. Unlike the neck chamber method, this method is unobtrusive and the subject (in this case, the dog) is not aware that the pressure is being perturbed (Eckberg and Sleight, 1992). No special equipment is needed except for a pressure transducer and the method does not require the co-operation of the subject. Furthermore, we performed the testing under conditions of constant mechanical hyperventilation to avoid the effects of breathing pattern and lung volume on BP (Eckberg and Sleight, 1992).

There are, however, a number of theoretical objections to the method used to test the arterial baroreflex. First, this method allows us to examine the effects of baroreflex influence on HR but not on peripheral vascular resistance. Second, the infusion of vasoactive agents evokes changes in pressure in cardiac and pulmonary circulation, and thus may elicit a response from non-arterial baroreceptors (Head, 1994). Third, the stimulus used is an artificial one.

We chose to administer the vasoactive drugs by infusion rather than by bolus injection for a number of reasons. First, the prominent sinus arrhythmia normally present in dogs tends to obscure the response to bolus injections of drugs. Second, the infusion method, titrated to specific BP levels, allows careful definition of the entire sigmoid systolic blood pressure- R-R interval relationship (Eckberg and Sleight, 1992). Third, the use of steady state BP and R-R intervals for the analysis overcomes the need for precise synchronization of arterial BP and R-R
interval measurements (Eckberg and Sleight, 1992). Finally, the steady state techniques estimates the contribution from both the cardiac sympathetic and vagus (Head, 1994); the cardiac vagus responds very rapidly and is thought to be mainly represented when the ramp method is used (Head, 1994).

The main disadvantage of the steady state method is that infusions of vasoactive agent may allow baroreceptor resetting to occur and thus alter the curve. To minimize this effect, we allowed 15-20 minutes between drug infusions and ensured that BP had returned to control levels before initiating the next infusion.

We used both pressor and depressor agents to study the arterial baroreflex control of HR. This method allowed us to define a greater portion of the sigmoid BP and R-R interval relationship, but the use of both phenylephrine and nitroprusside ignores the potential hysteresis of the baroreflex with rising versus falling pressures (Eckberg and Sleight, 1992). However, we did not observe evidence of hysteresis in the dogs (Figures 52 and 53).

In conclusion, the findings from this study demonstrate that the sustained daytime hypertension that accompanies OSA in the dog is associated with resetting of the baroreceptors to higher pressures but no change in sensitivity of the baroreflex.
8.2. Effects Of OSA on Ventilatory Response to Hypoxia and Hypercapnea

8.2.1. Introduction

The studies presented in section 8.2 describe the effects of OSA on ventilatory responses to progressive hypoxia and hypercapnea. The ventilatory changes associated with OSA are not a focus of this thesis; however, some of the results provide insight into the mechanisms that may be responsible for the pathogenesis of hypertension. In particular, the findings indicate abnormalities in chemoreceptor function that may contribute to the pathogenesis of hypertension.

The peripheral chemoreceptors have been implicated in the pathogenesis of hypertension in OSA in both acute and long-term studies, in humans and animals. Hypoxia, hypercapnea and asphyxia cause an increase in sympathetic nerve activity (Morgan et al., 1995) which further increases when apnea is imposed (Somers et al., 1988a; Hardy et al., 1994). The acute increases in sympathetic activity induced by asphyxia persist after return to normoxia and normocapnea (Morgan et al., 1995). Additional evidence for the role of chemoreceptors comes from a long-term model of episodic hypoxia in the rat (Fletcher et al., 1992b). Increase in diurnal BP were observed in rats with intact peripheral chemoreceptors after 35 days of exposure to episodic hypoxia, but not in carotid body denervated rats (Fletcher et al., 1992b). The BP increase was mediated through enhanced sympathetic activity (Fletcher et al., 1992c).

Given these considerations, this section will briefly outline the effects of OSA on ventilatory responses to hypoxia and hypercapnea. The findings point to an effect of OSA on peripheral but not central chemoreceptors. This work was performed in collaboration with Dr.
John Kimoff in Montreal. Data were collected on five dogs, three from our laboratory and two from Dr. Kimoff’s laboratory. The data from all five dogs will be presented to increase statistical power and allow more valid conclusions.

8.2.2. Methods

Experimental Protocol

OSA was produced in 5 dogs (4 female and 1 male) as described earlier (Chapter 2). Although two of the five dogs were studied in Dr. Kimoff’s laboratory, the method for induction of OSA was identical to that in our laboratory. In each dog, ventilatory responses to progressive hypoxia and hypercapnea were examined in the three phases of the study: before induction of OSA, during OSA, and after cessation of OSA. The control measurements were made 2-3 weeks prior to initiation of the model. OSA was produced for 1-3 months and ventilatory responses were examined during the last two weeks of this phase. Recovery measurements of ventilatory responses were made after at least 8 weeks of cessation of OSA. The dogs’ diet and daily routine remained constant throughout all phases of the study.

Respiratory Procedures and Measurements

Studies were performed during the same time of the day for each dog (between 1000 and 1300). During the studies, the dogs slept in the same position on a comfortable ‘bed’. The electroencephalogram (EEG) was monitored by way of two platinum coated subdermal needle electrodes placed in the scalp on either side of the midline. An electrode placed in the neck served as ground.

During the experiments, the dogs breathed through a cuffed endotracheal tube (10 mm internal diameter) inserted through the chronic tracheostomy. The endotracheal tube was
attached to the breathing circuit that consisted of a pneumotachograph (Fleish No. 2) and a three-way valve. Airflow was measured with the pneumotachograph and a differential pressure transducer (Validyne MP-45). The airflow signal was integrated (Beckman 9873B resetting integrator coupler) to provide tidal volume. Airway CO$_2$ and O$_2$ concentrations were measured with two analyzers (Beckman LB-2 for CO$_2$ and Ametek S-3A for O$_2$) that were calibrated daily with several precision gases. End-tidal CO$_2$ and O$_2$ concentrations were used to calculate end-tidal (alveolar) PCO$_2$ and PO$_2$. The Hill equation was used to calculate oxygen saturation from end-tidal PO$_2$ (Rosing and Cain, 1966). The airflow and the end-tidal CO$_2$ and O$_2$ concentrations signals were received by a microcomputer (model ATI, MEDAC) that provided a breath-by-breath analysis of tidal volume, respiratory frequency, minute ventilation, end-tidal PCO$_2$ and PO$_2$, and calculated S$_a$O$_2$. All signals were also recorded on paper (R612 recorder, Beckman) and on magnetic tape (3968A, Hewlett-Packard).

Hyperoxic progressive hypercapnea and isocapnic progressive hypoxia were induced in the dogs by use of standard rebreathing techniques, as described in detail previously (Phillipson et al., 1977; Phillipson et al., 1978). During progressive hypoxia, P$_A$CO$_2$ was maintained within ±1 mm Hg of the eucapnic control (i.e. pre-rebreathing) level. On any given day, several hypercapnic or hypoxic runs were performed, allowing sufficient time between successive trials for all variables to return to normal. The rebreathing runs were performed during wakefulness, nREM and REM sleep over two successive days during each of the phases. The rebreathing runs were continued until the point of arousal from sleep or movement of the head or body. Sleep stage was determined using behavioral and EEG criteria (Phillipson et al., 1976).
**Data Analysis**

The rebreathing tests were analyzed on a breath-by-breath basis, with calculation of minute ventilation and either $P_A CO_2$ or $S_a O_2$. The ventilatory responses to hypercapnia or hypoxia were then calculated by subjecting the breath-by-breath data to least-squares linear regression analysis over the linear portion of the response curve. The slope and Pearson correlation coefficient (r-value) were calculated. For each phase of study, multiple rebreathing tests were performed in each dog in wakefulness (n=5-6), nREM (n=5-6) and REM sleep (n=4-6).

Two questions were asked: (i) did OSA result in changes in the slope of the ventilatory response to hypoxia or hypercapnea, compared to control (pre-OSA and post-OSA)?, (ii) did OSA result in a change in arousal response to hypoxia or hypercapnea compared to control? Two-way ANOVA (conditions: dog, phase) was performed using commercially available software (*Sigmastat*, Jandel Scientific, San Rafael, CA) to answer these statistical questions. Differences were considered statistically significant if the null hypothesis was rejected at a level of $P<0.05$ using a two-tailed test and post-hoc analysis was performed using the Student-Newman-Keuls method.

### 8.2.3. Results

OSA resulted in little change in the slope of the hypercapnic response compared to control and post-OSA (Table 17, Figure 47). There were no statistically significant differences in the response of minute ventilation to hypercapnea (Figure 47). Furthermore, the arousal responses to progressive hypercapnea during n-REM and REM sleep did not change as a result of OSA (Table 17).
Figure 47: Relationship between end-tidal CO₂ ($P_{ET}CO_2$) and minute ventilation in 5 dogs pre-OSA (filled circles), during OSA (filled squares), and post-OSA (filled triangles). For each set of data, the measurements of $P_{ET}CO_2$ and minute ventilation made at the start and end of the linear portion of the response curve are shown.
Table 17: Mean values for 5 dogs for slope, r-values and $P_{A}CO_{2}$ at the termination of hypercapnic rebreathe

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<th>Phase</th>
<th>Awake Slope (S.E.)</th>
<th>r-value</th>
<th>$P_{A}CO_{2}$ at termination (S.E.)</th>
<th>nREM Slope (S.E.)</th>
<th>r-value</th>
<th>$P_{A}CO_{2}$ at arousal (S.E.)</th>
<th>REM Slope (S.E.)</th>
<th>r-value</th>
<th>$P_{A}CO_{2}$ at arousal (S.E.)</th>
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<td>57.37 (1.50)</td>
<td>2.17 (0.31)</td>
<td>0.84 (0.02)</td>
<td>55.11 (0.94)</td>
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<td>0.59 (0.09)</td>
<td>58.38 (1.38)</td>
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<tr>
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<td>57.29 (1.45)</td>
<td>2.00 (0.29)</td>
<td>0.86 (0.02)</td>
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<td>1.14 (0.18)</td>
<td>0.66 (0.07)</td>
<td>60.26 (1.23)</td>
</tr>
<tr>
<td>post-OSA</td>
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<td>1.83 (0.34)</td>
<td>0.86 (0.03)</td>
<td>55.72 (0.90)</td>
<td>1.16 (0.26)</td>
<td>0.59 (0.08)</td>
<td>59.82 (1.35)</td>
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</tbody>
</table>

In contrast to the responses to hypercapnea, OSA was accompanied by a significant decrease in the slope of the ventilatory response to hypoxia during wakefulness, and by a trend towards a decline during nREM sleep (Table 18 figure 48). There was also a significant increase in arousal threshold to hypoxia (i.e., lower arousal SaO2) in nREM and REM sleep during OSA compared to control (Table 18).
Figure 48: Relationship between calculated $S_{a}O_2$ and minute ventilation in 3 dogs pre-OSA (filled circles), during OSA (filled squares), and post-OSA (filled triangles). For each set of data, the measurements of $S_{a}O_2$ and minute ventilation made at the start and end of the linear portion of the response curve are shown.
Table 18: Mean values for 5 dogs for slope, r-values and \( S_aO_2 \) at the termination of hypoxic rebreathe

<table>
<thead>
<tr>
<th>Phase</th>
<th>Awake</th>
<th></th>
<th></th>
<th>nREM</th>
<th></th>
<th></th>
<th>REM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (S.E.)</td>
<td>r-value (S.E.)</td>
<td>( S_aO_2 ) at termination (S.E.)</td>
<td>Slope (S.E.)</td>
<td>r-value</td>
<td>( S_aO_2 ) at arousal (S.E.)</td>
<td>Slope (S.E.)</td>
<td>r-value</td>
</tr>
<tr>
<td>pre-OSA</td>
<td>-1.12 (0.16)</td>
<td>-0.82 (0.06)</td>
<td>68.55 (1.74)</td>
<td>-0.90 (0.11)</td>
<td>-0.91</td>
<td>64.98 (3.49)</td>
<td>-0.78 (0.10)</td>
<td>-0.72 (0.07)</td>
</tr>
<tr>
<td>OSA</td>
<td>-0.78** (0.12)</td>
<td>-0.83 (0.06)</td>
<td>57.76** (4.08)</td>
<td>-0.83 (0.15)</td>
<td>-0.87</td>
<td>60.56** (4.15)</td>
<td>-0.90 (0.11)</td>
<td>-0.80 (0.03)</td>
</tr>
<tr>
<td>post-OSA</td>
<td>-0.93 (0.29)</td>
<td>-0.85 (0.02)</td>
<td>66.36 (1.92)</td>
<td>-1.00 (0.28)</td>
<td>-0.89</td>
<td>68.07 (2.00)</td>
<td>-0.93 (0.12)</td>
<td>-0.73 (0.04)</td>
</tr>
<tr>
<td>p-value from ANOVA</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>0.054</td>
<td>&lt;0.001</td>
<td>not significant</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( S_aO_2 \) at the termination of hypoxic rebreathe; ** denotes statistically significant difference from baseline (pre-OSA)

8.2.4. Discussion

The findings of this study indicate that long-term application of the OSA model in the dogs did not result in changes in the ventilatory and arousal response to \( CO_2 \). However, OSA did produce a significant decline in the arousal response to hypoxia, and a decrease in ventilatory response to hypoxia during wakefulness and possibly nREM sleep. These findings strongly suggest that different mechanisms are involved in the impairment of hypoxic and hypercapnic ventilatory responses in OSA.

Sleep fragmentation during OSA may have contributed to the impaired hypoxic responsiveness with OSA. Several authors have examined the effects of sleep disruption on ventilatory control. In healthy subjects, sleep deprivation for 24–48 hours decreases the
ventilatory responses to hypoxia and hypercapnea (Cooper and Phillips, 1982; White et al., 1983). However, patients with OSA experience sleep fragmentation that may have different physiological effects than those associated with sleep deprivation. Short-term acoustic-induced sleep fragmentation does not affect the ventilatory responses to hypercapnea or hypoxia in sleeping dogs (Bowes et al., 1980) or the ventilatory responses to hypercapnea in normal subjects (Espinoza et al., 1991). Furthermore, in view of the unchanged hypercapnic responsiveness in our study, it is difficult to postulate an exclusive effect of sleep fragmentation on hypoxic but not hypercapnic responsiveness.

Metabolic factors may also contribute to the decreased hypoxic responsiveness. However, a decreased metabolic rate with OSA would have decreased the rate of induction of both hypoxia and hypercapnea during rebreathing trials. Since the changes in ventilatory responses were only seen for the hypoxic stimulus, it is unlikely that a major change in metabolic rate occurred with OSA.

The decreased responsiveness and delayed arousal threshold to hypoxia may represent a specific adaptation to a stimulus repeatedly generated during apneas. Such an adaptation could be the result of either changes in the peripheral chemoreceptors or central down-regulation of the response to hypoxia. The central effect may be mediated through an altered processing of carotid body stimuli or a change in responsiveness to direct central effects of hypoxia (e.g., hypoxia induced accumulation of neuroinhibitory peptides). Our data do not allow us to establish which of these mechanisms were responsible for the altered hypoxic responsiveness.
Ventilatory Control in OSA

There are several studies of ventilatory control in patients with OSA, but the results are controversial. Patients with the Pickwickian or obesity-hypoventilation syndrome, a subgroup of patients with OSA, have decreased hypoxic and hypercapnic ventilatory drive (Rochester and Enson, 1974; Zwillich et al., 1975). It has not been established whether these patients have congenitally low ventilatory responses that may contribute to the pathogenesis of OSA, or whether the responses are blunted secondary to chronic hypoxemia and hypercapnea (Marcus et al., 1994). However, obesity, a main feature of this syndrome, may be a confounding factor in most of these studies, since weight loss in such patients leads to marked improvement in ventilatory responsiveness to hypercapnea (Pederson and Torp-Pedersen, 1960). Another confounding variable in this patient population is the effect of abnormal baseline blood gases on the stimulus to the chemoreceptors. Nevertheless, the ventilatory response to hypercapnea appears to be depressed in hypercapnic patients with OSA (Garay et al., 1981; Radwan et al., 1995), but not in non-hypercapnic patients (Rajagopal et al., 1984). Similarly, children with OSA and normal blood gases also have normal ventilatory responses to hypercapnea and hypoxia (Marcus et al., 1994). In our study, the dogs had normal lung function prior to OSA and we observed no significant hypercapnea or hypoxia during wakefulness or sleep during the application of OSA.

Long-term administration of CPAP to hypercapnic patients with OSA produced a shift on the x-axis of the ventilatory responses to hypercapnea (Berthon-Jones and Sullivan, 1987; Lin, 1994), and hypoxia (Lin, 1994). No change in CO₂ response was noted in eucapnic patients with OSA after nasal CPAP (Berthon-Jones and Sullivan, 1987; Greenberg and Sharf,
1993); but tracheostomy results in improvement in ventilatory response to progressive hypercapnea even in non-hypercapnic OSA patients, manifested as either an increased slope (Albert-Tuken et al., 1980; Guilleminault and Cummiskey, 1982) or leftward shift without change in slope (Sullivan and Issa, 1980). Differences in the findings between CPAP and tracheostomy may be the indirect effects of tracheostomy on ventilatory control (independent of relief of obstruction) or differences in the rebreathing technique used (Berthon-Jones and Sullivan, 1987).

*Relevance to the Pathogenesis of Hypertension*

In this study, we found that OSA caused changes in the ventilatory response to hypoxia, but no change in the response to hypercapnea. This finding suggests possible alterations in the peripheral chemoreceptor function or decreased responsiveness to hypoxia in the central nervous system, as a result of OSA. Although we did not specifically examine the role of the chemoreceptors in control of BP, it is possible that changes in peripheral chemoreceptor function may play a role in the pathogenesis of hypertension. However, there is no clear evidence to-date, either from the literature or from this study, that such alterations will contribute to hypertension, and the potential mechanisms involved have not been clarified.

Among patients with OSA, hypertensive patients have a higher ventilatory response to hypoxia than normotensives (Vlachogianni et al., 1989; Tafil-Klawe et al., 1991). This finding suggests that the peripheral chemoreflex may contribute to the development of hypertension (Tafil-Klawe et al., 1991), although the mechanisms involved have not been
determined. Since all dogs developed elevated BP, we were not able to compare the ventilatory response between normotensive and hypertensive dogs.

In addition, chemoreceptors are involved in the cardiovascular effects of OSA. Hypoxia and hypercapnea cause a persistent increase in sympathetic nerve activity (Morgan et al., 1995) which is accentuated by lack of ventilation (Somers et al., 1988a; Hardy et al., 1994). This sympathetic response to hypoxia is exaggerated in borderline hypertensive patients compared to normotensives, possibly as a result of an impaired baroreflex (Somers et al., 1988b). In addition, hypertensive and normotensive patients with OSA demonstrate an exaggerated pressor response to hypoxia (Hedner et al., 1992). Thus, patients with OSA, especially those with borderline hypertension, may experience exaggerated sympathetic activation and BP surges during apnea, which may potentially lead to daytime hypertension (Somers and Abboud, 1993).

More direct evidence of a role for the peripheral chemoreceptors in the pathogenesis of hypertension in OSA can be derived from the model of episodic hypoxia in the rat (Fletcher et al., 1992b). Carotid body denervation prevented the increase in diurnal BP observed in intact rats after 35 days of exposure to episodic hypoxia (Fletcher et al., 1992b). It is interesting to note however that long-term steady state hypoxia does not seem to act as a stimulus for hypertension; for example, the prevalence of hypertension in patients with restrictive lung disease and comparable nocturnal desaturation to those with OSA is less than in patients with OSA (Shiner et al., 1990). It appears that the episodic nature of hypoxia in the rat model by Fletcher et al. (1992c) is important for the pathogenesis of hypertension mediated via the peripheral chemoreceptors. The exact mechanisms by which the
chemoreceptors contribute to the pathogenesis of hypertension in OSA remain to be determined.

In conclusion, we have demonstrated impaired hypoxic ventilatory and arousal responses in the canine model of OSA, but no change in the hypercapnic responses. We postulate that the nocturnal repetitive hypoxic events may contribute to impaired peripheral chemoreceptor function or decreased central responsiveness to hypoxia.
Chapter 9: Conclusions and Future Studies

In this final chapter, I aim to summarize the main findings of the methodological and physiological studies that I presented in Chapter 2-8. These studies have described a canine model of OSA and have shown that OSA causes changes in the acute responses to airway occlusions. In addition, the findings provide the first direct evidence of a cause and effect relationship between OSA and daytime hypertension that cannot be attributed to the sleep fragmentation associated with OSA. Future studies need to be performed to determine the stimuli, risk factors and mechanisms involved in the relationship between OSA and hypertension. The following discussion considers the results, limitations of the studies and outlines areas for future work.

Limitations of the Model of OSA

There are a number of limitations to this canine model of OSA. The site of occlusion in the dogs bypasses the nasopharynx and oro-pharynx, which is the site of occlusion in humans. This area of the upper airway has a rich afferent system that theoretically might alter the BP response to airway occlusion. Another limitation of this model is the rate of development of OSA in our dogs. We artificially increased the apnea index from 10 to 25 apneas per hour of sleep during the first 2 weeks of the protocol, to an index of 50 to 60 after 14 nights. This rate of apnea increase is likely much faster than occurs in human OSA. Finally, because of the small sample size, this study could not address the issue of variability in disease and disease consequences. Despite these limitations and the species differences, our findings are likely relevant to OSA in humans given the marked similarities between this canine model of OSA and the human condition (Kimoff et al., 1994).
Model of OSA

In Chapter 2, I described a unique induced model of OSA in the dog. With the use of biotelemetry and computers, the model allowed induction of OSA without any physical attachments between the animal and the monitoring apparatus. Furthermore, the model of OSA functioned independently of human intervention and required only routine monitoring.

The development of this long-term model of OSA represents a substantial development in the field of sleep-disordered breathing because it provides an effective tool for the study of the consequences of OSA. Previously, several investigators had produced short-term (≤ 48 hours) models of OSA in rats (Cragg and Phillips, 1984), new-born lambs (Fewell et al., 1988), pigs (Pinto et al., 1993) and dogs (Scharf et al., 1992; O'Donnell et al., 1994); all these models required constant monitoring by laboratory personnel. Kimoff et al. (1994), from our laboratory, had produced OSA in the dog for 5 consecutive days; however, these 5 day experiments were performed before the telemetry unit and remote-controlled occlusion valve were operational and required continuous human supervision.

Our model of OSA was applied to investigate one of the consequences of OSA, specifically hypertension. The results presented in Chapter 7 show that severe OSA (apnea index 50-60 apneas/hour of sleep) for 1-3 months resulted in sustained daytime hypertension. However, from the available data, it was not possible to determine the exact severity of OSA required to produce such hypertension.

Several factors related to the severity of the disease could be modified in this induced model. First, the occlusion-valve could be adjusted to produce only partial airway occlusion which would permit investigation of the consequences of increased airway resistance to
replicate snoring, without complete occlusion of the airway. Second, because the apnea index can be altered in the sleep detection program by changing the number of consecutive epochs of sleep required to generate the signal to close the occlusion valve, the degree of OSA needed to cause hypertension could be determined. Third, the application of the model as described in Chapter 2 was limited to less than 3 months because of the limited battery life of the telemetry unit. With an extended battery life, the effects of a longer period of OSA on cardiovascular function could be examined. Finally, using this animal model, the effects of the overlap of OSA and other respiratory conditions could be investigated. For example, there is considerable interest on the effects of the co-existence of OSA and chronic respiratory conditions (see section 1.8.5). Respiratory impairment could be created in the animals by surgically paralyzing the diaphragm or by increasing the respiratory load of the animal, and the cardiovascular and respiratory effects of the overlap of the two syndromes could be examined.

**Model of Sleep Fragmentation**

Similar to OSA, the induced model of sleep fragmentation described in Chapter 4 functioned independently and required minimal human intervention except for routine maintenance. The system was operational on a nightly basis for up to 2 months, allowing us to examine the long-term consequences of sleep fragmentation and to compare them to the long-term consequences of OSA.

Several models of short-term sleep fragmentation or deprivation have been described in the literature (Leiter et al., 1985; Cooper and Philips, 1982; White et al., 1983; Bowes et al., 1983; Espinoza et al., 1991). The unique features of our model are two-fold: first, sleep fragmentation was produced over several weeks; second, the system minimized
acclimatization of the dogs to acoustic stimuli that may occur with repetitive noise (Roehrs et al., 1994; Frederickson and Rechtschaffen, 1978; Williams et al., 1964). Specifically, if the dog failed to arouse at the point of activation of the alarm, the frequency of the sound progressively increased along a variable ramp until the dog aroused from sleep.

One area of interest is the effect of sleep disruption on cognitive function. We have collaborated with Dr. William Milgram in the Department of Psychology at the University of Toronto to perform preliminary studies in our dogs on the effects of sleep fragmentation and of OSA on cognitive function. An important experiment that can be performed is to modify the model of sleep fragmentation to determine the effects of fragmentation in one sleep stage, such as REM sleep only, on cognitive function.

**Stimuli for the Pathogenesis of Hypertension in OSA**

Speculation as to whether OSA is an independent risk factor for systemic hypertension has been prominent in several reviews (Stradling, 1989; Levinson and Millman, 1991; Carlson et al., 1993). In particular, considerable speculation has centered on the role of recurrent arousal from sleep. The results of the study described in Chapter 7 show that OSA per se leads to sustained daytime hypertension, and that recurrent arousals from sleep contribute to the nighttime increase in BP but not to the daytime changes. However, the studies in chapter 7 did not identify the specific stimulus that was responsible for the increase in daytime BP. The potential stimuli that have been postulated to cause daytime hypertension are arousal, episodic hypoxia, and the hemodynamic effects of cessation of breathing. The effects of these different stimuli in combination need to be examined. First, the effects of recurrent arousals and exposure to negative intrapleural pressure (without hypoxia) could be examined by subjecting the dogs to
airway occlusion during sleep while breathing sufficient oxygen to prevent oxygen desaturation during the apnea. Second, the effects of recurrent arousals and hypoxia (without negative intrapleural pressure swings) could be investigated by subjecting the dogs to recurrent hypoxic rebreathing during sleep to the point of arousal. These experiments, using modifications of the methods described in this thesis, may answer these important questions. With respect to hypoxia, Fletcher and colleagues (1992a) have shown that episodic hypoxia alone, regardless of sleep-wake state, causes diurnal hypertension in rats. These findings suggest that hypoxia may be critical in the pathogenesis of hypertension in patients with OSA.

**Potential Mediators of Hypertension in OSA**

The studies described in Chapter 8 indicate that the arterial baroreceptor reflex control of HR was reset to the higher pressure during OSA, without a change in slope. In addition, the ventilatory responses to progressive hypoxia were altered, suggesting a change in the activity of the peripheral chemoreceptors during OSA. There was no detectable change in plasma catecholamine levels with OSA. However, these studies did not identify the specific mediators that were involved in the pathogenesis of OSA. A crucial experiment that needs to be performed is to determine the role of the sympathetic nervous system in the pathogenesis of hypertension. In preliminary studies in collaboration with Dr. John Floras, we attempted to record sympathetic activity from the peroneal nerve by microneurography. However, consistent sympathetic nerve discharges could only be recorded for very short time periods. Further attempts to measure sympathetic activity, either from a peripheral nerve or from an implanted electrode in the renal sympathetic nerve may elucidate the role of the sympathetic nervous system in the pathogenesis of hypertension in OSA.
The results in Chapter 8 suggest changes in baroreceptor and chemoreceptor responses in OSA. To specifically determine the role of these responses, the effects of baroreceptor denervation or peripheral chemoreceptor denervation on the development of hypertension in OSA could be examined.

Risk Factors for the Pathogenesis of Hypertension in OSA

A logical speculation arising from the observation that OSA causes systemic daytime hypertension is that this relationship may be enhanced in the presence of other risk factors. In particular, it is reasonable to hypothesize that the degree of hypertension in the dogs with induced OSA may be augmented by age, obesity, glucose intolerance, and lipid abnormalities. In the studies described in Chapter 7, the youngest dog (age, 4 years) demonstrated the least change in daytime BP. However, the sample size was too small to make meaningful conclusions in this regard. Further experiments in older or obese dogs may answer these important questions.

Other Cardiovascular Consequences of OSA

During the series of experiments described in this thesis, we also collaborated with Dr. John Parker to examine the effects of OSA on left ventricular function. The findings indicate that long-term OSA results in decreased left ventricular ejection fraction and increased left ventricular mass. A logical extension of these studies is to examine the effects of OSA on right ventricular function and pulmonary artery pressure. In addition, the effects of OSA in dogs with compromised cardiovascular status (e.g., coronary artery occlusion) may elucidate the association between OSA and cardiovascular disease identified in several epidemiological studies (see Chapter 1).
**Acute Responses to Airway Occlusion in OSA**

The findings of the studies in Chapter 6 show that the long-term induction of OSA resulted in altered acute responses to airway occlusion, including prolonged duration of apnea, greater arterial hemoglobin desaturation, increased peak inspiratory effort, and greater surges in BP. These changes progressed over the course of the disease. In addition, this study showed that long-term sleep fragmentation, without airway obstruction, resulted in similar alterations in the acute responses to airway occlusion. This later finding indicates that the changes in the responses to airway occlusion with OSA are primarily a function of sleep fragmentation, rather than of the other stimuli such as hypoxia, hypercapnea or repeated respiratory efforts against a closed airway. However, the studies did not determine the mechanisms responsible for the changes in the acute responses. The longer duration of apnea may be the result of decreased ventilatory responsiveness to the respiratory stimuli, particularly hypoxia (White et al., 1983; Cooper and Phillips, 1982; Schiffman et al., 1983).

**Management Recommendations**

The results of the studies in Chapter 7 showing a direct relationship between OSA and hypertension have considerable clinical implications. Many hypertensive patients may have OSA that contributes to their high BP. Although routine sleep studies of all hypertensive patients may not be warranted, a careful sleep history is indicated in all hypertensive patients, including attention to snoring, witnessed apneas and daytime sleepiness. The presence of symptoms of OSA may indicate the need for further sleep investigation in specific patients (Carlson et al., 1993).
Concluding Remarks

The studies described in this thesis were designed to determine whether OSA, per se, causes sustained daytime hypertension. The results described herein provide the first direct evidence that OSA causes hypertension. In addition, the development of hypertension could not be attributed to the recurrent arousals from sleep that characterize the OSA syndrome. However, the studies described in this thesis have raised several additional questions related to the OSA syndrome, and have pointed to further experiments that would be needed to answer these questions.
APPENDIX 1

Illustration of the Modified Interval Histogram Method for EEG Analysis
APPENDIX 2

Sleep Detection Program

This program was originally written by Hideo Makino; however, I modified the program by introducing a second EMG threshold to improve accuracy.

DECLARE SUB ADTHR (EMGTHR!)
DECLARE SUB DSAVE ()
DECLARE SUB TC2 ()
DECLARE SUB DISPCAL (CHANNEL!)
DECLARE SUB DTREAD (VMAX, VMIN)
DECLARE SUB TC1 ()
DECLARE SUB AD (CHN, TOT)
DECLARE SUB FRQ ()
DECLARE SUB WINTVL (DXB!, DOLD, RC!)
DECLARE SUB CALB (CHANNEL, CAL1, AVR)
DECLARE SUB GETDATA (AMP, AMP2, EMGSUM)

' FILE NAME "APNEAI0.txt"
' 1) In hardcopy file, record No. was changed to 1200 from 500.
' 2) On data display, the first line is kept the same position.
' 3) file name of the datafile was changed to "#"mmd-N0.dat
' 4) At first menu, THAMP1, 2 were removed. INITIAL input was added.
' 5) When AMP < 10, display NO SIGNAL
' 6) On April 26, 1994, changed parameters to maximize detection in Tina.

DIM INDATA(2, 3000), DT(801), INTVL(801), WFRQ(6) FOR EEG AND EMG
COMMON SHARED INDATA(), MAXREC, CAL, CAL1, D100, AVR1, AVR2, DT(), INTVL(),
WFRQ(), ADDRESS, D200.
VOLT, PSTAT, MAXCLOSE, VWAIT, PAGE, B2D1, B2D1EXT, SWSEMG, EMGEXT1, INITIAL$ FOR EEG AND EMG

' COMMON INDATA(), MAXREC, CAL, CAL1, D100, AVR1, AVR2, DT(), INTVL()
' WFRQ(), ADDRESS, D200. VOLT, PSTAT, MAXCLOSE, THAMP1, THAMP2, SWSEMG,
EMGEXT1, VWAIT

SCREEN 9: CLS : PSTAT = 1
' PSTAT : past status of judgment DISPLAY
time = 5: MAXREC = time * 300
' TIME : acquisition time, MAXREC: total record
VWAIT = 3: MAXCLOSE = 18
' VWAIT : OBSERVATION TIME 4* 6 sec
INITIAL$ = "T"
' INITIAL : first character of datafile
ADDRESS = 256 * 7 + 16
' BASIC ADDRESS = 710H, SAMPLE 3.3 msec
PAGE = 1: B2D1EXT = 3.9
' DEFAULT value for B2/D1
PAGE = 1: EMGEXT1 = 55
' DEFAULT FOR EMGTHR

***** FIRST MENU *****

350 CLS : LOCATE 5, 5: PRINT " PARAMETER CHANGE ? "
PRINT
PRINT " 1) INPUT ONE CHARACTER FOR DATA FILE NAME ; default= T" FOR
datafile: changed 06/16/93
PRINT " 2) CHANGE MAX VALVE CLOSING TIME - now MAXCLOSE= 108 sec;"
PRINT " 3) NEXT STEP -- START CALIBRATION & MEASUREMENT"
PRINT " 4) CHANGE B2/D1 FACTOR, now B2/D1=; B2D1EXT: B2D1 = B2D1EXT' corrected 05/29/92
PRINT " 5) CHANGE THRESHOLD FOR EMG TO DISTINGUISH non-REM and Moving W=";
EMGEXT1: SWSEMG = EMGEXT1: CORRECTED 04/28/93
PRINT " 6) EXIT" PRINT
PRINT *** TO EXIT PROGRAM DURING OPERATION, PLEASE HIT SPACE BAR **** % ANY KEY to SPACE BAR

355 SEL$ = INKEY$: IF SEL$ = "" THEN GOTO 355
  IF SEL$ = "1" THEN GOTO 360 ' CHANGE left char of datafile
     IF SEL$ = "2" THEN GOTO 370 ' CHANGE MAX VALVE CLOSING TIME
     IF SEL$ = "3" THEN CLS : GOTO 400 ' NEXT STEP
     IF SEL$ = "4" THEN GOTO 380 ' CHANGE B2/D1
     IF SEL$ = "5" THEN GOTO 390 ' CHANGE EMG THRESHOLD
     IF SEL$ = "6" THEN GOTO 180 ' EXIT
     GOTO 355
   
360 CLS
   INPUT "INPUT ONE INITIAL CHARACTER = "; INITIALS
   GOTO 350

370 CLS
   MAXCLS = MAXCLOSE ' VWAIT = 3; default factor VWAIT * 6 = 30 sec
   -- observation time
   PRINT "INPUT MAXIMUM VALVE CLOSING TIME. NOW IT IS "; MAXCLOSE * 6; " sec"
   INPUT " ONE COUNT = 6 sec COUNT 7 = 42 sec COUNT = "; MAXCLS
   IF MAXCLS > 0 THEN MAXCLOSE = MAXCLS
   GOTO 350

380 INPUT " INPUT B2/D1=; B2D1EXT " &amp;&amp; changed form B2D1 to B2D1EXT (05/29/92)
   GOTO 350

390 INPUT " THRESHOLD FOR EMG AMPLITUDE TO DETECT SWS="; EMGEXT1 ' ADDED 01/04/93
   GOTO 350

' ***** SECOND MENU *****

400 VIEW PRINT 1 TO 25: LOCATE 1, 1
   PRINT "*** Please select No. *** 1) EEG CAL. 2) EMG CAL. 3) SLEEP DETECT 4) EXIT "
401 SEL$ = INKEY$: IF SEL$ = "" THEN GOTO 401
  IF SEL$ = "1" THEN GOTO 403 'EEG CAL
  IF SEL$ = "2" THEN GOTO 420 'EMG CAL
  IF SEL$ = "3" THEN GOTO 450 'SLEEP DETECT
  IF SEL$ = "4" THEN GOTO 180 'EXIT
   GOTO 400
CLS : LOCATE 2. 1: PRINT " Calibration for EEG. IF READY, HIT ANY KEY"
CALL TC2 'SAMPLE=3.3msec
405 Q$ = INKEY$: IF Q$ = "" THEN GOTO 405 ELSE CALL CALB(0, CAL, AVR)
AVR1 = AVR: CALL DSAVE
GOTO 400
420 CLS : LOCATE 15. 1: PRINT " Calibration for EMG. IF READY, HIT ANY KEY"
CALL TC1 'SAMPLE=1.0msec
425 Q$ = INKEY$: IF Q$ = "" THEN GOTO 425 ELSE CALL CALB(1, CAL1, AVR)
AVR2 = AVR: CALL DSAVE
GOTO 400

****** SLEEP DETECTION PROGRAM START ******
OPEN "CALIB.DATU" FOR INPUT AS #4
CAL : EEG 50μV value, AVR1 baseline
INPUT #4, CAL, AVR1, CAL1, AVR2
CAL1: EMG 100μV value, AVR2 baseline
CLOSE #4
D100 = CAL1 / 100: IF D100 = 0 THEN D100 = 1 'Normalize EMG to 100
IF CAL < 1000 THEN D200 = 1: GOTO 1
D200 = CAL / 200 'Normalize EEG to 200

1 VOLT = 100 / CAL 'Correction to 50uV
FOR I = 1 TO 2: FOR J = 1 TO MAXREC 'Clear data input area
INDATA(I, J) = 0
NEXT J: NEXT I

10 CLS
160 CALL TC2: CALL DISPLAY(time)
GOTO 350

180 END '*** END ***
SUB AD (CHN, TOT) STATIC
30 IF INP(ADDRESS + 4) < 128 THEN GOTO 30 'WITHOUT THIS
LO = INP(ADDRESS + 5): HI = INP(ADDRESS + 6) 'TIMER COUNT BECAME STRANGE
TOT = 256 * HI + LO
IF TOT > 32767 THEN TOT = TOT - 65536!
END SUB

SUB ADTHR (EMGTHR) STATIC 'stop timer and
OUT ADDRESS + 4, 129 '129 = 128 + 1; GAIN * 2
OUT ADDRESS + 5, 2 'select A/D ch.2
OUT ADDRESS + 6, 0 'start conversion
CALL AD(2, EMGTHR) 'get data EMG THRESHOLD
IF EMGTHR > 0 THEN EMGTHR = INT(EMGTHR / 10.24) / 10 ELSE EMGTHR = 0 'avoid DC offset of A/D
EMGTHR = EMGTHR * 2
CALL TC2 'start timer 3.3msec again
END SUB

SUB CALB (CHANNEL, CALX, AVR) STATIC
DMAX = 32766: DMIN = 32767: SUM = 0: RC = 3000: THBGN = 5
IF CHANNEL = 0 THEN RC = 1000 'RC : 3 sec sample for calibration signal
CNL = CHANNEL + 1 'EEG 3.3msec/sample, EMG 1 msec/sample
LOCATE 2 + CHANNEL * 13, 1
PRINT " DATA INPUT...wait ' wait for rising edge of signal
OUT ADDRESS + 5, CHANNEL: CALL AD(0, TOT) DUMMY INPUT
110 CALL AD(0, TOT): INDATA(CNL, 1) = TOT ' get data
CALL AD(0, TOT): INDATA(CNL, 2) = TOT
BEGIN = ABS(TOT - INDATA(CNL, 1)) ' check the rising edge
IF BEGIN < THBGN THEN GOTO 110 ' start data sampling
LOCATE 2 + CHANNEL * 13, 1: PRINT " DATA INPUT...start "; SUM = INDATA(CNL, 1) + INDATA(CNL, 2)
' SUM for baseline value
FOR RCRD = 3 TO RC
CALL AD(0, TOT): INDATA(CNL, RCRD) = TOT ' get p-p voltage
IF DMAX < TOT THEN DMAX = TOT
IF DMIN > TOT THEN DMIN = TOT ' DMAX : maximum value
SUM = SUM + TOT ' DMIN : minimum value
NEXT RCRD
AVRX = (DMAX + DMIN) / 2 ' get mean voltage
AVR = CJN(SUM / RC) ' get baseline value
CALX = ABS(DMAX - DMIN) ' get p-p voltage
CALY = ABS(DMAX): IF ABS(DMIN) > CALY THEN CALY = ABS(DMIN) ' get peak voltage
IF CALX < 40 THEN CALY = AVR * 100
FOR J = 1 TO RC ' Normalize for display
INDATA(CNL, J) = INDATA(CNL, J) / CALY * 50 ' value becomes if 50
NEXT J
CALVAL = 50 + 50 * CHANNEL
IF CHANNEL = 0 THEN CAL = CALX: AVR1 = AVR
IF CHANNEL = 1 THEN CAL1 = CALX * 2: AVR2 = AVR
20 LOCATE 2, 1
PRINT " Calibration data 50": CHR$(230); "V="; CAL; " Average=": ; " (MAX-MIN)/2="; AVRX
LOCATE 15, 1
PRINT " Calibration data 100": CHR$(230); "V="; CAL1; " Average=": ; " (MAX-MIN)/2="; AVRX
AVRX
CALL DISPCAL(0): CALL DISPCAL(1)
END SUB

SUB DISPCAL (CHANNEL) STATIC
A = 50: B = 500: C = 100 + CHANNEL * 180: JUMP = 6: CNL = CHANNEL + 1
IF CHANNEL = 0 THEN JUMP = 2
LINE -(A, C), , 0 ' waveform display
LINE -STEP(B, 0)
LINE -STEP(-B, -50), , 0 ' waveform display
LINE -STEP(0, 100)
LINE -STEP(0, -50)

FOR M = 1 TO B
LINE -(A + M, C - INDATA(CNL, M * JUMP)), 10
NEXT M
LINE -STEP(0, 50), , 0
END SUB

SUB DISPLAY (time) STATIC
DIM JUDGES$(9) ' judgment data
JUDGES$(1) = " --WAKE--": JUDGES$(2) = " SWS "
JUDGES$(3) = " SWS " : JUDGES$(4) = " SWS "
JUDGES$(5) = " SWS " : JUDGES$(6) = " **REM** "
JUDGES$(7) = " NOSIGNAL"
*** EMGTHR: EMG THRESHOLD (on-line input from control box) ***

\[ T_1 = 1; \text{PDISP} = 0; T_1 \text{LIMIT} = 1200 \]
\[ \text{PDISP: previous display changed 06/16/93} \]
\[ T_1: \text{segment count} \]
\[ \text{CSWS} = 1 \]
\[ \text{COUNTER FROM BEGINNING OF SWS} \]

\[ \text{FILENAMES} = \text{RIGHTS(INITIALS, 1)} + \text{MIDS(DATES, 4, 2)} + \text{LEFTS(DATES, 2)} \]

900 IF PAGE > 9 THEN SECDIGS = "" ELSE SECDIGS = "0"
\[ \text{HDCOPYS} = \text{FILENAMES} + "." + \text{SECDIGS} + \text{LTRIM(RIGHTS(STR$(PAGE), 2))} + ".\text{DAT}" \]
OPEN HDCOPYS FOR OUTPUT AS #2 ' EXP. DATA STORAGE (SAME OUTPUT TO CRT'

CLS : VIEW PRINT 1 TO 25: LOCATE 1,1
\[ \text{TITLES} = "\text{TIME SEG D2 D1 TH AL B1 B2 EMG THR AMP B2/D1 JUDGEMENT}" \]

PRINT "TIME INTERVAL ="; time; " sec "; PRINT NAMS; " "; DATES, TIMES
PRINT TITLE$
VIEW PRINT 3 TO 25

PRINT #2, "TIME INTERVAL ="; time; " sec "; PRINT #2, NAMS; " "; DATES, TIMES;
"DATAFILE= ":
HDCOPYS
PRINT #2, TITLES

1000 CALL GETDATA(AMP. AMP2, EMGSUM) ' DATA INPUT FOR 5 sec
CALL ADTHR(EMGTHR) ' EMG THRESHOLD LEVEL INPUT

*** JUDGMENT OUTPUT ***

PRINT " "; TIME; " "; PRINT USING "##ff#"; T1; : PRINT " "; FOR I = 1 TO 6: PRINT USING "##ff#"; WFRQ(I); : NEXT I IF WFRQ(2) > 0 THEN SLP = WFRQ(6) / WFRQ(2) ELSE SLP = 0
PRINT USING "###.#": EMGSUM; : PRINT USING "###.#": EMGTWR;
PRINT " "; : PRINT USING "###.#": AMP; SLP;

******* JUDGMENT SECTION ***********

'STATE IS FOR DISPLAY WORDS
IF SLP = 0 OR AMP < 10 THEN STATE = 7: GOTO 999
IF SLP < B2D1 THEN GOTO 1010

GOTO 1015

1010 B2D1 = B2D1EXT: SWSEMG = EMGEXT1: CSWS = 1
\[ \text{IF EMGSUM} \geq \text{SWSEMG} \text{THEN STATE} = 1: \text{GOTO} 999 \]
\[ \text{IF AMP} > 550 \text{THEN STATE} = 1: \text{GOTO} 999 \]
\[ \text{IF AMP} \leq 550 \text{THEN STATE} = 2: \text{CSWS} = \text{CSWS} + 1: \text{GOTO} 999 \]

1015 B2D1 = B2D1EXT: SWSEMG = EMGEXT1: CSWS = 1
\[ \text{IF EMGSUM} < 1 \text{THEN STATE} = 7: \text{GOTO} 999 \]
\[ \text{NO SIGNAL} \]
\[ \text{IF EMGSUM} > \text{EMGTHR} \text{THEN STATE} = 1 \text{ELSE STATE} = 6: \text{GOTO} 999 \]
IF CSWS > 2 THEN B2D1 = B2D1EXT

'Valve control section
VALVE = 0
IF STATE = 1 THEN COUNT = VWAIT: GOTO 2000   % 0 to VWAIT "WAKE"
IF STATE = 7 THEN COUNT = VWAIT: GOTO 2000  % 0 to VWAIT "no signal"
COUNT = COUNT - 1   % + to -   "decrease COUNT"
IF COUNT >= 0 THEN GOTO 2000  % < to > and from VWAIT to 0  : VWAIT: OBSERVATION

PERIOD
IF COUNT < -MAXCLOSE THEN COUNT = VWAIT - 1: VALVE = 0: GOTO 2000
% > to < and from MAXCLOSE to -Maxclose

VALVE = 7: Disp$ = "**"

2000   OUT ADDRESS + 1, VALVE: OUT ADDRESS, 0  'D/A CH.0 OUTPUT for VALVE CONT.

'DISPLAY SECTION
PRINT JUDGE(STATE): : PRINT USING "###": COUNT;
OUT ADDRESS + 3, 4 - STATE: OUT ADDRESS + 2, 255  'D/A CH.1 output as a marker (each 6 sec)
IF VALVE = 7 THEN PRINT Disp$ ELSE PRINT   'PRINT "**" when valve is closed

'DISK OUTPUT
PRINT #2, " ", TIME$: "; : PRINT #2, USING "###": T1; : PRINT #2, " ";
FOR I = 1 TO 6: PRINT #2, USING "#####": WFRQ(I): : NEXT I
PRINT #2, USING "#####": EMGSUM; EMGTHR; : PRINT #2, USING "#####": AMP; SLP;
PRINT #2, JUDGE(STATE); : PRINT #2. USING "#####": COUNT;
IF VALVE = 7 THEN PRINT #2, " "; Disp$ ELSE PRINT #2, " "; 'PRINT "**" when valve is closed

OUT ADDRESS + 3, 4 - STATE: OUT ADDRESS + 2, 0  'second D/A output, real JUDGEMENT
T1 = T1 + 1
IF T1 > T1 LIMIT THEN CLOSE #2: : OUT ADDRESS + 1, 0: OUT ADDRESS, 0: PAGE = PAGE + 1:
T1 = 1: GOTO 900

B$ = INKEY$: IF B$ = "" THEN GOTO 1000
BX = CVI(B$ + ") - 8240  '++ conver B$ to a number. ".0"=8240
IF 0 < BX AND BX < 20 THEN VWAIT = BX  '++ on-line parameter change; VWAIT
IF B$ <> " " THEN GOTO 1000  '++ if inkey$ is not "space", goto next step
CLOSE #2
' else, end of measurements
OUT ADDRESS + 1, 0: OUT ADDRESS, 0  'when exit, open valve

END SUB

SUB DSAVE STATIC
OPEN "CALIB.DAT" FOR OUTPUT AS #3  'file name for calibration data
PRINT #3, CAL, AVR1, CAL1, AVR2  'store calibration data
CLOSE #3
END SUB

SUB DREAD (VMAX, VMIN) STATIC
SUM = 0: VMAX = -32766: VMIN = 32767
' clear data area INDATA()
FOR I = 1 TO 2: FOR J = 1 TO MAXREC: INDATA(I, J) = 0: NEXT J: NEXT I

OUT ADDRESS + 5, 0  'CHANNEL SELECT
CALL AD(0, TOT)  'DUMMY READ
FOR J = 1 TO MAXREC
CALL AD(0, TOT); TOT = TOT - AVR1
IF TOT >= VMAX THEN VMAX = TOT ELSE IF TOT < VMIN THEN VMIN = TOT
IF TOT > CAL THEN TOT = CAL ELSE IF TOT < -CAL THEN TOT = -CAL
INDATA(1, J) = CINT(TOT / D200 / 10) + 401
OUT ADDRESS + 5. 1 ' channel select, ch.1
OUT ADDRESS + 6. 0 ' start conversion
CALL AD(2, TOT) ' get EMG value
OUT ADDRESS + 5. 0 ' set channel to 0 to save conversion time

LIMITEMG = CAL1 / 10
IF EMGCOUNT>1 THEN EMGCOUNT=EMGCOUNT-1: GOTO
TOTA = TOT - AVR2: IF TOTA > LIMITEMG THEN TOTA = LIMITEMG ' voltage limit
IF TOTA < -LIMITEMG THEN TOTA = -LIMITEMG ' lower voltage limit
INDATA(2, J) = CINT(TOTA / D100): SUM = SUM + TOT
NEXT J
AVR2 = CINT(SUM / 1500) ' change baseline level to avoid drift
END SUB ' 1500 is a number of 5 sec data; 300 * 5 sec

SUB FRQ STATIC
FOR I = 1 TO 6: WFRQ(I) = 0: NEXT I: total = 0 ' interval histogram
FOR J = 10 TO 15 ' Beta 2: 20-30 Hz
WFRQ(6) = WFRQ(6) + INTVL(J)
NEXT J
FOR J = 16 TO 22 ' Beta 1: 13.5-20 Hz
WFRQ(5) = WFRQ(5) + INTVL(J)
NEXT J
FOR J = 23 TO 40 ' Alpha: 7.5-13.5 Hz
WFRQ(4) = WFRQ(4) + INTVL(J)
NEXT J
FOR J = 41 TO 76 ' Theta: 7.5-4 Hz
WFRQ(3) = WFRQ(3) + INTVL(J)
NEXT J
FOR J = 77 TO 149 ' Delta 1: 2-4 Hz
WFRQ(2) = WFRQ(2) + INTVL(J)
NEXT J
FOR J = 150 TO 200 ' Delta 2: 0.5-2 Hz
WFRQ(1) = WFRQ(1) + INTVL(J)
NEXT J

FOR K = 1 TO 6: total = total + WFRQ(K): NEXT K
IF total <= 10 THEN GOTO 98 ' get % component ' cancel because of noise
FOR M = 1 TO 6: WFRQ(M) = WFRQ(M) * 100 / total: NEXT M
' WFRQ(2) = WFRQ(1) + WFRQ(2); WFRQ(1) = 0
' WFRQ(5) = WFRQ(5) + WFRQ(6); WFRQ(6) = 0
GOTO 99
98 FOR K = 1 TO 6: WFRQ(K) = 0: NEXT K
99 END SUB

SUB GETDATA (AMP, AMP2, EMGSUM) STATIC
FOR J = 1 TO 80: DT(J) = 0: INTVL(J) = 0: NEXT J ` INTVL: interval
AMP = 0: AMP2 = 0: EMGSUM = 0

CALL DTREAD(VMAX, VMIN) ' data are in INDATA(), 1 is for EEG, 2 is for EMG
DOLD = 0: SUM = 0 'DATA STORAGE FOR WAVE COMPARISON
FOR RC = 1 TO MAXREC
DXB = INDATA(1, RC)
IF DXB = DOLD THEN GOTO 300 'JUMP IF WAVE IS FLAT
IF RC < 5 THEN GOTO 290 'JUMP TO AVOID START EDGE INTERVAL COUNT
IF DXB > DOLD THEN CALL WINTVL(DXB, DOLD, RC)
290 DOLD = DXB
300 SUM = SUM + ABS(INDATA(2, RC))
90 NEXT RC
AMP = (VMAX - VMIN) * VOLT 'ACTUAL VOLTAGE VOLT = 0.33
AMP2 = VMAX: IF -VMIN > VMAX THEN AMP2 = -VMIN
AMP2 = AMP2 * VOLT 'actual voltage for AMP2
MAX10 = MAXREC / 10: EMGSUM = SUM / MAX10  'get EMG mean value
CALL FRQ
END SUB

SUB TC1 STATIC 'program for 1 msec counter
ADDGAIN = 1 'GAIN * 2
X = INP(ADDRESS + 6)
OUT ADDRESS + 4, 128 'set timer
OUT ADDRESS + 9, 23
OUT ADDRESS + 8, 0
OUT ADDRESS + 8, 128
OUT ADDRESS + 9, 5 'SET COUNTER 5
OUT ADDRESS + 8, 49
OUT ADDRESS + 8, 13 'MAIN FRQ 13 = 10 kHz
OUT ADDRESS + 8, 16 '16=10H FOR 1.0msec INTERVAL
OUT ADDRESS + 8, 0
OUT ADDRESS + 9, 112 'START COUNTERING
OUT ADDRESS + 4, 132 + AD DGAIN 'external conversion start.
END SUB

SUB TC2 STATIC 'program for 3.3 msec timer
ADDGAIN = 1 'GAIN * 2
X = INP(ADDRESS + 6)
OUT ADDRESS + 4, 128 'set parameters
OUT ADDRESS + 9, 23
OUT ADDRESS + 8, 0
OUT ADDRESS + 8, 128
OUT ADDRESS + 9, 5 'SET COUNTER 5
OUT ADDRESS + 8, 49
OUT ADDRESS + 8, 13 'MAIN FRQ = 10 kHz
OUT ADDRESS + 8, 51 'FOR 3.3 msec OUTPUT
OUT ADDRESS + 8, 0
OUT ADDRESS + 9, 112 'START COUNTERING
OUT ADDRESS + 4, 132 + AD DGAIN 'start external conversion
END SUB

SUB WINTVL (DXB, DOLD, RC) STATIC
B1 = DOLD + 1
IF DT(DXB) = 0 THEN GOTO 60
XINT = RC - DT(DXB)
IF XINT > 600 THEN GOTO 60 '< 0.5 Hz limit
IF XINT > 150 THEN XINT = 151 '< 2 Hz limit
INTVL(XINT) = INTVL(XINT) + 1 'get time interval
60 FOR DXC = B1 TO DXB
DT(DXC) = RC
NEXT DXC
' DT(DXC,1) = TIMER COUNTER
END SUB
APPENDIX 3

Detailed Schematic of the Alarm
APPENDIX 4

Validation of Apparatuses Common to Chapters 6-8

Properties of the Chart Recorder

Three properties were considered: the voltage linearity, the frequency response, and the response time. To test the voltage linearity, we connected a signal generator to the recorder, inserted a sine wave signal of 0.1-3 volts and observed the displacement on chart paper. The linearity of the chart recorder over this range of signals is shown in figure I(A). Linear regression analysis of these data showed that the measured displacement on chart paper was strongly correlated with the input voltage (correlation coefficient=0.99). The 0-90% response time of the chart recorder was less than 8 msec. Similarly, input signals of 0-80 Hz were used to determine the frequency response of the chart recorder. The upper limit of the frequency response of the pen of the chart recorder was identified as the frequency at which the amplitude of the output was considerably compromised, and this was found to occur at 80 Hz (figure I(B)).

5.2.1b. Measurement of Airflow

A very low range differential pressure transducer was used to measure the pressure across the mesh in the pneumotachograph. We examined the frequency response, linearity and resonant frequency of this differential pressure transducer. Linearity of the pressure transducer was tested by providing different pressures and recording the displacement on a chart recorder. Linear regression analysis of the data showed that the pressure transducer was linear over the range of -1 to +1 cm of H₂O (correlation coefficient=0.99) (figure II). The 0-90% response time
Figure I: Linearity and frequency response of chart recorder. Graph A represents the relationship between input voltage and the displacement on chart paper. Solid line, calculated linear regression. Graph B shows that the upper limit of the frequency response of the pen was 70 Hz.
Figure II: Linearity of the differential pressure transducer used for measurement of airflow. The displacement on chart paper in response to applied pressure demonstrates that the pressure transducer was linear over the range of -1 to +1 cm of H_2O. Solid line, calculated linear regression.
of this transducer to "instantaneous" pressure change (i.e. bursting a balloon) was 0.5 msec at a resonant frequency of 25 Hz.

The linearity of the pneumotachograph and differential pressure transducer for the measurement of air flow was validated by connecting the system to a rotameter and recording the displacement on chart paper for different values of fixed flow from the rotameter. The linearity of the pneumotachograph for the recording of known flow signals is shown in figure III. To simulate inspiration and expiration, the rotameter was connected to each end of the pneumotachograph. Linear regression analysis of these data showed that the displacements observed on chart paper were highly correlated to the flow rates (correlation coefficient=1.00).

**Measurement of Oxygen Saturation**

We validation the accuracy of the oximeter in one dog. For this experiment, the dog was anaesthetized with halothane and a catheter was inserted into a carotid artery. The dog's temperature was also recorded with a rectal probe. The dog was then made progressively hypoxic by rebreathing a mixture with low oxygen, during which samples of carotid arterial blood were drawn quickly (within 1-3 breaths) into heparinized syringes. The arterial blood samples were analyzed within 2 minutes of collection for $P_aO_2$, $P_aCO_2$, temperature, and calculated $S_aO_2$ using a commercially available arterial blood gas machine. The results are shown in figure IV and demonstrate close agreement between $S_aO_2$ measured with the ear oximeter and that determined in arterial blood (correlation coefficient=0.993, slope=1.0, p<0.001).
Figure III: Linearity of the pneumotachograph used for measurement of airflow. The displacement on chart paper in response to known flow rates demonstrates that the pneumotachograph was linear over the range of inspiratory and expiratory flows. **Solid line**, calculated linear regression.
Figure IV: Relationship between arterial O₂ saturation (SₐO₂) measured simultaneously by ear oximeter and by sampling carotid arterial blood in one dog. *Solid line*, line of identity.
**Measurement of Airway Pressure**

The changes in airway pressure were measured with a strain gauge pressure transducer (Statham P23Db transducer, Gould Inc.). The ability of this system to accurately record pressure was tested by subjecting the transducer to different positive (expiratory) and negative (inspiratory) pressures and recording the displacement on chart paper. Linear regression analysis of these data

**Measurement of BP**

The manometer-tipped catheter was calibrated with a mercury manometer referenced to atmosphere for a pressure range of 0 to 250 mm Hg. The linearity of the manometer-tipped catheter was examined on three occasions and is shown in figure VII. Linear regression analysis of these data showed that the manometer-tipped catheter was linear over the range of values measured (correlation coefficient=1.00). The 0-90% response time of this transducer to an “instantaneous” pressure change (i.e. bursting the balloon) was less than 0.5 msec at a resonant frequency of 50 kHz. Therefore, the properties of this manometer-tipped catheter were adequate for accurate measurements of physiological BP signals, and for validation of the implanted system.

showed that the relationship between applied pressure and displacement was linear (figure V) (correlation coefficient=1.00). The 0-90% response time of this pressure transducer to an instantaneous pressure change (i.e. bursting a balloon) was <50 msec, which is adequate for the measurement of changes in airway pressure, in response to an airway occlusion.
Figure V: Linearity of the pressure transducer used for measurement of airway pressure. The displacement on chart paper in response to applied pressure demonstrates that the pressure transducer was linear over the range of positive and negative pressures. Solid line, calculated linear regression.
Figure VI: Relationship between input frequency and the output frequency of the cardiotachometer used to measure heart rate. *Solid line*, line of identity.
Figure VII: Linearity of the manometer-tipped catheter used to validate the implanted BP measurement system. The displacement on chart paper in response to applied pressure demonstrates that the manometer-tipped catheter was linear over the range of pressures. **Solid line**, calculated linear regression.
Measurement of HR

In addition to the recording the raw ECG signal, the instantaneous heart rate was also derived (Cardiotachometer Coupler, Beckman, type 9857). The ability of this system to accurately record the instantaneous heart rate was tested by connecting a signal generator to the Cardiotachometer and inserting square wave signals in the range of 0.5 to 3 Hz (equivalent to 30-180 beats per minute). The efficacy of this system is shown in figure VI. Linear regression analysis of these data showed that the correlation between the input and measured frequency was highly significant and that the system was accurate in detecting heart rate (correlation coefficient=1.00; slope=1.01, p<0.001). This relationship was reproducible for input signals of 1 to 50 mV.
APPENDIX 5

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References:


Sleight, P. Role of baroreceptor reflexes in circulatory control, with particular reference to hypertension. Hypertension 18: III31-34, 1991


