EFFECT OF AROUSAL STATE AND INCREASED RESPIRATORY DRIVE ON CARDIO-RESPIRATORY VARIABLES IN THE HARBOUR SEAL (*Phoca vitulina richardsi*)

by

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Abstract

In this study, I examined the cardio-respiratory patterns of Pacific harbour seal pups (*Phoca vitulina richardsi*) under normoxic/normocarbic (air), hypoxic/normocarbic (15.22% O_2 ± 0.47 SEM, 11.99% O_2 ± 0.29 SEM, and 8.63% O_2 ± 0.22 SEM in air) and normoxic/hypercarbic (2.04% CO_2 ± 0.06 SEM, 4.16 %CO_2 ± 0.06 SEM, and 5.66% CO_2 ± 0.07 SEM in air) conditions while awake and sleeping on land. Animals were chronically instrumented to record electroencephalogram (EEG), electromyogram (EMG), and electrocardiogram (EKG) signals, and recordings of all variables, plus respiration (whole body plethysmography) and metabolic rate (O_2 consumption and CO_2 production), were made on each gas for two to four hours on separate days.

My results show that, on air, metabolic rate was lower in sleep (7.71 ml O_2 min^{-1} kg^{-1} ± 0.93 SEM) than wakefulness (WAKE) (8.79 ml O_2 min^{-1} kg^{-1} ± 0.70 SEM), with the fall in metabolism accompanied by a decrease in breathing frequency (18.76 breaths/minute ± 0.23 SEM in WAKE to 10.70 breaths/minute ± 0.49 SEM in sleep) and an increase in the incidence of periods of apnea. The maximum apnea length recorded (~ 3 minutes) was similar in length to the average dive time of harbour seals (~3.2 minutes). Breathing was rarely seen in REM sleep, appearing in only one or two animals, during one or two REM sleep episodes.

Tachypnea was present at all levels of increased respiratory drive, both hypoxia and hypercarbia, however hypoxia induced a dramatic bradycardia (up to 50% decrease in heart rate [f_H]) on 8.63% O_2 ± 0.22 SEM regardless of arousal state, while hypercarbia produced a small tachycardia in slow-wave sleep (SWS) only. Metabolic rate ($\dot{V}_{O_2}$) was relatively unchanged in both hypoxia and hypercarbia, while total ventilation ($\dot{V}_E$)

increased significantly in both WAKE and SWS. The hypoxic and hypercarbic chemosensitivities of the harbour seal pups, therefore, are similar to those of terrestrial mammals. Unlike the situation seen in terrestrial mammals however, where hypoxic and hypercarbic sensitivities are often reduced in SWS, the sensitivity of harbour seal pups to both gases appears unchanged during the change in arousal state from WAKE to sleep.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

EEG	electroencephalogram
EMG	electromyogram
EKG	electrocardiogram
WAKE	wakefulness
SWS	slow wave sleep
REM	rapid-eye movement sleep
F_{O_2}	fractional composition of O_2
F_{CO_2}	fractional composition of CO_2
F_{EO_2}	fractional expired O_2 content
F_{ECO_2}	fractional expired CO_2 content
F_{IO_2}	fractional inspired O_2 content
F_{ICO_2}	fractional inspired CO_2 content
f_R	breathing frequency
f_{Rinst}	instantaneous breathing frequency
V_T	tidal volume
$\dot{V}_E$	total ventilation
$\dot{V}_{O_2}$	rate of oxygen consumption
$\dot{V}_{CO_2}$	rate of carbon dioxide production
$\%E_{O_2}$	% oxygen extraction
ACR	air convection requirement
P_{O_2}	partial pressure of O_2
P_{CO_2}	partial pressure of CO_2

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Chapter 1

General Introduction

Sleep and Respiration in Phocid Seals

1.1 Sleep

Sleep is a state or period of reduced physical and neuronal activity accompanied by a suspension of voluntary movements and a complete or partial unconsciousness (Horne 1988; McGinty and Szymusiak 1994). Early scientists defined sleep and its different states through either physiological variations in brain wave activity, detected via electroencephalogram (EEG) patterns, or changes in behaviour of the animal. EEG patterns are useful in identifying the different arousal states as they measure the amount of brainwave activity in both the right and left cerebral hemispheres, thereby acting as a measure of alertness of the brain. Aside from wakefulness, the two main states of arousal identified are slow-wave sleep (SWS) and rapid eye-movement (REM) sleep, or paradoxical sleep (PS). Although EEG patterns are useful and convenient in identifying the different arousal states, there are some instances, such as during certain drug treatments or after neocortical lesions, where an animal is considered asleep behaviourally, but shows minimal sleep-like EEG patterns (McGinty and Szymusiak 1994). Because of this, both behavioural and electrical brain activity are used in conjunction to accurately identify and define WAKE and sleep states which, in most terrestrial mammals, cycles from WAKE to SWS to REM sleep (Lydic 1987).

Electromyograms (EMG), or physiological measures of muscle tone, are used in conjunction with EEG patterns to identify and distinguish the different states of arousal. This is especially useful in differentiating REM sleep from WAKE, where REM sleep is associated with muscle atonia (see below), while wakefulness is associated with relatively constant muscle tone (Lydic 1987; Horne 1988; McGinty and Szymusiak 1994).

1.1.1 Slow-wave Sleep

SWS (Fig. 1.1) is identified by low frequency, high amplitude, synchronized EEG patterns which typically comprise 75-80% of the average sleep cycle (Lydic, 1987; Horne 1988; McGinty and Szymusiak 1994; Hunter and Milsom 1998). In humans, four different stages of SWS have been described (stage 1 to 4), each varying slightly in EEG pattern. As the level of alertness decreases in an individual, EEG patterns become progressively slower in frequency and higher in amplitude, with the most slow-wave activity associated with the deepest stages of SWS. In non-human mammals, SWS is not typically separated into the four different stages but is considered one stage.

In addition to its distinctive EEG pattern, SWS is associated with some very distinctive physiological characteristics. During SWS, it is not uncommon to see an increase in the release of growth hormones and gonadotropic hormones from the anterior pituitary, or a decrease in blood pressure, heart rate, respiratory rate, and metabolic rate over WAKE state levels (Lydic 1987). Other physiological characteristics commonly observed during SWS include a drop in hypothalamic temperature sensitivity and body temperature as well as reduced cerebral blood flow, which can be correlated to the reduced cerebral metabolism and the reduced activity in a majority of brain neuronal sites (McGinty and Szymusiak 1994).

1.1.2 Rapid eye-movement sleep

The second state of sleep commonly identified is rapid eye-movement (REM) sleep, or paradoxical sleep (PS). The name "rapid eye-movement sleep" originates from the fact that when this state was first identified in humans, it was characterized by rapid

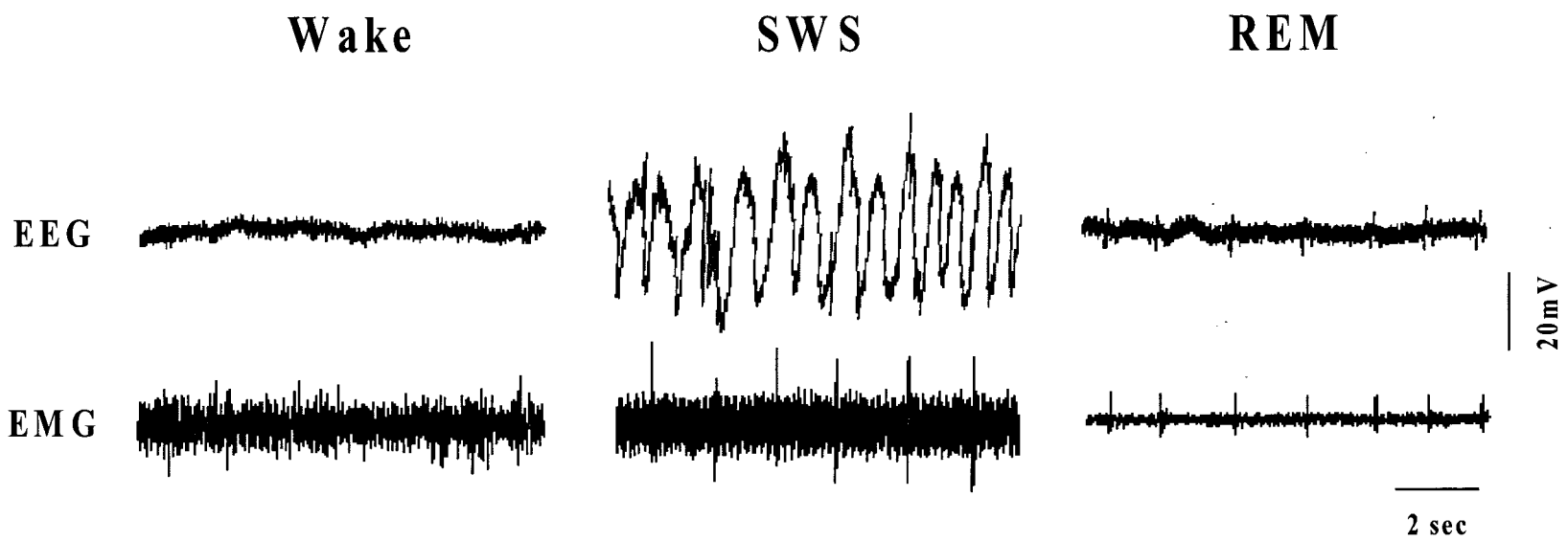


Figure 1.1. Representative recordings of EEG and EMG during the three primary states of arousal: WAKE, SWS, and REM sleep. During SWS, there is an increase in amplitude and decrease in frequency of the EEG waves, compared to those of WAKE and REM sleep. The muscle tone of REM sleep (shown through the EMG trace) is greatly reduced compared to both WAKE, and SWS. Heart rate signals are evident in the REM sleep EEG signal, as well as in the SWS and REM sleep EMG signals.

eye-movements. Not all animals, however, show the rapid eye-movements characteristic of human REM sleep. Because of the desynchronized or activated EEG patterns associated with REM sleep (Fig. 1.1), identifiable through the high frequency, low amplitude EEG signals, similar to those observed during wakefulness (Fig. 1.1), a more universal classification may be paradoxical sleep, paradoxical in the sense that the EEG signal looks like WAKE, but the body is asleep (Horne 1988).

REM sleep, as with SWS, is associated with many unique physiological characteristics. Body temperature is not constant due to reductions in thermoregulatory control, while blood pressure, heart rate, and respiratory rate are irregular, with metabolic rate and cerebral blood flow increased compared to SWS levels (McGinty and Szymusiak 1994). The increased cerebral blood flow leads to an increase both in cerebral metabolism and hypothalamic temperature. REM sleep is also associated with an increased discharge in brain neuronal sites, leading to the characteristic activated WAKE-like EEG patterns (Horne 1988; McGinty and Szymusiak 1994).

As with SWS, there are different stages associated with REM sleep, specifically tonic REM sleep and phasic REM sleep, also referred to as tonic and phasic events, which are present in most mammals. The term "event" suggests these are not successive changes in arousal, as in SWS, rather they appear consistently throughout the REM sleep episode. Tonic REM sleep lasts the duration of the REM sleep episode and is characterized by the desynchronization of the EEG signal (similar to WAKE) and muscle atonia (Orem 1980; Horne 1988). The degree of muscle atonia present and the amount of REM sleep observed is species dependent. Some species, such as humans and other

animals with high degrees of encephalization (i.e. highly advanced cerebrum), exhibit a relatively high amount of REM sleep, while animals with relatively low degrees of encephalization, such as ungulates, exhibit minimal amounts of REM sleep (Horne 1988). Regardless of the amount of REM sleep present, or the degree of encephalization, muscle atonia, especially of head and neck muscles, is a constant feature of tonic REM sleep. To allow for muscle atonia, without injury to the animal, REM sleep is often observed in individuals lying down, and in the case of animals that sleep standing up (e.g. ungulates), individuals will lie down for a portion of the sleep episode, allowing for REM sleep to occur (Horne 1988). There are some exceptions to the overall muscle atonia. The respiratory muscles, and in some instances, the eye muscles leading to the rapid eye-movements of some animals, maintain both muscle tone and the ability to contract (Orem 1980; Horne 1988).

Phasic REM sleep is episodic in appearance and is characterized by the occurrence of pontogeniculooccipital (PGO) waves in the EEG signal, as well as myoclonic twitches, and in some animals, eye movements (Orem 1980; Horne 1988; Hunt et al. 1998). PGO waves are 200-300μV peaks found in the EEG signal, approximately 100ms in length, occurring at a frequency of 1 to 2 per second (Orem 1980; Hunt et al. 1998). PGO waves, aside from triggering bursts of firing from geniculate and cortical neurons of the brain, as well as other signals originating in the brainstem that damp out sensory input and motor output, are associated with the activation of muscles surrounding the eye, and are positively correlated with respiratory neuronal activation (Orem 1980; Orem 1988; Hunt et al. 1998).

1.1.3 Sleep in Marine Mammals - Family Phocidae

Although the exact reasons are unknown, sleep is important to survival (Horne 1988) and to ensure that the physiological needs of the individual are met, and different strategies of sleep and sleep patterns have evolved in animals living in different environments. Sleep in marine mammals, especially those that cannot or do not spend any appreciable amount of time on land, present an interesting challenge. How can an animal living completely submerged in water sleep? It is a well-known fact that sleep consists of various levels of consciousness and that motor ability is dampened considerably during sleep (McGinty and Szymusiak 1994). During certain portions of sleep (tonic event of REM sleep), postural muscles experience atonia, while at the same time, in marine mammals, some level of consciousness or motor ability has to be present to prevent sinking or drifting in the water and to allow the sleeping individual to breathe.

The nineteen different species of seal from family Phocidae occupy numerous different niches, from estuarine and coastal to deep sea, spending an appreciable amount of time both on land and in water. Because of this, one can closely examine the evolution of some of the different sleep strategies developed in marine mammals.

One of the more interesting strategies of sleep in marine mammals occurs in bottlenose dolphins *Tursiops truncates*), harbour porpoises (*Phocoena phocoena*), beluga whales (*Delphinapterus leucas*) and otariid seals, both on land and in water (Cape fur seal [*Arctocephalus pusillus pusillus*], northern fur seal [*Callorhinus ursinus]*, Stellar sea lion [*Eumetopias jubatus]*, and southern sea lion [*Otaria byronia*], where sleep occurs in one cerebral hemisphere at a time (Horne 1988; Lyamin and Chetyrbok 1992; Lyamin et al. 2002a; Lyamin et al. 2002b). Studies of sleep involving phocid seals reveal a

different, albeit equally interesting strategy. These animals follow the pattern of SWS and REM sleep commonly associated with sleep in terrestrial mammals; both cerebral hemispheres sleep at the same time, regardless of arousal state, and individuals alter breathing pattern as an adaptation to sleep in water (see below). Of the nineteen different species of phocid seal, patterns of sleep have been extensively examined in gray seals (*Halichoerus grypus*) (Ridgway et al. 1975), Caspian seals (*Pusa caspica*) (Mukhametov et al. 1984), harp seals (*Pagophilus groenlandicus)* (Lyamin 1993; Lyamin et al. 1993), and northern elephant seals (*Mirounga angustirostris*) (Blackwell and Le Boeuf 1993; Castellini et al. 1994a; Castellini et al. 1994b; Castellini 1996; Milsom et al. 1996), while sleep in other phocid seals, such as southern elephant seals (*Mirounga leonina*) (Falabella et al. 1999), hooded seals *(Cystophora cristata*) (Castellini 1996), and Weddell seals (*Leptonoychotes weddelli*) (Castellini et al. 1992) has been observed behaviourally, although not examined at the neurophysiological level.

While the pattern of cerebral brainwave activity is similar during sleep in all phocid seal species studied to date, the actual order of arousal state that have been reported varies. The Caspian seal, harp seal, and northern elephant seal undergo an arousal state pattern similar to terrestrial mammals: WAKE - SWS - REM sleep (Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1994a). Studies of sleep in the gray seal however, reveal a different pattern of arousal state: WAKE - REM sleep - SWS (Ridgway et al. 1975). This arousal state pattern has not been observed in any other mammal, marine or terrestrial (Ridgway et al. 1975).

Because arousal state patterns have only been intensively studied in four species of phocid seal (gray seal, Caspian seal, harp seal, and northern elephant seal) and two

different patterns have been observed, it is difficult to predict the arousal state pattern of other phocid seals and to determine the underlying reasons for the variation in arousal state pattern. It is possible that the evolutionary relationships and ecological similarities of the phocid species in question affect the sleep state pattern.

1.2 Respiration

1.2.1 Breathing Pattern

1.2.1.1 WAKE

Phocid seals are diving mammals. They live primarily in aquatic environments and remain submerged for extended periods of time. While at the water's surface, individuals are most vulnerable to predation and must maximize the total amount of time spent underwater. The length of the dives and consequential apneas (cessation of breathing), both average and maximum, vary from species to species, with some animals, such as the harp seal, exhibiting maximum apneas of 13 minutes (Lydersen and Kovacs 1993) while other animals, such as the southern elephant seal, exhibit maximum apneas as long as 120 minutes (Hindell et al. 1992). During dive bouts, breathing cannot be continuous due to repeated prolonged submergences, and therefore, is considered episodic with breaths clustered into distinct episodes separated by pauses of variable lengths (Irving et al. 1935; Butler and Jones 1982; Castellini 1996). This ability to maintain prolonged apneas, and exhibit episodic breathing patterns, suggests that phocid seals have specific adaptations allowing for either increased oxygen storage, decreased oxygen consumption, or both, and it leads to intriguing questions regarding the mechanisms of breathing in these animals.

Unlike other marine mammals (cetaceans and otariids), phocid seals take many breaths before re-submergence and are therefore considered "slow" breathers while at the surface (Kooyman 1973). The tidal volume (V_T), or the volume of an average breath, of phocid seals is similar in size to its total lung capacity (TLC), such that with each breath, most of the air in the lungs is renewed. This aids in quickening the removal of respiratory waste products such as carbon dioxide, as well as replenishing the used oxygen stores (Kooyman 1973). Because the tidal volume of phocid seals is so large and is near its maximum value (40-90% of TLC), the primary mechanism through which seals can meet increases in metabolic oxygen requirements is through increasing respiratory frequency, and hence, the total amount of time spent eupneic (Gallivan 1981; Butler and Jones 1997).

Although primarily considered aquatic mammals, phocid seals also spend an appreciable amount of time on land, with the amount of time spent in water versus time spent on land varying greatly from species to species. It has been shown in many studies, on a variety of species of phocid seal (including the northern elephant seal [Castellini et al. 1994a; Castellini et al. 1994b; Milsom et al. 1996], the Weddell seal [Castellini et al. 1992], the harp seal [Lyamin 1993], the gray seal [Ridgway et al. 1975], the hooded seal [Castellini 1996], the leopard seal (*Hydrurga leptonyx*) [Williams and Bryden 1993], and the Caspian seal [Mukhametov et al. 1984]) that breathing patterns while on land and in an awake state are not episodic, but continuous and irregular (i.e. arrhythmic).

1.2.1.2 Sleep

Research has shown that while on land, breathing in phocid seals is not always continuous. During certain states of sleep, breathing becomes episodic in pattern, similar to the diving animal (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1992; Castellini et al. 1994a; Milsom et al. 1996). Changes in breathing pattern associated with changes in arousal state are not unique to marine mammals. In terrestrial mammals, SWS is primarily accompanied by slower, yet regular breathing patterns, and changes in tidal volume (both increasing and decreasing), as well as decreases in total ventilation ($\dot{V}_E$) and breathing frequency (f_R) over WAKE state levels (Lydic 1987). REM sleep is associated with increases in breathing frequency, as well as the presence of apneas and hypopneas (decreased lung ventilation) (Lydic 1987).

Current information regarding breathing patterns during sleep in marine mammals in general, and phocid seals in particular, is limited and ambiguous. From the four species of phocid seal that have been studied with regard to breathing patterns and different states of arousal (gray seal, Caspian seal, harp seal, and northern elephant seal) it appears that breathing pattern is not only specific to arousal state, but is possibly also specific to species (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1994a; Milsom et al. 1996). As mentioned previously, breathing during periods of wakefulness is either episodic (dive situations) or continuous and irregular (on land). During SWS, breathing becomes episodic in all phocid seal species studied to date, with the animal either on land or in the water (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Lyamin et al. 1993; Castellini et al. 1994a; Castellini et al.

1994b; Milsom et al. 1996). The variability arises in the breathing patterns seen in REM sleep.

In phocid seals, a conflict arises between sleep and swimming. It is known that during REM sleep, in all mammals studied to date, muscle atonia occurs (Lydic 1987; Horne 1988). If seals were to also experience muscle atonia during REM sleep, while submerged, their swimming ability would be impeded. Therefore, if a seal cannot swim to the surface to breathe during REM sleep, it can do one of two things: 1) fail to enter REM sleep or 2) alter its breathing pattern such that it can remain submerged for much, if not all, of the REM sleep episode. Because REM sleep is so important to the survival of animals (Orem 1980; Horne 1988), it would be difficult to remove it completely from the sleep cycle; therefore all species of phocid seal currently studied alter their breathing pattern, body posture, or both, during REM sleep.

In the gray seal, breathing during REM sleep is continuous and regular, therefore the animal must be on land or positioned in the water such that it can maintain a continuous breathing pattern (i.e. floating with nostrils just above the surface of the water) (Ridgway et al. 1975). During REM sleep in the ice-dwelling Caspian and harp seals, on land and fully submerged, breathing is sporadic and rare, with most REM sleep episodes occurring within one respiratory pause (Mukhametov et al. 1984; Lyamin 1993; Lyamin et al. 1993). The northern elephant seal, an offshore species, does not breathe during REM sleep. It maintains prolonged apneas for the duration of the REM sleep episode, regardless of location, on land or in water (Castellini et al. 1994a; Castellini et al. 1994b; Milsom et al. 1996).

Thus, of the four phocid seal species studied to date, there are three very different state dependent patterns of breathing. It is difficult to determine, without further studies, whether different breathing patterns arise from differences in ecology, phylogeny, or adaptations.

1.2.2. Oxygen Consumption

Oxygen consumption rate is a variable commonly used to determine the energy (metabolic) requirements of animals (i.e. how much energy must be consumed for an animal to survive). During dive and breath-hold situations, there are limited amounts of O_2 available to the cells, tissues, and organs of phocid seals. According to Hochachka (1988) there are two ways in which to deal with finite oxygen stores during these apneic situations: 1) turn to alternate sources of energy such as anaerobic metabolism (Pasteur effect), or 2) decrease oxygen consumption rates in hypoxia tolerant tissues, such as skeletal muscle (reversed Pasteur effect or hypometabolism).

Many studies have shown that during natural dives, phocid seals do not turn to anaerobic metabolism unless the dive is exceptionally long, as is evident by the lack of lactate build-up (see Hochachka and Guppy 1987, and Hochachka 1988 for reviews). Thus, hypometabolism of hypoperfused tissues allows the hypoxia-sensitive tissues (heart, brain, and lungs) to maintain normal, or near-normal oxygen consumption rates, as well as allows the seal to remain apneic (diving or voluntary) for prolonged periods without the build-up of waste byproducts.

In this context, the changes in breathing pattern associated with changes in arousal state seen in phocid seals leads to some intriguing questions regarding oxygen

consumption rates. Why is breathing on land in an awake animal continuous and irregular, while breathing in the same animal during SWS is episodic? Why is the breathing pattern during REM sleep species dependant?

It has been suggested that when metabolic rate and oxygen requirements are low, the need to breathe continuously is eliminated and breathing becomes slow and/or episodic (White et al. 1985; Milsom et al. 1997). This implies that during wakefulness on land, where breathing is slow but continuous and irregular, phocid seals have a much higher metabolic rate than during SWS on land and in the water, where breathing is episodic. Breathing during REM sleep shows no apparent pattern or relationship to metabolic rate. In the Caspian and harp seals, although sporadic and rare during REM sleep, breathing remains episodic (Mukhametov et al. 1984; Lyamin 1993). If breathing patterns are a reflection of metabolic rate, the change in state of arousal from SWS to REM sleep in these species does not appear to significantly affect the metabolic rate. In the gray seal, where REM sleep occurs after WAKE and before SWS, breathing is continuous (Ridgway et al. 1975), again suggesting no change in metabolic rate with the onset of REM sleep. It also suggests a decrease in metabolic rate as the animal enters SWS, illustrated by the change in breathing pattern from continuous to episodic. The complete absence of breathing during REM sleep in the northern elephant seal (Castellini et al. 1994a; Castellini et al. 1994b; Milsom et al. 1996) suggests a dramatic decrease in metabolic rate as arousal state decreases. These data lead to the question: is the metabolic rate of phocid seals linked to state of arousal and breathing patterns?

In terrestrial mammals, it is hypothesized that breathing during wakefulness is governed by both metabolic rate and behaviour (Phillipson 1978). As arousal state

decreases, the factors affecting breathing change. During SWS, it is suggested that breathing pattern is controlled primarily through metabolic demands, while during REM sleep, it is thought that behaviour, as well as the absence of many physiological control mechanisms, play the primary role and appear to act independent of the metabolic system (Phillipson 1978; Lydic 1987). According to Heller (1988), whole body metabolic rate is lowered during the change of arousal state from WAKE to SWS by about 10%, on average, supporting the hypothesis of Phillipson (1978).

Because of its easy accessibility in the wild, the Weddell seal is one species in which numerous field metabolic studies have been performed (Kooyman et al. 1973; Castellini et al. 1992; Ponganis et al. 1993; Davis and Kanatous 1999). Castellini et al. (1992) closely examined the changes in the metabolic rate of Weddell seals in response to changes in activity, and behaviourally defined arousal levels. They found that as the level of activity decreased from relatively short dives to resting at the surface (wakefulness), metabolic rates remained relatively constant despite the decrease in activity. Interestingly, metabolic rates of short dives appeared to be 1.5 times higher than the metabolic rate observed in behaviourally defined sleep, while the metabolic rate of resting seals was no different from sleeping seals.

Studies by Lavigne et al. (1986a and 1986b) and Worthy (1987) indicate that the resting metabolic rate of phocid seals, in an awake state, is the same, regardless if the seal is at the surface of the water or on land, allowing for comparisons of the patterns of change in metabolic rate between different arousal states of marine and terrestrial mammals, regardless of location. As mentioned previously, in terrestrial mammals, a decrease in both arousal and alertness levels is typically accompanied by a decrease in

metabolic rate (Phillipson 1978; Heller 1988). Data on the changes in metabolic rate of marine mammals, due to changes in arousal state, contradict this. Castellini et al. (1992) conclude that the metabolic rate of a behaviourally sleeping Weddell seal is no different from an awake, resting individual. Worthy (1987) examined changes in metabolic rate of harp and gray seals and found a 40.8 ± 10.6% decrease in metabolic rate during behavioural sleep when compared to resting, WAKE state levels. The differences in magnitude of change in metabolic rate between the different states of arousal, raises many questions regarding the control mechanisms. One possibility is that there are physiological differences associated with differences in behaviour, allowing for the different magnitudes of change in metabolic rate, associated with arousal state. Before any concrete conclusions or predictions can be made however, the effects of neuro-physiologically defined sleep on metabolic rates in phocid seals need to be examined.

1.2.3 Oxygen Storage

One of the most important adaptations of phocid seals, allowing them to remain apneic for prolonged periods of time, is their ability to store excess oxygen for use during long breath-hold periods. There are three potential sources for oxygen storage: lungs, muscle, and blood. In phocid seals, the lungs are not an optimal store for oxygen during dives. First, an increase in lung oxygen stores would increase the buoyancy of the animal, thereby increasing the energy expenditure required to dive below the surface (Kooyman 1973). Secondly, if an animal diving deep beneath the surface of the water were to store large quantities of air in its lungs, the nitrogen gas that comprises most of that air (79%) would be forced into the blood as the lungs collapsed from the pressure of

the dive (1 atmosphere absolute for each 10m below sea level), resulting in nitrogen narcosis upon re-surfacing, a potentially deadly state (Gooden 1990). In diving phocid seals therefore, the lungs contain only a small portion of total oxygen stores, and in fact, most dives occur on exhalation with the lung volume 20 to 60% of the total lung capacity (for reviews, see Kooyman 1973 and Burggren 1988). While awake and resting quietly on the surface of the water, or on land, periodic, short-duration apneas are common. In situations such as these, the lungs would be beneficial as oxygen stores and, in fact, to utilize the oxygen stores of the lung, apneas in awake, resting phocid seals usually occur on inspiration (Kooyman 1973).

Muscle, although not a major store for oxygen in most terrestrial mammals, is known for its increased oxygen storage capacity in seals (Lenfant et al. 1970), with approximately 3-10 times more oxygen stored than in terrestrial mammals (Polasek and Davis 2001). During apneic situations, vasoconstriction brought about by stimulation of carotid body chemoreceptors, a result of the dive response, decreases the amount of blood oxygen stores available to the hypoperfused tissues (Elsner et al. 1977; Daly et al. 1977). The increased myoglobin oxygen stores (MbO_2) and mitochondrial densities of these hypoperfused muscle tissues however, prevent complete ischemia and provide sufficient oxygen to the tissues, allowing them to function with minimal reliance on anaerobic metabolism during prolonged periods of apnea (Kanatous et al. 1999).

The primary oxygen store in phocid seals is the blood. There are many different physiological aspects of the blood that can be changed to increase its overall oxygen carrying capacity. Increases in blood volume, hematocrit, and hemoglobin concentration, compared to levels seen in terrestrial mammals, all aid in increasing the total oxygen

stores available to the diving animal during apneic situations (Lenfant et al. 1970; Kooyman 1973; Burggren 1988). Studies have shown that seals that experience the longest breath-holds have the highest blood oxygen stores, with the highest hemoglobin concentrations and hematocrit levels (Lenfant et al. 1970), thus indicating that increasing the oxygen storage capacity of the blood can be very beneficial to the animal without negative effects on its diving capability (Kooyman 1973; Burggren 1988).

If we compare the average and maximum recorded dive times of various species of phocid seal with the total oxygen stores (Fig. 1.2), it is interesting to note that the longer the maximum attainable dive time, the less the lungs are used as an oxygen store and the more the blood and muscles are utilized.

1.3 Respiratory Drive

Oxygen is required by all air-breathing vertebrates to maintain aerobic metabolism. If oxygen supplies are depleted, either internally or externally, aerobic metabolism cannot be utilized and the animal must turn to anaerobic metabolism, an inefficient source of ATP. When hypoxic conditions, or conditions of low O_2 arise, carotid body peripheral chemoreceptors, stimulated by low blood oxygen partial pressures (P_{O_2}), stimulate respiratory control centres to increase breathing frequency as a mechanism to increase O_2 uptake and blood P_{O_2} levels. The rate of oxygen consumption however, is decreased during hypoxic conditions to conform to oxygen availability, while the rate of oxygen delivery to the blood, or total ventilation ($\dot{V}_E$), is increased (Frappell et al. 1992; Mortola 1999).

In hypercarbic conditions, where ambient levels of carbon dioxide are increased, the partial pressure of CO_2 in the blood (P_{CO_2}) increases, with immediate results of decreasing both blood and intracellular pH levels (Cameron 1989; Henderson-Smart 1994). This is primarily due to the bicarbonate reaction:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

where any increase in CO_2 shifts the equation to the right, increasing the overall $[H^+]$, and decreasing blood pH.

Although there is some input from the peripheral chemoreceptors, the majority of the hypercarbic response is due to central chemoreceptor input (Cameron 1989; Henderson-Smart 1994). Breathing frequency, total ventilation, and tidal volume all increase significantly during hypercarbia, while oxygen consumption rates remain relatively unchanged (Mortola and Lanthier 1996).

Studies on the effects of hypoxia and hypercarbia in marine mammals are primarily limited to phocid seals in the WAKE state (Irving 1938; Robin et al. 1963; Bainton et al. 1973; Påsche 1976a; Påsche 1976b; Craig and Påsche 1980; Parkos and Wahrenbrock 1987; Mortola and Lanthier 1989; Milsom et al. 1996; Kohin et al. 1999). From the initial studies on restrained animals, and the more recent studies on unrestrained animals, it is apparent that there is no difference in sensitivity to hypoxia or hypercarbia when most phocid seals are compared to terrestrial mammals, however there are increased thresholds for the hypoxic and hypercarbic ventilatory responses (i.e. animals react with the same intensity but do not show a response until higher levels of respiratory drive are reached) (Bainton et al. 1973; Påsche 1976a; Påsche 1976b; Gallivan 1980;

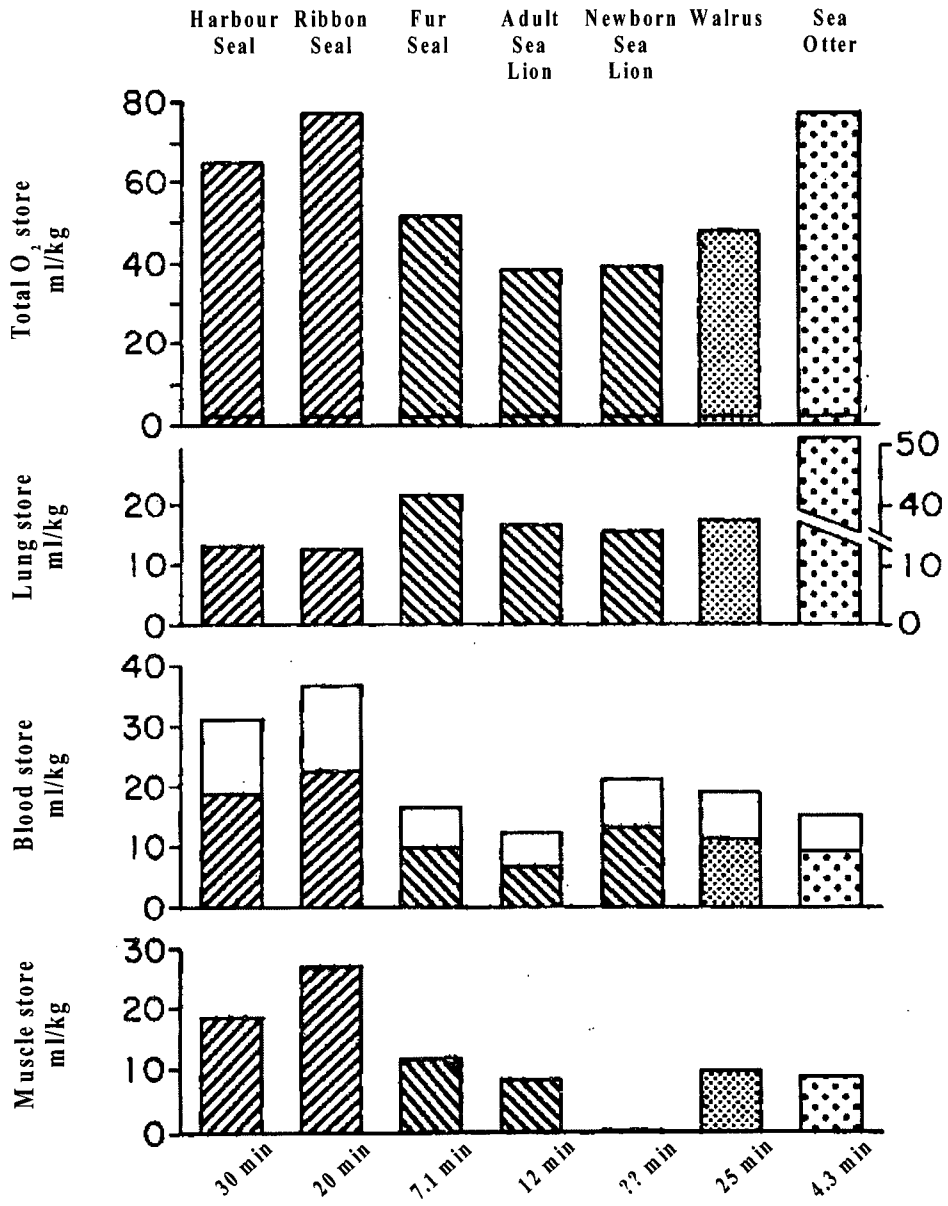


Figure 1.2. Oxygen stores at the beginning of a dive in the sea otter and various species of pinniped. Top row gives the total body store; the lower rows give the distribution between lung, blood (arterial-top half, venous-bottom half), and muscles respectively (figure adapted from Lenfant et al. 1970). Maximum dive times are noted at the bottom of the figure. Data for the harbour seal, South American fur seal (*Arctocephalus australis*), and walrus (*Odobenus rosmarus*) are from Riedman (1990), data for the California sea lion (*Zalophus californianus*) are from Reeves et al. (2002), data for the ribbon seal (*Histriphoca fasciata*) are from Burt and Grossenheider (1980), and data for the sea otter (*Enhydra lutris*) are from Estes and Bodkin (2002).

Parkos and Wahrenbrock 1987; Milsom et al. 1996).

The responses of respiratory sensitivity to hypoxia in juvenile Weddell seals are different from all other phocid seals studied to date. These data indicate that juvenile Weddell seals have a decreased hypoxic sensitivity compared to terrestrial mammals and other species of phocid seal (Parkos and Wahrenbrock 1987). Parkos and Wahrenbrock (1987) hypothesized that the decreased hypoxic sensitivity of the Weddell seal could be due to the exposure of the pups to hypoxia during diving early in life and therefore could be an adaptation for prolonged dives. No other phocid seal studied to date however, even the more precocial harbour seal, shows the decreased hypoxic ventilatory sensitivity that the Weddell seal does (Bainton et al. 1973; Påsche 1976a; Påsche 1976b; Gallivan 1980; Milsom et al. 1996).

During periods of sleep, the threshold for medullary neuronal activation is increased over WAKE state levels, and although there is some discrepancy over the level of change in sensitivity associated with arousal state, lower levels of blood P_{O_2} (higher degree of hypoxia) and higher levels of blood P_{CO_2} (higher degree of hypercarbia) are required to stimulate increases in respiratory variables (Netick et al. 1984; Khoo et al. 1995; Daly 1997). The northern elephant seal is the only phocid seal in which the effects of hypoxia and hypercarbia have been examined during different states of arousal. Using f_R as an indicator of sensitivity, Milsom et al. (1996) concluded that hypoxic sensitivity was increased and hypercarbic sensitivity was unchanged during SWS, when compared to WAKE state levels in this species. The increase in hypoxic sensitivity is similar to that seen in rats and ground squirrels, and the unchanged hypercarbic sensitivity is similar to that seen in goats (Milsom et al. 1996). Although there are similarities in the hypoxic and

hypercarbic sensitivities of phocid seals during the WAKE state, the species-specific differences in breathing pattern, related to changes in arousal state in phocid seals, make it difficult to predict the chemosensitivities of the different species of phocid seal, during SWS.

1.4 Heart Rate

Heart rate is not static. As the internal and/or external environment in which the animal resides changes, physiological variables such as breathing and metabolism also change. These changes typically lead to simultaneous changes in heart rate; specifically tachycardia (increased heart rate) and bradycardia (decreased heart rate). An electrocardiogram (EKG) is a tool designed to measure the variations in the electrical potential of the heart from various locations on the surface of the body. Using the EKG signal (Fig. 1.3a), there are two common techniques for calculating heart rate: 1) counting the number of beats per unit time, or 2) using the R-R peak interval (seconds) to determine instantaneous heart rate (1/R-R interval) (Fig. 1.3b).

Although heart rate varies from species to species and individual to individual, average heart rate values can be used as an estimate of a normal heart rate for that species. In most terrestrial mammals, as well as marine mammals, heart rate accelerates during inhalation and decelerates during exhalation, leading to the presence of a respiratory sinus arrhythmia (Daly 1997). This is especially prevalent in phocid seals during both dive and sleep-related apneas. It is known that when seals dive, there is a subsequent bradycardia, similar to that present during resting and sleep apneas (Kooyman 1985; Castellini et al. 1994a). Both the diving and sleep-apneic bradycardias are

therefore thought to be associated with the changes in respiratory pattern, and in fact, it has been suggested that the bradycardia of short dives and apneas (on land and in water) are extensions of the normal respiratory sinus arrhythmia and therefore are not "true" bradycardias (Castellini et al. 1994a). Kooyman (1985) suggests that in Weddell seals, a "true" diving bradycardia does not occur until dives longer than ten minutes, at which point the heart rate is lower than that observed in a resting apneic state.

Although there is no correlation between swim speed and heart rate in phocid seals, there is a strong relationship between the strength of the bradycardia and the length of the apneic period, such that the lower the heart rate, the longer the dive and/or subsequent apnea (Kooyman 1985; Hill et al. 1987; Thompson and Fedak 1993). In reference to this, Thompson and Fedak (1993) suggested that phocid seals have a finite number of heart beats in apneic situations, such that once that number is reached, the seal must recommence respiratory activity: i.e. the lower the heart rate during the apneic period, the longer the animal can breath-hold. This appears true only for short dives and apneas (Hill et al. 1987; Butler and Jones 1997). During forced and prolonged dives, as well as extended sleep-related apneas, the bradycardia in some species of phocid seal can reach values as low as 2-3 beats min^{-1}, however most diving heart rates typically stay above 10 beats min^{-1} (Andrews 1999).

In addition to lowering the heart rate during apneic periods, phocid seals also vasoconstrict blood vessels to selective, hypoxia resilient tissues (see Butler and Jones 1982 for review). This serves to increase the peripheral resistance to blood flow, allowing for the maintenance of constant blood pressure and blood flow to hypoxic

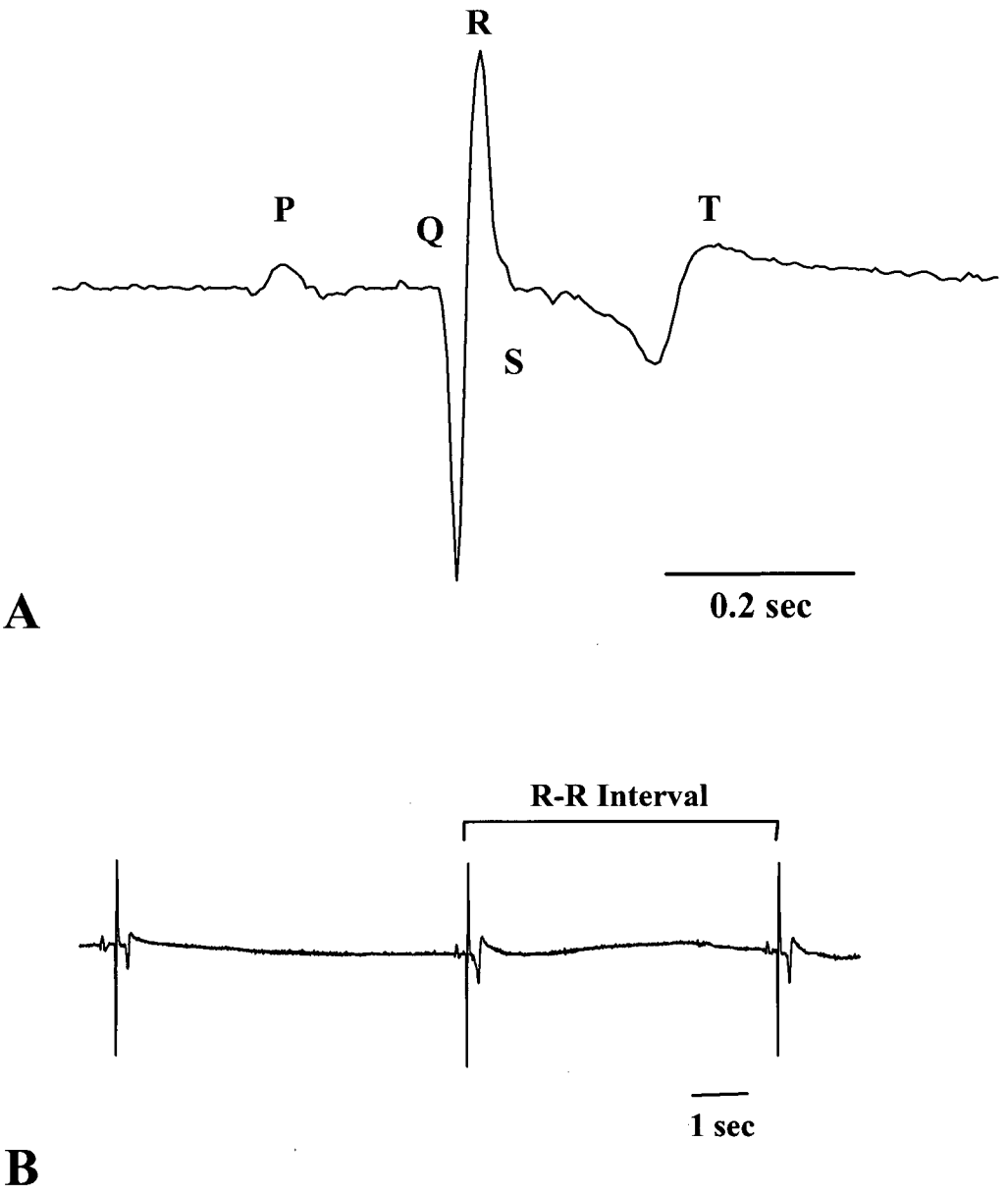


Figure 1.3. Electrocardiogram (EKG) signal of an 11-month old harbour seal. Gold-disc EKG electrodes were sutured just beneath the panniculus, one caudal and one cranial to the heart, on opposite sides of the dorsum of the seal. A) illustrates the important deflections of the EKG signal, while B) illustrates the R-R interval commonly used for determining instantaneous heart rate.

sensitive tissues such as the brain, lungs, and heart (Hill et al. 1987; Thompson and Fedak 1993; Castellini and Zenteno-Savin 1997).

As phocid seals surface, they show an anticipatory tachycardia and vasodilation of the hypoperfused tissues. It is thought that the increase in heart rate, and the increased blood flow act to remove waste products such as lactate and CO_2 from the hypoperfused tissues, as well as allowing for the uptake of residual O_2 from the blood (Fedak et al. 1988). The benefit of collecting respiratory waste byproducts and reducing blood Po_2 levels prior to any respiratory event is to minimize surface time due to increased efficiency in removing the waste products and oxygen loading (Fedak et al. 1988; Thompson and Fedak 1993; Butler and Jones 1997).

Because heart rate is so intimately linked to respiration and metabolism, factors that affect these variables also affect heart rate. In terrestrial mammals, as arousal state decreases from WAKE to SWS, there is a subsequent decrease in heart rate due to decreases in both breathing frequency and vasomotor tone (Lydic 1987). In phocid seals, as arousal state decreases from WAKE to SWS, heart rate is also observed to drop, however in northern elephant seal pups, harp seal pups, and yearling gray seals, the decrease in overall heart rate is due to significant decreases in apneic heart rate only (Ridgway et al. 1975; Lyamin 1993; Castellini et al. 1994a).

Hypoxia and hypercarbia also significantly increase breathing frequency in terrestrial mammals, thereby causing tachycardia and hypertension, increasing cardiac output, and reducing systemic vascular resistance (Daly 1997). To maintain a ventilation-perfusion match, allowing optimal delivery of oxygen to body tissues, heart rate increases with breathing frequency in both WAKE and sleep states, as respiratory

drive increases (Daly 1997). In phocid seals, the cardiovascular response to increased respiratory drive (hypoxia and hypercarbia) is seldom studied. In the northern elephant seal, Kohin et al. (1999) found an increase in heart rate during wakefulness in both hypoxia and hypercarbia, while Milsom et al. (1996) found no change in heart rate associated with hypoxia and an increase in heart rate during hypercarbia, while awake. Opposite to terrestrial mammals, there was no increase in heart rate during SWS in both hypoxia and hypercarbia (Milsom et al. 1996) in the northern elephant seal.

The matching of the heart rate to breathing frequency in phocid seals is rarely studied, especially under conditions of increased respiratory drive and decreased states of arousal. To date there has only been one species examined, thus providing information regarding the cardiovascular response of that phocid seal, the northern elephant seal, to hypoxia and hypercarbia. From the studies on northern elephant seals (Milsom et al. 1996; Kohin et al. 1999), there are two different patterns of response, possibly due to low N-values. It is therefore difficult to understand and predict the mechanisms behind the cardiovascular response to variations in respiratory drive.

1.5 Purpose of Study

Due to their aquatic and terrestrial lifestyles, as well as their ability to remain apneic for prolonged periods of time, phocid seals have many unique physiological adaptations. Because they spend so much of their time at sea, little is known about the respiratory physiology of phocid seals during the different states of arousal. Studies of gray seals, Caspian seals, harp seals, and northern elephant seals on land and in water reveal that the changes in respiratory variables, in response to arousal state, are species

dependent (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin et al. 1993; Castellini et al. 1994a). The data currently available however, do not provide enough information allowing for a potential model, possibly relating the cardio-respiratory physiology of the animal to its environment and/or ecology.

Sensitivity to increasing levels of respiratory drive (i.e. hypoxia and hypercarbia) has been studied in phocid seals and data indicate that, although they are capable of prolonged breath-holds, seals are just as sensitive to changes in blood gas composition as terrestrial mammals. Due to animal and/or equipment constraints, none of the aforementioned arousal state studies, or hypoxic/hypercarbic respiratory drive studies, completed to date, give a complete picture of the changes in cardio-respiratory variables and breathing pattern. They focus rather, on one or two of these variables, typically $\dot{V}_E$ and/or $\dot{V}_{O_2}$.

This thesis, therefore, sets out to determine the cardio-respiratory and breathing patterns of the harbour seal, a small, short-duration diver, both during different states of arousal and different levels of respiratory drive. Specifically, male harbour seal pups were examined in each arousal state, under conditions of hypoxia and hypercarbia. A stimulus response curve, depicting the effects of increased levels of respiratory drive, on the cardio-respiratory and breathing patterns, during the different arousal states was produced.

With the completion of these experiments, three main questions regarding state of arousal, increased respiratory drive, and the cardio-respiratory/breathing patterns of harbour seal pups were answered:

- What is the sleep state pattern of the harbour seal? Does it follow the typical mammalian pattern of WAKE-SWS-REM sleep?

- What is the breathing pattern of the harbour seal during different states of arousal? Is it state specific? How does it compare with other species of phocid seal?

- How do harbour seals respond to changes in respiratory drive during the different states of arousal? Do sleep and breathing patterns change during different levels of respiratory drive (i.e. in normoxia versus hypoxia versus hypercarbia)?

1.5.1 Specific Hypotheses

Hypothesis #1. The sleep state pattern will follow the pattern typically seen in terrestrial mammals (awake-SWS-REM sleep).

Hypothesis #2. The breathing pattern of the harbour seal, on land, will be state specific. During periods of wakefulness, breathing will be continuous and irregular, during periods of SWS breathing will be episodic, and during periods of REM sleep, breathing will be sporadic and rare, similar to that seen in the Caspian and harp seals (Mukhametov et al. 1984; Lyamin 1993).

Hypothesis #3. Metabolic rate will decrease as arousal state decreases, such that WAKE $\dot{V}_{O_2}$ values will be greater than SWS $\dot{V}_{O_2}$ values.

Hypothesis #4. Awake harbour seals will respond to increases in respiratory drive (i.e. hypoxia and hypercarbia) in a manner similar to terrestrial mammals. In hypoxia, $\dot{V}_{O_2}$ will decrease, f_R and $\dot{V}_E$ will increase and V_T will remain unchanged. In hypercarbia, $\dot{V}_{O_2}$ will remain unchanged, while f_R, $\dot{V}_E$, and V_T increase significantly.

Hypothesis #5. During SWS and REM sleep, both hypoxic and hypercarbic sensitivity will be reduced compared to WAKE state levels, however the change in sensitivity will remain similar to terrestrial mammals.

Chapter 2

Effects of arousal state and respiratory drive on cardio-respiratory variables in harbour seal pups (*Phoca vitulina richardsi*).

2.1 Introduction

Since Irving (1938) suggested that the extreme dive capabilities of marine mammals were in part due to a reduction in their sensitivity to hypoxia and hypercarbia, numerous studies on phocid seals have suggested that this is not the case, at least while animals are awake and not diving. Under these conditions, phocid seals appear just as sensitive to increases in CO_2 (hypercarbia) as terrestrial mammals, although the threshold level of CO_2 required to elicit a response may be higher (Påsche 1976b; Craig and Påsche 1980; Parkos and Wahrenbrock 1987; Milsom et al. 1996; Kohin et al. 1999). Northern elephant seal pups (*Mirounga angustirostris*) (Milsom et al. 1996; Kohin et al. 1999), juvenile harbour seals (*Phoca vitulina*) (Craig and Påsche 1980), and hooded seal pups (*Cystophora cristata*) (Påsche 1976a) also appear just as sensitive to hypoxia as terrestrial mammals (with an increased threshold level for eliciting a response), while juvenile Weddell seals (*Leptonyhotes weddelli*) exhibit a decreased hypoxic sensitivity (Parkos and Wahrenbrock 1987).

The effects of arousal state on breathing and responses to changes in respiratory drive in marine mammals have not been studied widely. Sleep is a state or period of reduced physical and neuronal activity accompanied by a suspension of voluntary movements and a complete or partial unconsciousness (Horne 1988; McGinty and Szymusiak 1994). As such, it is also accompanied by a reduction in metabolic rate and breathing. In mammals, there are three main states of arousal: wakefulness (WAKE), slow-wave sleep (SWS), and rapid eye-movement (REM) sleep. One of the key characteristics of SWS is a decrease in muscle tone, while in REM sleep there is a complete loss of muscle tone (Lydic 1987). Many marine mammals sleep for prolonged

periods while completely submerged in water suggesting they possess adaptations that either allow them to overcome the reduction or loss of muscle tone and float to/at the surface and breathe during sleep, or prolong breath-holding. The latter could entail a reduction in hypoxic/hypercarbic sensitivity. While there is a growing body of information regarding breathing patterns during sleep in phocid seals, information regarding sensitivity to changes in respiratory drive during different arousal states is limited.

During routine dive bouts in awake phocid seals, breathing is episodic with periods of apnea during diving followed by short periods of breathing at the water's surface (Butler and Jones 1982). Studies on northern elephant seal pups (Bartholomew 1954; Castellini et al. 1994; Milsom et al. 1996), harp seal pups (*Pagophilus groenlandicus*) (Lyamin 1993; Lyamin et al. 1993), adult Caspian seals (*Phoca caspica*) (Mukhametov et al. 1984), and juvenile gray seals (*Halichoerus grypus*) (Ridgway et al. 1975; Mortola and Lanthier 1989) have shown that while awake on land, breathing is continuous and irregular with only short periods of apnea present. In all phocid seal species studied to date, SWS has been shown to occur both on land and in the water, and breathing during SWS is always episodic with submerged individuals floating to the surface to breathe (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1994a). Gray seals (juvenile and newborn) (Ridgway et al. 1975; Mortola and Lanthier 1989), northern elephant seal pups (Castellini et al. 1994), harp seal pups (Lyamin 1993), and adult Caspian seals (Mukhametov et al. 1984) all exhibit REM sleep both in and out of the water. Breathing is continuous during REM sleep in gray seals, therefore animals must be positioned such that they can breathe, while in both harp seal

pups and adult Caspian seals, breathing is rare during REM sleep, and when present, it is sporadic and irregular (Ridgeway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Lyamin et al. 1993). In northern elephant seal pups, breathing appears never to occur during REM sleep (Castellini et al. 1994; Milsom et al. 1996).

To the best of my knowledge, the information concerning respiratory sensitivity in phocid seals during sleep is restricted to one study. Milsom et al. (1996) examined the effects of both hypoxia and hypercarbia on breathing in northern elephant seal pups. In their study, animals were subjected to levels of hypoxia and hypercarbia that roughly doubled their breathing frequency (reported average: 13.3% O_2 ± 0.15 SEM and 5.6% CO_2 ± 0.31 SEM). No study to date has performed a detailed examination of the slopes and thresholds of the ventilatory responses to hypoxia and hypercarbia during sleep in phocid seals. The purpose of my study, therefore, was to produce a more complete stimulus response curve depicting the effects of hypoxia and hypercarbia on the cardio-respiratory and breathing pattern responses of the harbour seal during different states of arousal. Specifically I wished to determine whether the responses seen in harbour seal pups differed from those seen in terrestrial mammals, particularly during the transition from WAKE to sleep.

2.2 Methods

2.2.1 Animals and Housing

Three male harbour seal pups, wild caught off the west coast of BC, under a license issued by the Department of Fisheries and Oceans, Canada, were housed in two connecting outdoor tanks located at the University of British Columbia (UBC), under

conditions of natural photoperiod and ambient temperature. They were raised in captivity and at the time of the experiments were 6 to 12 months old, weighing 36-42 kg. A large, wooden deck on which the seals could haul out surrounded the two tanks. The tanks were 3 meters in diameter and 1.5 meters deep, and fresh water continuously flowed through each. All seals were fed a diet of herring (*Clupea sp.*), which was supplemented daily with a vitamin tablet (Mazuri Vita-Zu Mammal Tablet, PMI Nutrition International) placed under the operculum of one fish. Experiments were conducted between January and June of 2000.

2.2.2 Surgical Procedures

The following experiments were performed under the guidelines established by the Canadian Council for Animal Care, and were approved by the UBC Animal Care Committee (Permit # A98-0290). Each seal was manually restrained and anesthetized with 5% Isoflurane (Janssen) in oxygen (5 L/minute) administered via face mask. When a sufficient level of anesthesia was obtained, such that jaw muscle tone was minimal, the animal was intubated and anesthesia was maintained with 1 to 3% Isoflurane. Since apnea is common in seals under anesthesia, all animals were manually ventilated throughout the entire surgery.

All surgical procedures were performed aseptically. Surgical sites were shaved and disinfected with 70% alcohol and an iodine-based solution (polyvinylpyrrolidine-iodine complex 10%, Iodovet, Rougier Pharma). To measure cerebral activity (electroencephalogram [EEG]), four cutaneous incisions were made on the dorsal aspect of the head, two above the frontal and two above the parietal bones of the skull. At each

incision point, a small, stainless steel screw with accompanying lead wire was fixed into the skull, and secured with dental acrylic (Flexacryl, Lang Dental). To measure postural muscle tone (electromyogram [EMG]), two Teflon-coated, stainless steel wires were sutured into the right and left nuccal muscles respectively. On opposite sides of the dorsum of the seal, gold disc electrocardiogram (EKG) electrodes (Grass Instruments) (one caudal and one cranial to the heart) were sutured just beneath the panniculus (blubber layer). The leads from the EEG, EMG and EKG electrodes were then tunneled between the skin and panniculus to an exit point located on the back of the animal between the scapulae, where they were subsequently soldered to an eight-pin underwater computer connector (USI square miniconn, Underwater Systems) which was then secured to the fur on the back of the seal with epoxy glue (Evercoat Ten Set Epoxy Adhesive and Filler, Fibre Glass-Evercoat Co., Inc.). A diagrammatic representation of the electrode placement is shown in Figure 2.1.

All incision sites were sutured and infiltrated with 1 ml of Bubivacaine hydrochloride (Marcaine, Sanofi Winthrop Pharmaceuticals) to provide post-operative analgesia. After surgery, seals were given one intra-muscular injection of an anti-inflammatory (1 mg/kg of Ketoprofen, Anafen, Merial) and allowed to recover in a dry pool for 24 hours. For two weeks post-surgery, seals were placed on oral antibiotics (2.5 mg/kg of Enroflaxacin; Baytril 50 mg/ml, Bayer Inc., Toronto, Ontario, Canada) to prevent infection of the cutaneous wounds.

Of the three study animals, only one developed a minor infection at one of the incision sites. This was successfully treated with oral antibiotics (2.5 mg/kg of Enroflaxacin; Baytril 50 mg/ml, Bayer Inc.) with no long-term effects on the health of the animal.

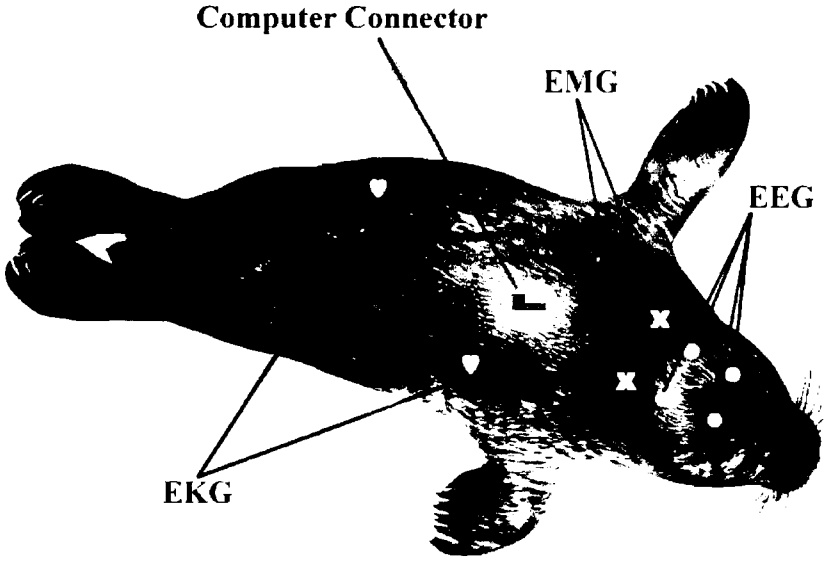


Figure 2.1. Electrode placement. Four EEG electrodes were placed in the skull of each seal, two above the frontal and two above the parietal bones. EMG electrodes were sutured into the right and left nuccal muscles respectively, and on opposite sides of the dorsum of the seal, gold disc EKG electrodes (one caudal and one cranial to the heart) were sutured just beneath the panniculus. The leads from the EEG, EMG and EKG were then tunneled between the skin and panniculus to an exit point located on the back of the animal between the scapulae, where they were subsequently soldered to an eight-pin underwater computer connector which was then secured to the back of the seal with epoxy glue.

2.2.3 Experimental Protocol

Prior to each series of experiments, seals were habituated to a 120 litre, custom built, clear, plexi-glass metabolic dome until they exhibited periods of sleep and quiet wakefulness with no signs of stress. Two to four hours before an experiment, seals were fed approximately 500g of herring. Just prior to the experiment, the relaxed, satiated seal was coaxed onto deck and entered a kennel, in which they were subsequently weighed (± 0.2 kg). After returning to the deck, the computer connector on the back of the seal was attached to the lead to a computer, and the seal was secured in the metabolic dome. Seals were allowed a ten-minute adjustment period before data recording began, and the length of the recording session varied from two to four hours, depending on co-operation levels of the seal. Preliminary observations indicated that the most optimal time in which to conduct experiments was at night, however due to limitations in visual acuity of the observer, experiments were performed during the second most optimal time, between 10am and 2pm. A hypnogram of this time period for one seal in one session (Fig. 2.2) illustrates that there was still a large proportion of time spent in sleep. Ambient air was pulled through the metabolic dome via a vacuum (6.5 Gallon, 3.0 peak horsepower Wet/Dry Vacuum, Shop Vac) at a rate of 28 to 30 litres/minute. Small holes placed at the back of the dome allowed for controlled inflow of ambient air, as well as the manipulation of that air by the addition of various gases. Sub-samples of the outflow air were dried with anhydrous calcium sulfate (Drierite, Drierite Company) and analyzed for fractional composition of oxygen (F_{O_2}) and carbon dioxide (F_{CO_2}) using medical gas analyzers (Beckman OM-11 oxygen analyzer [calibrated with ambient air] and Beckman LB-2 CO_2 analyzer [calibrated with a 5% CO_2, 95% N_2 mixture]) (Fig. 2.3). Temperature

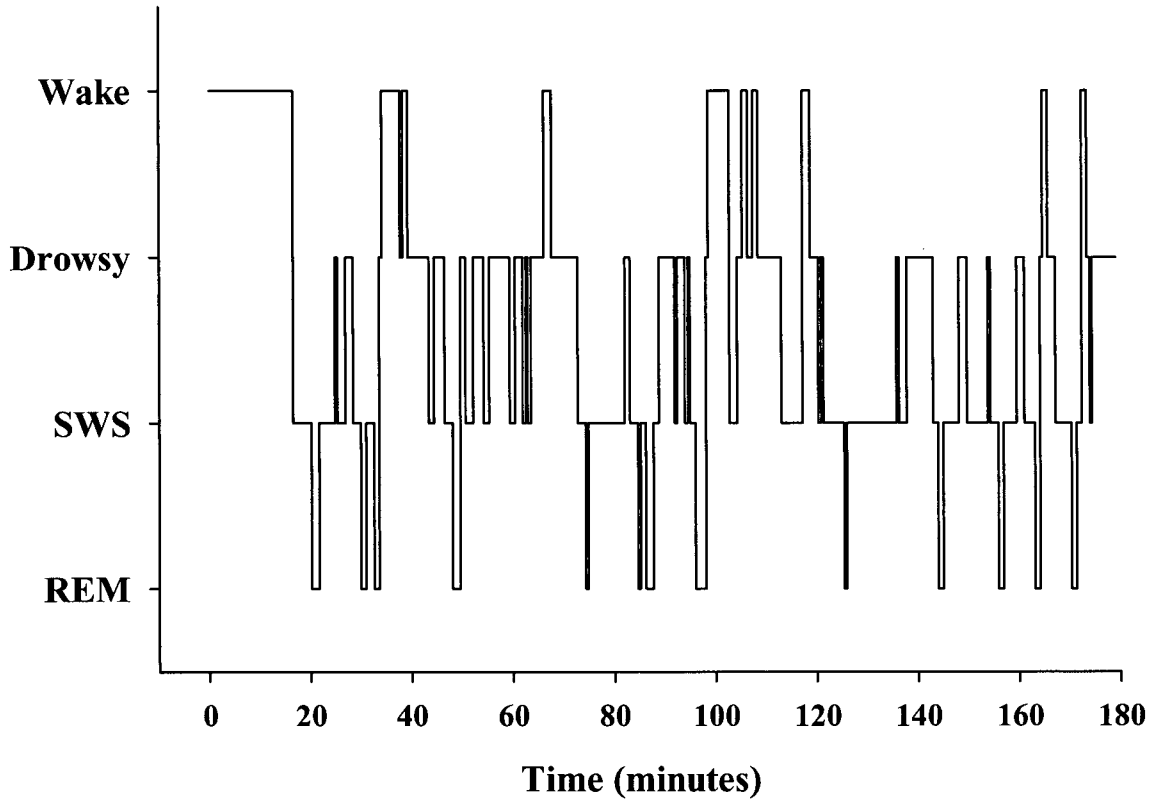


Figure 2.2. Wake-Sleep Hypnogram. The amount of time an animal spends in each state of arousal, during a specified period of time, is visible in this four-hour hypnogram of one harbour seal pup. Note that the appearance of REM sleep is rare, and in all cases, is preceded by SWS.

within the metabolic dome was continuously monitored, and maintained near ambient temperature (10°C to 25°C), well within the thermal neutral zone for juvenile harbour seals (–2.5°C to 25.1°C, Hansen et al. 1995).

EEG, EKG, and EMG signals, as well as a breathing trace (measured as the differential pressure between the metabolic dome and the outside) were amplified and filtered using Gould AC amplifiers, and, along with F_{O_2}, and F_{CO_2} were recorded continuously on computer using a data acquisition system (DataQ Instruments 1998; DI-400, DataQ Instruments) sampling at a rate of 222 Hz/channel. Behaviour, monitored through visual observation, was also recorded for the duration of the experiment.

To increase respiratory drive, the environment within the metabolic dome was made either hypoxic or hypercarbic. To decrease the oxygen content of the inflow air (F_{IO_2}) to the dome, 100% medical grade nitrogen was added to the metabolic dome at a known flow rate, and mixed with ambient air to give three different levels of hypoxia: 15.22% O_2 ± 0.47 SEM, 11.99% O_2 ± 0.29 SEM, and 8.63% O_2 ± 0.22 SEM. To increase the carbon dioxide content of the inflow air (F_{ICO_2}) to the dome, 100% medical grade carbon dioxide was added to the metabolic dome and mixed with ambient air to give three different levels of hypercarbia: 2.04% CO_2 ± 0.06 SEM, 4.16% CO_2 ± 0.06 SEM, and 5.66% CO_2 ± 0.07 SEM. Each gas level was presented to the animal on separate days, in semi-random order.

2.2.4 Data analysis

Arousal states were scored using standard sleep scoring techniques, as outlined by Rechtschaffen et al. (1968). Data traces were analyzed in 30-second epochs and divided

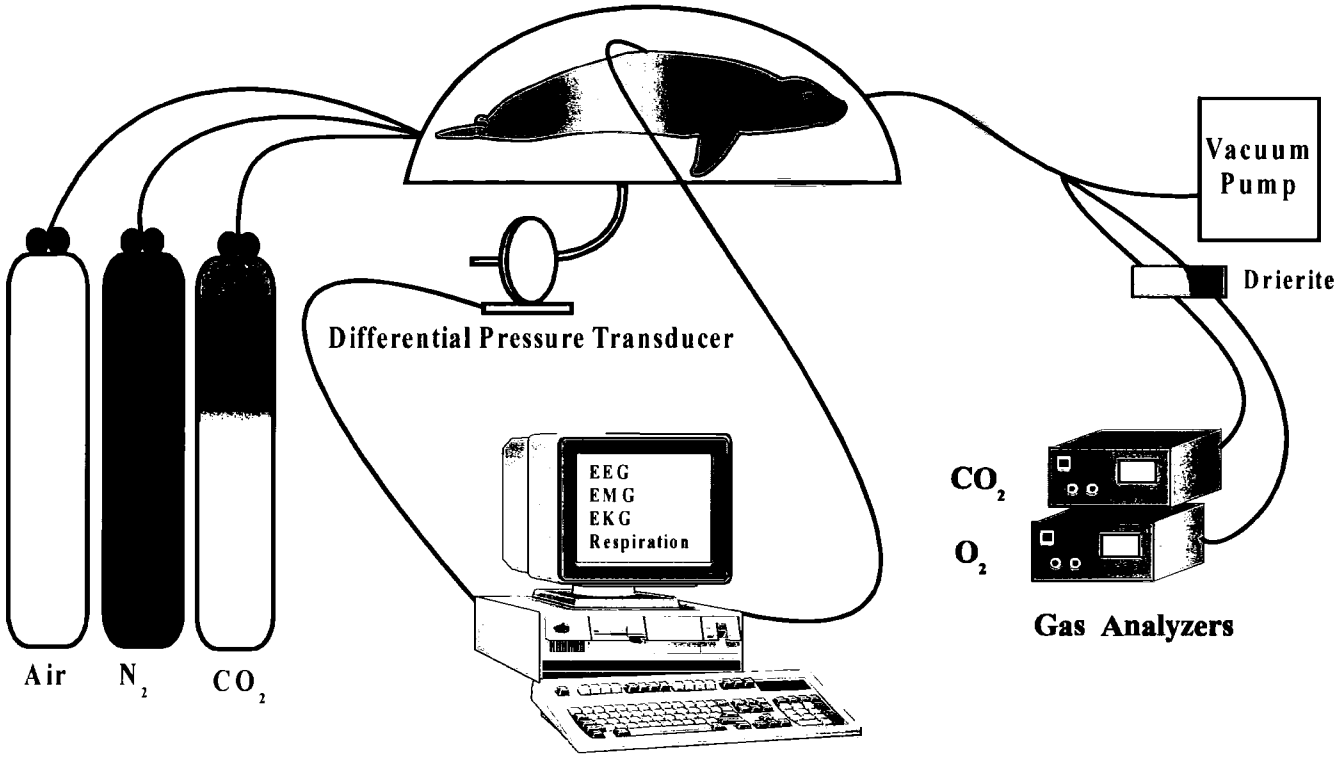


Figure 2.3. Experimental set-up. After being weighed, the seal was secured in a 120-litre clear, plexi-glass, metabolic dome with the eight-pin computer connector, attached to the back of the seal connected to the lead to a computer, where EEG, EMG, and EKG signals were recorded, along with a breathing trace, F_{O_2}, and F_{CO_2}. Behavioural observations were made throughout the experiment via visual observation and were subsequently noted. Air was pulled through the metabolic dome at a rate of 28-30 litres/minute with a vacuum, and sub-samples of the outflow air were removed, dried, and analyzed for F_{O_2}, and F_{CO_2}. 100% N_2 was mixed with ambient air at a known flow rate as it entered the dome to give hypoxic values of 15.22% $O_2 \pm 0.47$ SEM, 11.99% $O_2 \pm 0.29$ SEM, and 8.63% $O_2 \pm 0.22$ SEM. 100% CO_2 was mixed with ambient air at a known flow rate, as it entered the dome, to give hypercarbic values of 2.04% $CO_2 \pm 0.06$ SEM, 4.16% $CO_2 \pm 0.06$ SEM, and 5.66% $CO_2 \pm 0.07$ SEM.

into WAKE, drowsy, SWS, and REM sleep based on EEG and EMG traces (Fig. 1.1), as well as behaviour. Only well-established WAKE, SWS, and REM sleep periods were analyzed further.

The breathing trace was analyzed for total frequency (f_R) and tidal volume (V_T), which were subsequently used to calculate total ventilation ($\dot{V}_E$) in ml air/kg/minute.

eq. 2.1 $$\dot{V}_E = f_R \times V_T$$

To determine total breathing frequency (breaths/minute) for each state of arousal, the number of breaths per total time was calculated for each section of trace analyzed. Tidal volume (ml/kg) was calculated using the area under the differential pressure transducer trace, which is proportional to the product of (flow) X (time), for each breath. For each animal, ten breaths were analyzed in each state, at each level of respiratory drive, and V_T was calculated using the following equation, adapted from McArthur and Milsom (1991).

eq. 2.2 $$V_T = \frac{\left\{ \frac{(\text{area of breath}) \times (\text{volume of calibration breath}) \times T_b(PSH_2O - PCH_2O)}{(\text{area of calibration breath}) \times [T_b(PSH_2O - PCH_2O) - T_c(PSH_2O - PCH_2O)]} \right\}}{\text{weight of seal}}$$

where T_b is body temperature (°K), PSH_2O is water vapor pressure at sea level (760mmHg), PCH_2O is water vapor pressure of the metabolic dome (corrected for dome temperature), and T_c is temperature inside the metabolic dome (°K).

Because of the presence of prolonged end-inspiratory pauses or apneas during normal breathing, animals could increase breathing frequency by either reducing or eliminating the periods of apnea, or by reducing the length of individual breaths, and hence the inter-breath interval. To distinguish between these options, apnea length and inter-breath intervals were also determined from the breathing trace. An apnea is defined as any pause in breathing exceeding the length of two missed breaths, in this case, any

pause greater than 5 seconds. The inter-breath interval is defined as the time, in seconds, between any two breaths not separated by an apnea, and was used to determine the instantaneous breathing frequency (f_{Rinst} = 60/inter-breath interval). The total time spent breathing (eupneic) versus not breathing (apneic) at each level of respiratory drive was determined as a percentage of the total time analyzed.

Oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) values were calculated for WAKE and SWS states, on air, using equation 2.3 and 2.4 (adapted from Frappell et al. 1992, equations A5 and A8 respectively). All $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values are recorded in ml O_2/kg body weight, STPD. The average respiratory quotient (RQ) for each seal, on air, was determined from the calculated values of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Table 2.1).

eq. 2.3

$$\dot{V}_{O_2} = \frac{(F_{IO_2} - F_{EO_2})\text{air flow}}{\frac{1 - F_{IO_2}}{\text{weight of seal}}}$$

eq. 2.4

$$\dot{V}_{CO_2} = \frac{(F_{ECO_2} - F_{ICO_2})\text{air flow}}{\frac{1 - F_{ICO_2}}{\text{weight of seal}}}$$

eq. 2.5

$$RQ = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}$$

To determine $\dot{V}_{O_2}$ during hypoxic conditions, the average RQ value for that animal on air, and the calculated $\dot{V}_{CO_2}$ (equation 2.6) for the gas level in question were used (equation 2.7):

eq. 2.6

$$\dot{V}_{CO_2} = \frac{(F_{ECO_2} - F_{ICO_2})\text{air flow}}{\text{weight of seal}}$$

Table 2.1. Mean $\dot{V}_{O2}$, $\dot{V}_{CO2}$, and RQ values on air, during both WAKE and SWS states. The calculated RQ values in air were used to calculate $\dot{V}_{O2}$ during hypoxic conditions.

	WAKE	SWS
$\dot{V}_{O2}$ ml O_2/kg/min	8.79 ± 0.70	†7.71 ± 0.93
$\dot{V}_{CO2}$ ml CO_2/kg/min	6.37 ± 0.35	†4.97 ± 0.28
RQ	0.73 ± 0.03	0.66 ± 0.03

Mean values ± SEM. † indicates a significant difference between WAKE and SWS values. (N=3)

eq. 2.7

$$\dot{V}_{O_2} = \frac{\dot{V}_{CO_2}}{RQ}$$

where F_{ICO_2} is assumed to be 0.003% in ambient air. To determine $\dot{V}_{O_2}$ during hypercarbic conditions, F_{IO_2} values (0.2094 in ambient air) and recorded values of F_{EO_2} were used in the following equation:

eq. 2.8

$$\dot{V}_{O_2} = \frac{(F_{IO_2} - F_{EO_2})\text{air flow}}{\text{weight of seal}}$$

Equations 2.6 and 2.8 are adapted from Frappell et al. (1992; equations A6 and A3 respectively).

It is assumed, when calculating $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, that air is dried and CO_2 free (Withers 1977; Frappell et al. 1992). Although air was dried prior to analysis in the current study, CO_2 was not removed. According to Withers (1977), the errors associated with failure to remove CO_2 are negligible under normal conditions. Failure to dry the outflow air prior to the measurement of flow rate (as was done in the present experiment) results in a maximum error of 6.2% in the calculated values of $\dot{V}_{O_2}$ (Withers 1977, Frappell et al. 1992). It should be noted however, that the air flow rates were recorded using a mass flow meter in litres/minute STPD, and therefore any errors associated with failure to dry the air prior to recording flow rate are minimal.

Oxygen consumption values were also used to calculate percent oxygen extraction ($\%E_{O_2}$) and air convection requirement (ACR) (ml air/ml O_2) at each level of respiratory drive for each state of arousal using the relationships:

eq. 2.9

$$\%E_{O2} = \frac{\dot{V}_{O_2}}{\dot{V}_E \times F_{IO_2}}$$

and

eq. 2.10

$$ACR = \frac{\dot{V}_E}{\dot{V}_{O_2}}$$

To determine average heart rate (f_H), the number of beats per total time (beats/minute) was calculated for each section of the trace. Both eupneic and apneic heart rates were also calculated independently (i.e. total # beats in eupnea/total time eupneic and total #beats in apnea/total time apneic). To assess the relative change in heart rate due to increases in respiratory drive, the % change in heart rate was calculated using air values as a baseline.

2.2.5 Statistical Analysis

Statistical analyses were performed using Sigma Stat software (Jandel Scientific, version 1.0, 1993). Data for the different levels of respiratory drive were analyzed and compared to air values using a one-way Repeated measures ANOVA, followed by the Bonferroni t-test method of all pairwise multiple comparison. Data for SWS were compared to WAKE values using similar statistical analyses. Data were considered significantly different if $P<0.05$. All results are presented as means ± SEM.

2.3 Results

2.3.1 Hypoxia

2.3.1.1 Respiration

Decreasing F_{IO_2} produced increases in f_R during both wakefulness and SWS (Fig. 2.4), however the increase was only significant at levels of 8.63% O_2 ± 0.22 SEM. f_{Rinst} remained relatively constant in both states as F_{IO_2} decreased (Table 2A) indicating that the increases in f_R were due solely to decreases in the length (Fig. 2.4) and frequency

(Table 2A) of the periods of apnea. As a consequence, the percent time apneic decreased dramatically in both WAKE and SWS (Table 2A). There was no change in V_T in WAKE or SWS during hypoxia and, as a consequence, the changes in total ventilation ($\dot{V}_E$) during hypoxia reflected the changes in f_R (Fig. 2.4D).

f_R was significantly lower in SWS than WAKE during air and 15.22% O_2 ± 0.47 SEM due primarily to increases in the length and frequency of occurrence of apneas, and thus increases in the percent time apneic (Fig. 2.4, Table 2.2A). V_T, on the other hand, increased in animals as state of arousal decreased from WAKE to SWS (this increase was however, not significant) (Fig. 2.4C) and thus, in general, levels of total ventilation remained constant regardless of arousal state at all levels of hypoxia Fig. 2.4D). Thus while there was no decrease in hypoxic sensitivity associated with the transition from WAKE to sleep (there was a strong hypoxic ventilatory response present in both states), breathing was always slower but deeper during SWS.

2.3.1.2 Metabolic rate

Oxygen consumption ($\dot{V}_{O_2}$) was unaffected by hypoxia within each state (Fig. 2.5A). Although the total amount of oxygen available in each breath decreased substantially in hypoxia, there was no significant change in the relative amount of oxygen extracted per breath ($\%E_{O_2}$) in WAKE or SWS (Fig. 2.5B). Thus, since the amount of O_2 in the ambient air decreased, while the $\dot{V}_{O_2}$ and $\%E_{O_2}$ levels remained constant, the air convection requirement (ACR) or total amount of ventilation required to meet oxygen demands, increased, although not significantly, during both arousal states (Fig. 2.5C).

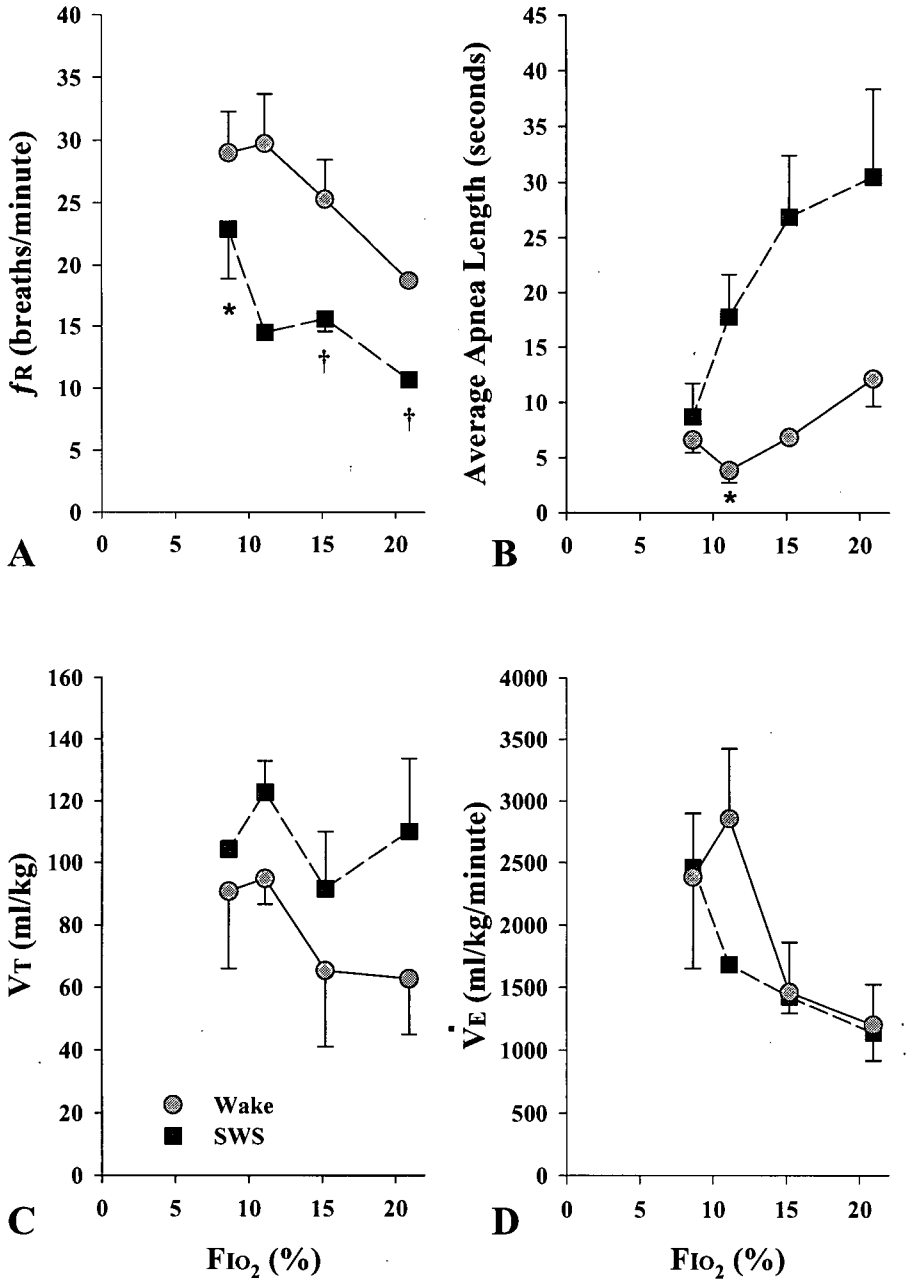


Figure 2.4. The effect of hypoxia on breathing frequency (f_R) (A), average apnea length (B), tidal volume (V_T) (C), and total ventilation ($\dot{V}_E$) (D) as a function of state of arousal. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE and SWS values at that level of respiratory drive. (N=3 in all states of arousal, at all level of hypoxia, except 15.22% O_2 ± 0.47 SEM where N=2).

Table 2.2. Values for various respiratory variables during WAKE and SWS in A) an hypoxic and B) an hypercarbic environment. Mean values ± SEM . * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE and SWS values at that level of respiratory drive.

A	WAKE				SWS			
	Air	15.22% O_2 ±0.47	11.99% O_2 ±0.29	8.63%O_2 ±0.22	Air	15.22% O_2 ±0.47	11.99% O_2 ±0.29	8.63%O_2 ±0.22
#apneas/hr	146.40 ±29.30	98.40 ±27.30	*58.60 ± 29.60	*61.70 ±19.10	102.70 ±31.00	111.10 ±31.70	117.40 ±26.60	78.30 ±29.30
#apneas/hr >20sec	16.227 ±7.10	*1.01 ± 0.583	*0.33 ±0.33	*0.46 ±0.46	† 34.43 ±5.398	*† 20.25 ±5.03	25.04 ±9.84	*7.75 ± 6.88
Maximum apnea (sec)	24.82 ±6.75	10.64 ±1.03	9.00 ±1.60	11.38 ±0.96	† 74.43 ±4.76	50.29 ±12.10	47.91 ±17.62	* 19.59 ±9.00
Time in apnea (%)	44.10 ±1.17	*21.20 ±4.48	*11.20 ±5.45	*14.00 ±2.81	† 62.30 ±1.46	† 48.70 ±1.00	† 50.50 ±2.81	*24.40 ±3.90
% Time spent breathing	55.90 ±1.17	78.8 ±4.47	66.5 ±26.220	86.00 ±3.90	† 37.70 ±1.47	† 51.40 ±0.981	49.50 ±2.82	*75.60 ±13.24
Instantaneous *f*R (breaths/minute)	33.80 ±0.45	32.30 ±2.02	33.50 ±2.16	32.30 ±2.48	29.60 ±1.97	30.40 ±1.56	29.40 ±0.52	30.80 ±3.76

B	WAKE				SWS			
	Air	2.04% CO_2 ±0.06	4.16% CO_2 ±0.06	5.66% CO_2 ±0.07	Air	2.04% CO_2 ±0.06	4.16% CO_2 ±0.06	5.66% CO_2 ±0.07
#apneas/hr	146.40 ±29.33	79.22 ±20.46	*23.65 ±10.46	*7.84 ±5.00	102.67 ±30.97	81.38 ±27.72	37.91 ±4.72	14.59 ±14.36
#apneas/hr >20sec	16.23 ±7.10	8.40 ±8.40	7.93 ±7.93	*0.82 ±0.82	† 34.43 ±5.40	†22.64 ±10.83	17.28 ±10.40	*2.04 ±2.04
Maximum apnea (sec)	23.55 ±6.63	15.60 ±4.01	12.02 ±7.76	*6.59 ±4.04	† 74.43 ±4.76	* 46.90 ±13.27	*24.93 ±5.96	*7.79 ±4.22
Time in apnea (%)	44.13 ±1.17	20.91 ±6.35	*10.89 ±8.71	*2.24 ±1.75	†62.28 ±1.46	*40.79 ±7.12	*20.81 ±7.22	*4.48 ±4.43
% Time spent breathing	55.90 ±1.17	*77.20 ±8.06	*89.10 ±8.70	*97.80 ±1.76	†37.70 ±1.47	*†59.60 ±6.70	*79.20 ±7.22	*94.30 ±5.17
Instantaneous *f*R (breaths/minute)	33.80 ±0.45	34.40 ± 2.49	34.90 ±2.45	35.90 ±1.27	29.60 ±1.97	29.00 ±3.20	33.40 ±4.68	35.40 ±3.17

N=3 in all states of arousal, at all level of hypoxia, except 15.22% O_2 ± 0.47 SEM where N=2).

$\dot{V}_{O_2}$ was significantly reduced during SWS in animals breathing air and 11.99% O_2 ±0.29 SEM compared to levels during WAKE (Fig. 2.5A). There was, however, no difference in $\%E_{O_2}$ or ACR as arousal state decreased from WAKE to SWS (Fig. 2.5B, C).

2.3.1.3 Heart Rate

With increasing levels of hypoxia, f_H *decreased* during WAKE and SWS (Fig. 2.6A). In general these decreases were only significant at the lowest levels of F_{IO_2}. While the decrease was not significant during REM sleep, I have no data at the lowest level of F_{IO_2} (Fig. 2.6A). Interestingly, the relative decrease in f_H was the same regardless of arousal state (Fig. 2.6B). f_H was always higher during periods of breathing than during periods of apnea, and hypoxia led to decreases in heart rate under both conditions (breathing and apnea) (Fig. 2.6C, D).

f_H was significantly lower in SWS and REM sleep compared to WAKE in air and at all levels of hypoxia tested. Since there were no significant differences between the f_H observed during breathing in WAKE and SWS, this reflected the lower f_H seen during periods of apnea in SWS and REM sleep.

2.3.2 Hypercarbia

2.3.2.1 Respiration

Hypercarbia also produced significant increases in f_R in both WAKE and SWS (Fig. 2.7A). The response was, however, proportionately much greater in SWS compared to WAKE (a 220% versus an 88% increase). f_{Rinst} (Table 2.2B) did not change in either state as CO_2 levels

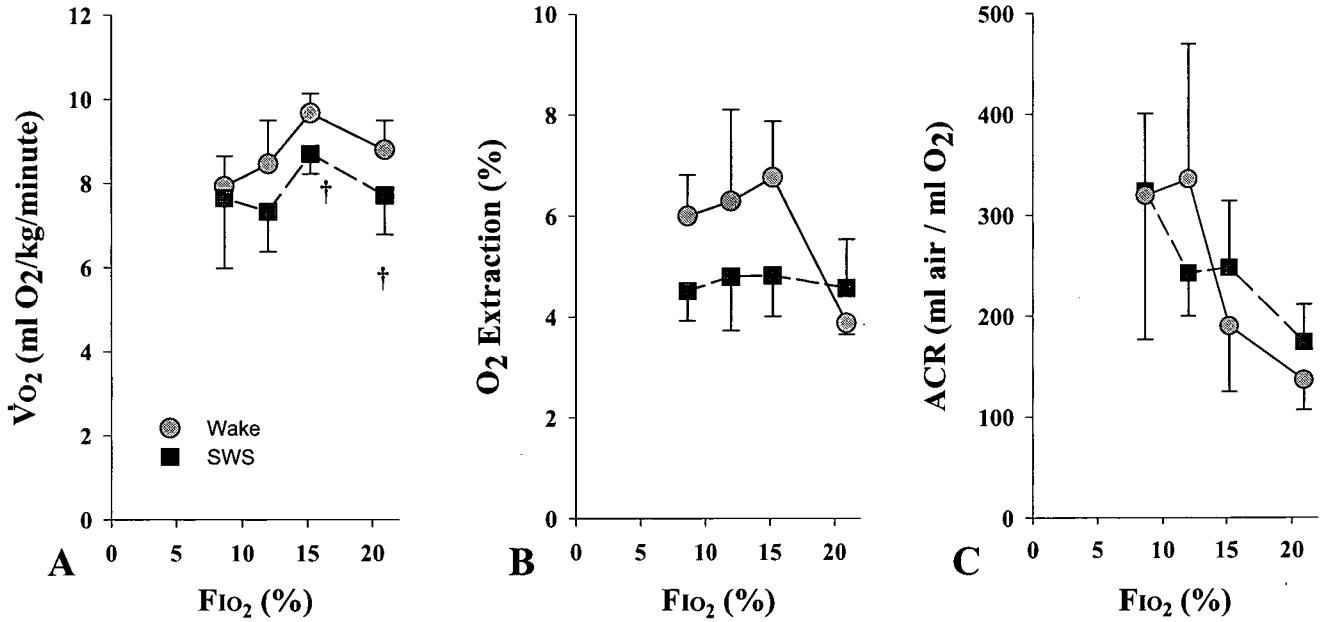


Figure 2.5. The effect of hypoxia on levels of oxygen consumption ($\dot{V}O_2$) (A), percent oxygen extraction (%E_{O_2}) (B), and air convection requirement (ACR) (C) as a function of state of arousal. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE and SWS values at that level of respiratory drive. (N=3 in all states of arousal, at all level of hypoxia, except 15.22% O_2 ± 0.47 SEM where N=2).

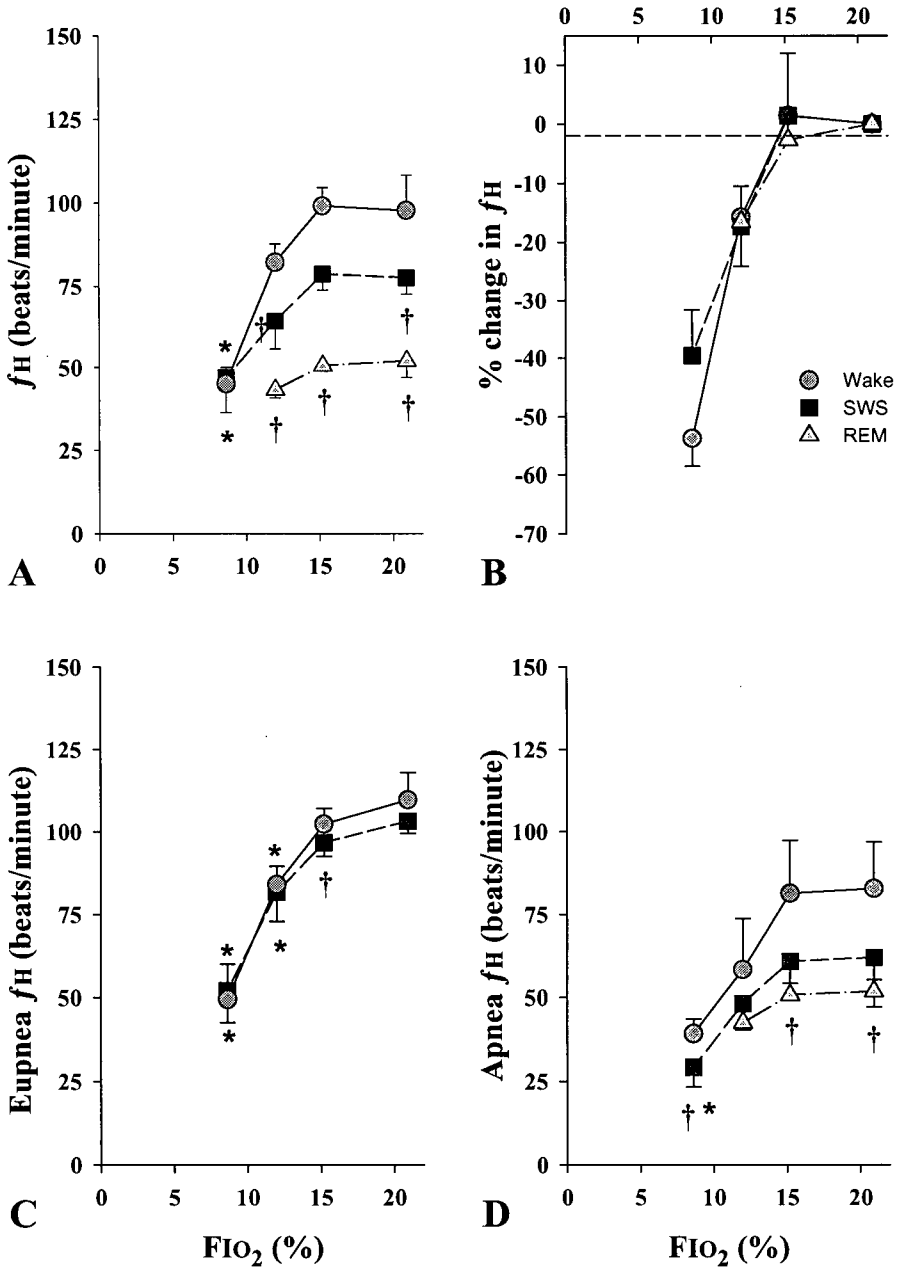


Figure 2.6. The effect of hypoxia on total heart rate (f_H) (A), % change in f_H (B), eupneic f_H (C), and apneic f_H (D) as a function of state of arousal. Data for the % change in f_H are calculated using air values as baseline levels. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE, SWS, and REM sleep values at that level of respiratory drive. (N=3 in all states of arousal, at all level of hypoxia, except 15.22% O_2 ± 0.47 SEM where N=2).

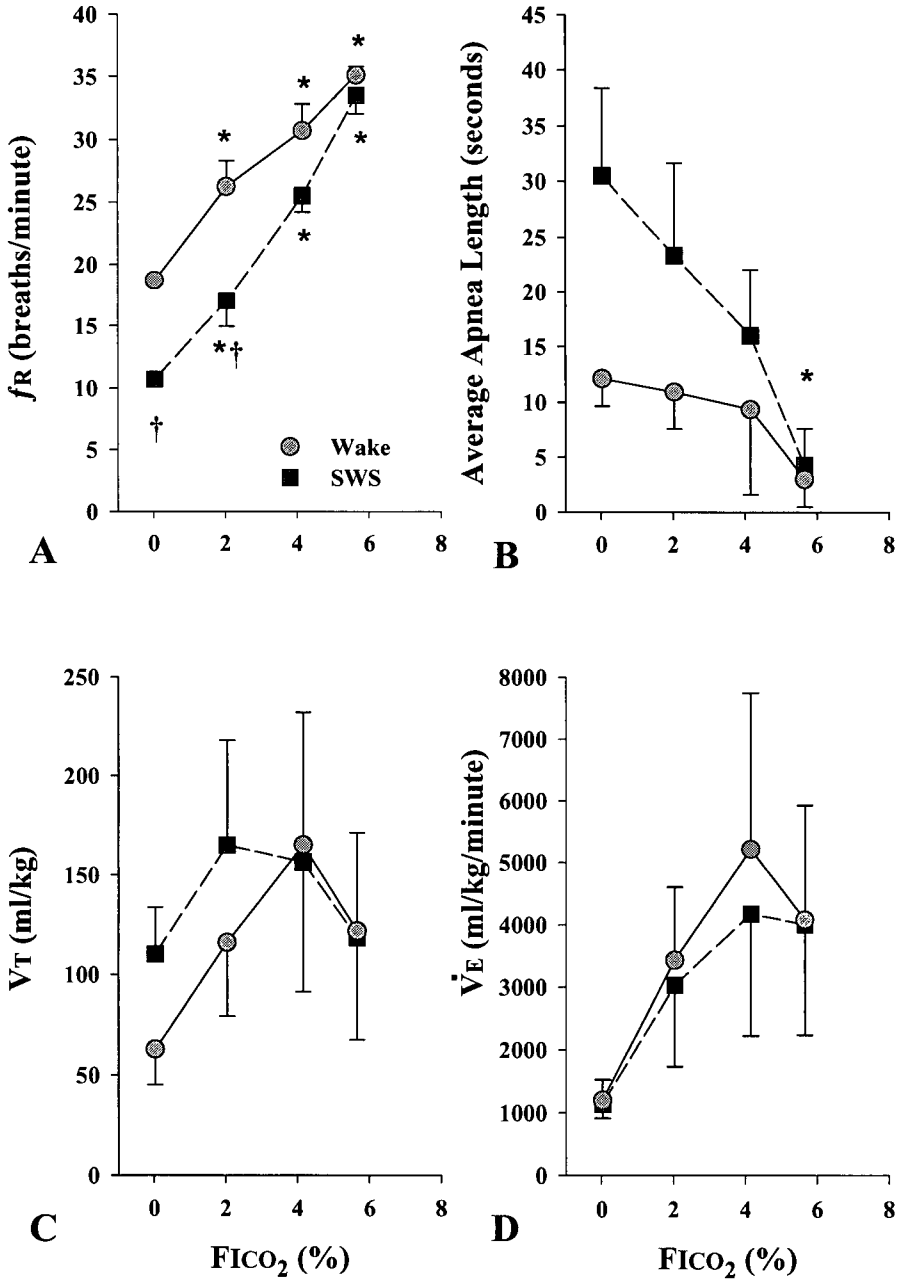


Figure 2.7. The effect of hypercarbia on breathing frequency (f_R) (A), average apnea length (B), tidal volume (V_T) (C), and minute ventilation ($\dot{V}_E$) (D) as a function of state of arousal. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE and SWS values at that level of respiratory drive. (N=3).

rose. Thus, during wakefulness the increase in f_R was due to a significant decrease in the frequency of apneas while during SWS it was due to decreases in both the length and frequency of periods of apnea (Fig.2.7, Table 2.2B). As a consequence, there was a significant decrease in the percent time apneic in both WAKE and SWS (Table 2.2B). V_T also increased during hypercarbia during both arousal states, as did $\dot{V}_E$, however due to low N-values, these increases were not significant (Fig. 2.7).

As state of arousal decreased from WAKE to SWS, there were significant decreases in f_R in air and 2.04% CO_2 ± 0.06 SEM (Fig. 2.7A). The average length of apnea in SWS, although always greater than WAKE values, was never significantly greater (Figure 2.7B). Correspondingly, there were no differences in the frequency of occurrence of apneas between the different states of arousal, although the frequency of long apneas (>20 seconds) was significantly higher in SWS (Table 2.2B). Levels of V_T were not different between the different arousal states. The net result of these changes was that $\dot{V}_E$ was not significantly different between the two arousal states (Fig. 2.7D) regardless of the level of CO_2 present. Thus there was also no decrease in hypercarbic sensitivity associated with the transition from WAKE to sleep (there was a strong hypercarbic ventilatory response present in both states), although breathing was slower but deeper during SWS at low levels of inspired CO_2.

2.3.2.2 Metabolic Rate

$\dot{V}_{O_2}$ did not change during hypercarbia in either state (Fig. 2.8A). As the level of CO_2 in air increased, and f_R increased significantly, there were significant decreases in the amount of

oxygen extracted from each breath ($\%E_{O_2}$) SWS only (Fig. 2.8B). This resulted in large, although not significant, increases in the ACR (Fig. 2.8C).

$\dot{V}_{O_2}$ was significantly reduced during SWS in animals breathing air, while $\%E_{O_2}$ was not significantly different between states and neither was the ACR (Fig. 2.8B, C).

2.3.2.3 Heart Rate

There were no significant changes in f_H during hypercarbia in awake animals or animals in SWS (Fig. 2.9A, B) but there were during REM sleep. This increase in f_H only occurred at 2% CO_2. f_H was always higher during periods of breathing than during periods of apnea while heart rate in hypercarbia remained constant during both eupniec and apneic periods (Fig. 2.9C, D).

On average, f_H was significantly reduced during SWS only in animals breathing air but was always reduced in REM sleep. This primarily reflected the higher heart rate seen during periods of breathing in awake animals and the fact that animals rarely breathed in REM sleep (Fig. 2.9D).

2.3.3 REM sleep

REM sleep was observed at all gas levels, but not in all animals (Table 2.3). At low levels of hypoxia there was a progressive increase in the percent of sleep episodes containing REM sleep but no change in the average length of REM sleep (Table 2.3). At the most severe level of hypoxia, however, there were reductions in both the incidence of REM sleep and the length of the REM sleep bouts. During hypercarbia, there was a progressive decrease in the percent of sleep episodes containing REM sleep but the length of REM episodes did not vary.

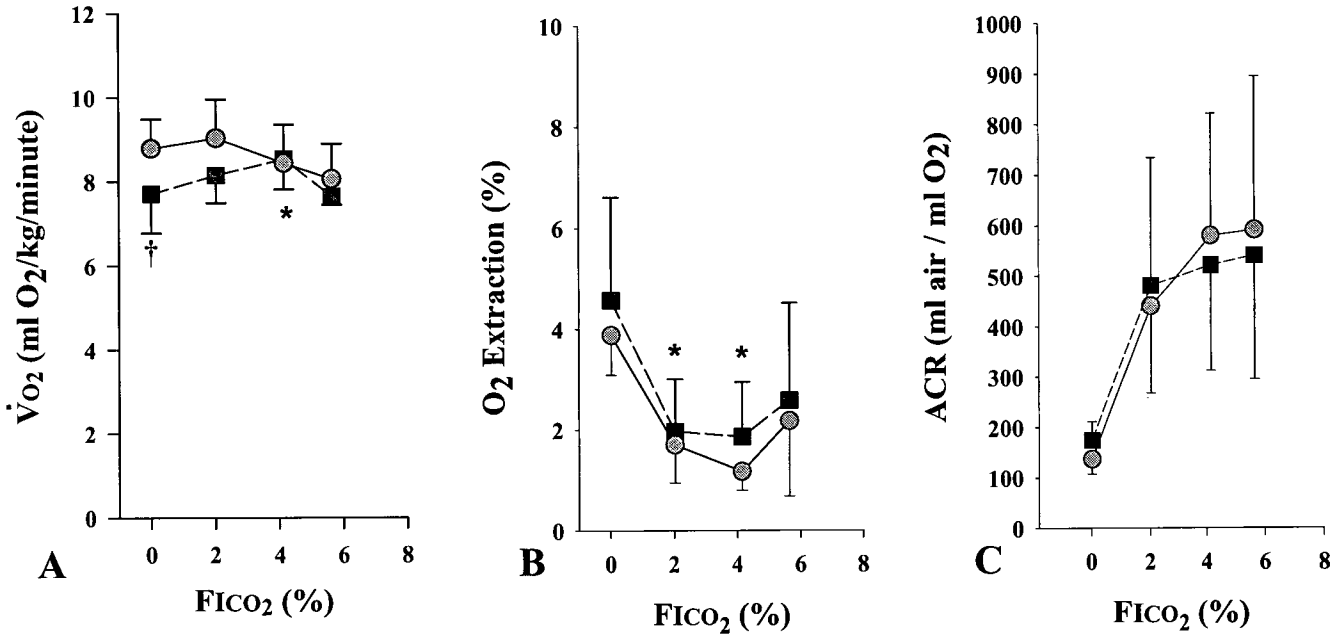


Figure 2.8. The effect of hypercarbia on levels of oxygen consumption ($\dot{V}O_2$) (A), percent oxygen extraction (%E_{O_2}) (B), and air convection requirement (ACR) (C) as a function of state of arousal. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE and SWS values at that level of respiratory drive. (N=3).

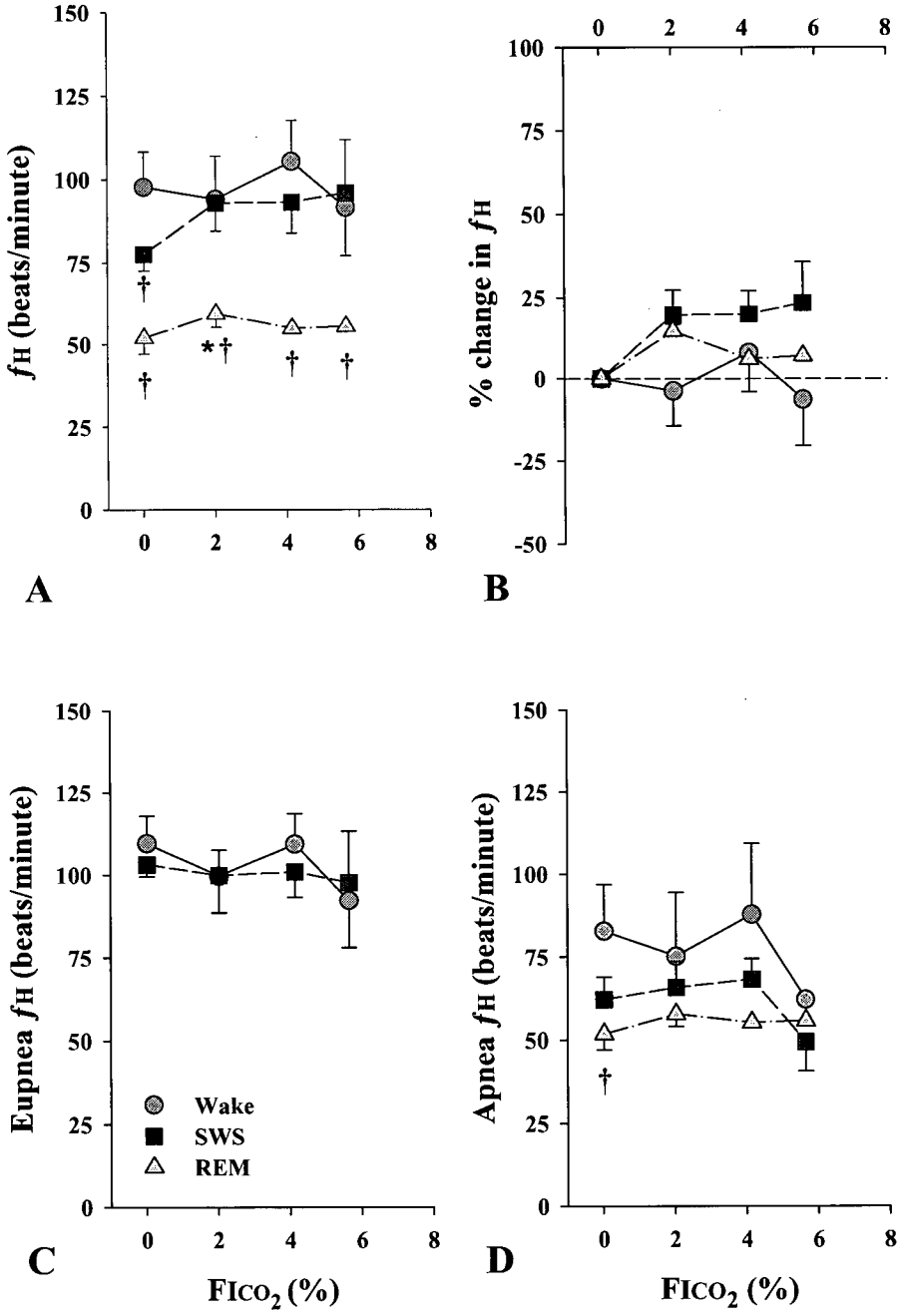


Figure 2.9. The effect of hypercarbia on total heart rate (f_H) (A), % change in f_H (B), eupneic f_H (C), and apneic f_H (D) as a function of state of arousal. * indicates a significant difference between a specific level of respiratory drive and air. † indicates a significant difference between WAKE, SWS, and REM sleep values at that level of respiratory drive. (N=3).

Table 2.3 Frequency of occurrence of REM sleep at different levels of respiratory drive.

	Air	Hypoxia			Hypercarbia		
		15.22 ± 0.47 $\%O_2$	11.99 ± 0.29 $\%O_2$	8.63 ± 0.22 $\%O_2$	2.04±0.06 $\%CO_2$	4.16±0.06 $\%CO_2$	5.66±0.07 $\%CO_2$
# animals exhibiting REM sleep	3	2	2	1	3	3	1
% sleep episodes with REM sleep	35.3	41.7	80.0	20.0	46.7	15.0	7.0
Average length REM sleep (sec)	29.4 ± 5.6	23.2 ± 2.6	33.8 ± 4.4	13.4 ± 2.8	22.0 ± 4.2	22.0 ± 4.2	20.30

Mean values ± SEM. (N=3)

Breathing was rare during REM sleep, occurring in 20% of trials in one animal breathing air, and in 25% and 33% of trials in two animals breathing 11.99% O_2 ± 0.29 SEM and 2.04% CO_2 ± 0.06 SEM (Table 2.4). Both f_R and V_T were highly variable on those rare occasions when breathing did occur during REM sleep (Table 2.4).

2.3.4 Breathing Pattern

The inter-breath intervals on air, and at the most extreme levels of hypoxia and hypercarbia are plotted on Poincaré plots (N versus N-1) for both awake animals and animals in SWS in Figure 2.10. While breathing air, the inter-breath interval was quite variable both in WAKE and SWS. During wakefulness, most inter-breath intervals were less than 50 seconds (Fig. 2.10A) while in SWS they were more variable and could be up to 110 seconds long. The increased variability of inter-breath intervals in SWS reflected the greater incidence of apneas and the presence of a more episodic breathing pattern compared to WAKE (Fig. 2.11). As the level of respiratory drive increased, in both hypoxia and hypercarbia, the length of the inter-breath interval was reduced and the amount of variability decreased. They were reduced to less than 20 seconds in length during WAKE and SWS in hypercarbia but could still last up to 70 seconds during SWS in hypoxia. Breathing during REM sleep was too rare to include in these comparisons.

2.4 Discussion

2.4.1 State of Arousal

The changes in respiration that accompany changes in arousal state in phocid seals have only been quantified in four species; yearling gray seals (Ridgway et al. 1975),

Table 2.4. Effect of hypoxia and hypercarbia on respiratory variables during REM sleep.

	Air	11.99 ± 0.29% O_2	2.04 ± 0.006% CO_2
fR (breaths/minute)	7.88	5.54 ± 0.80	9.59 ± 1.04
VT (ml/kg)	255.00 ± 190.00	60.90 ± 28.00	120.80 ± 27.90
$\dot{V}_E$ (ml/kg/minute)	1144.60 ± 1497.40	332.90 ± 136.10	1144.60 ± 253.60
% time spent breathing	0.95 ± 0.95	13.00 ± 10.03	10.80 ± 6.99

Mean values ± SEM (N=3)

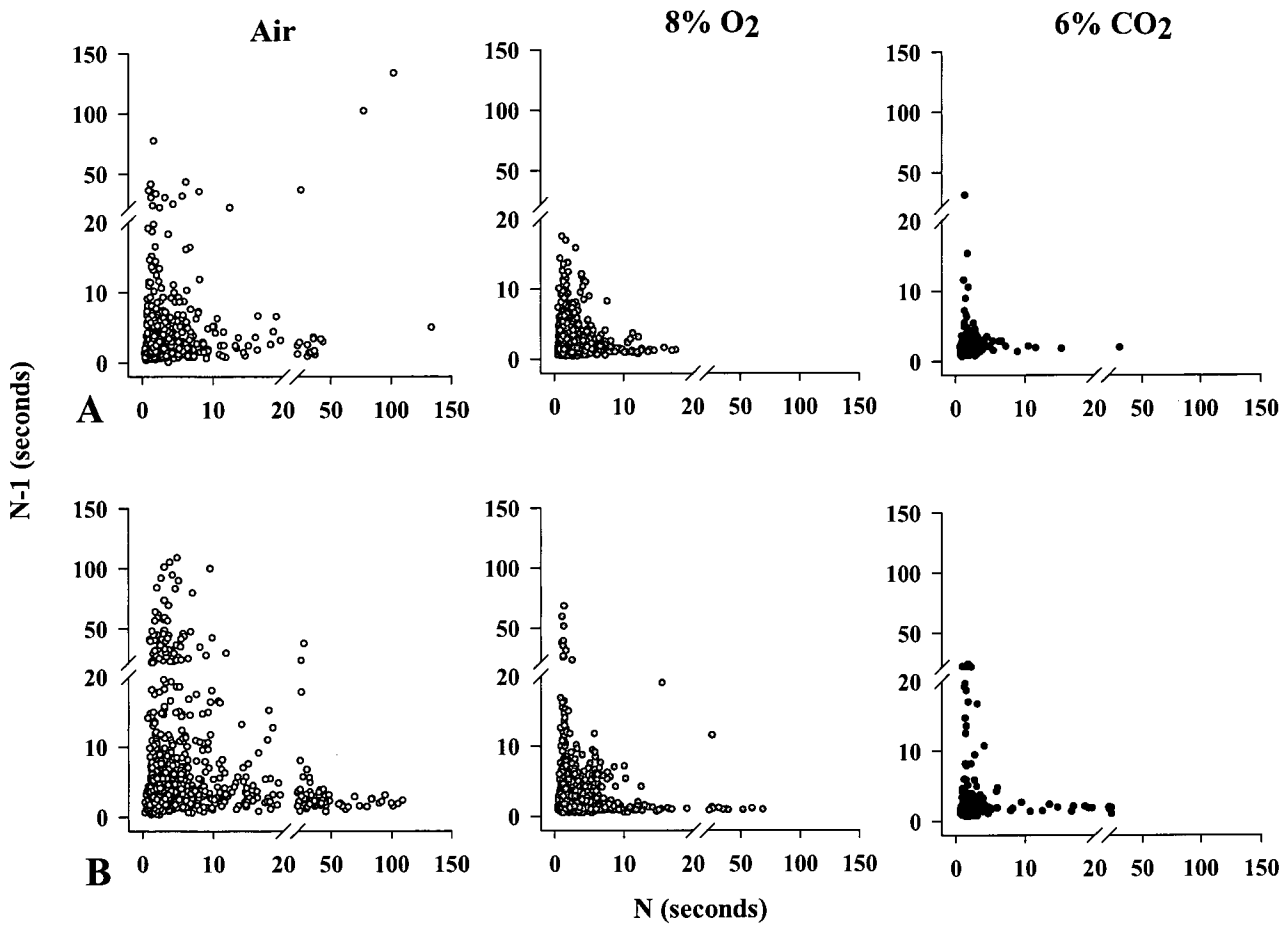
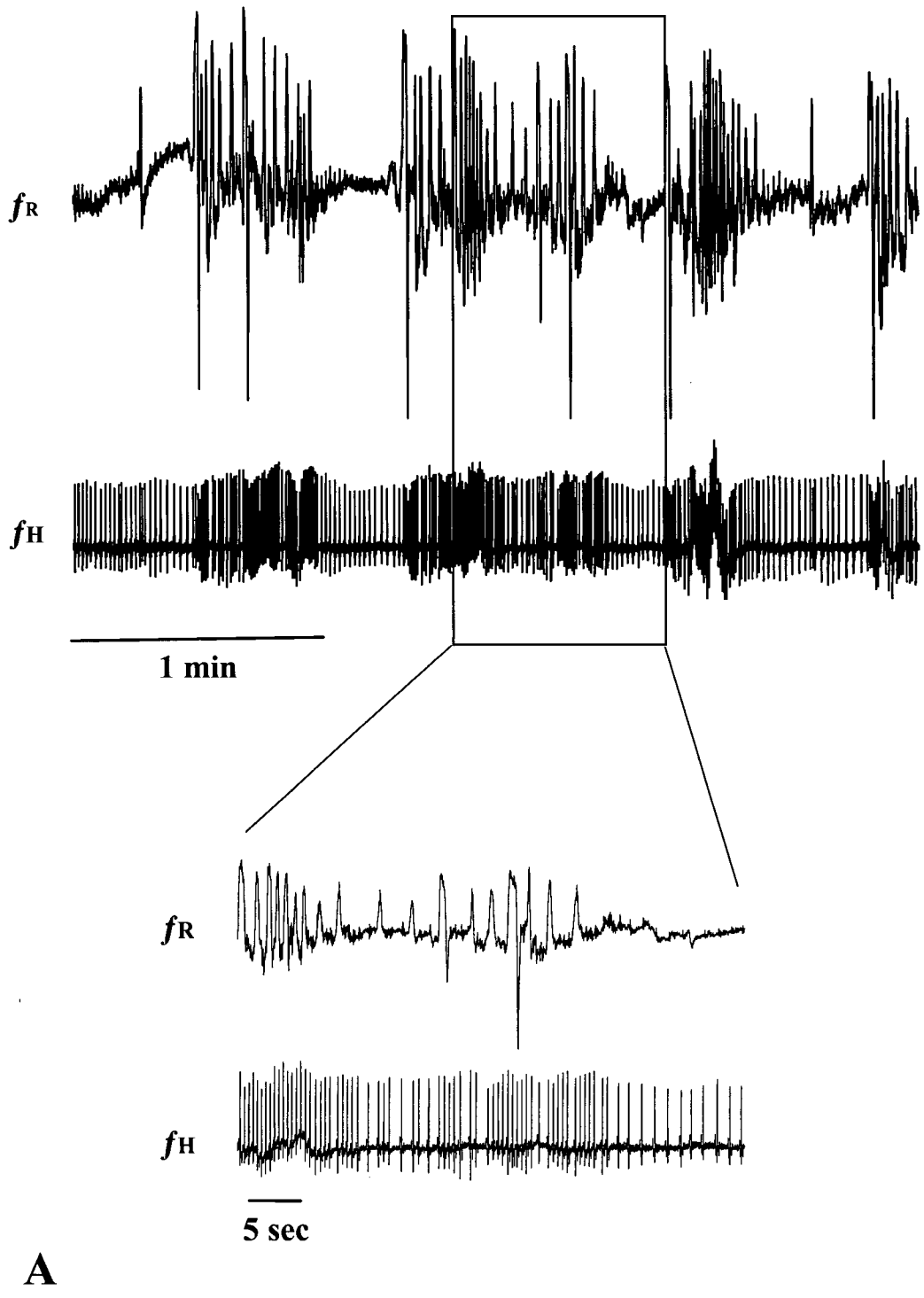


Figure 2.10. Poincaré plots illustrating the variability in inter-breath intervals during wakefulness (A), and SWS (B) as a function of respiratory drive (N=3) (see text for details).



A

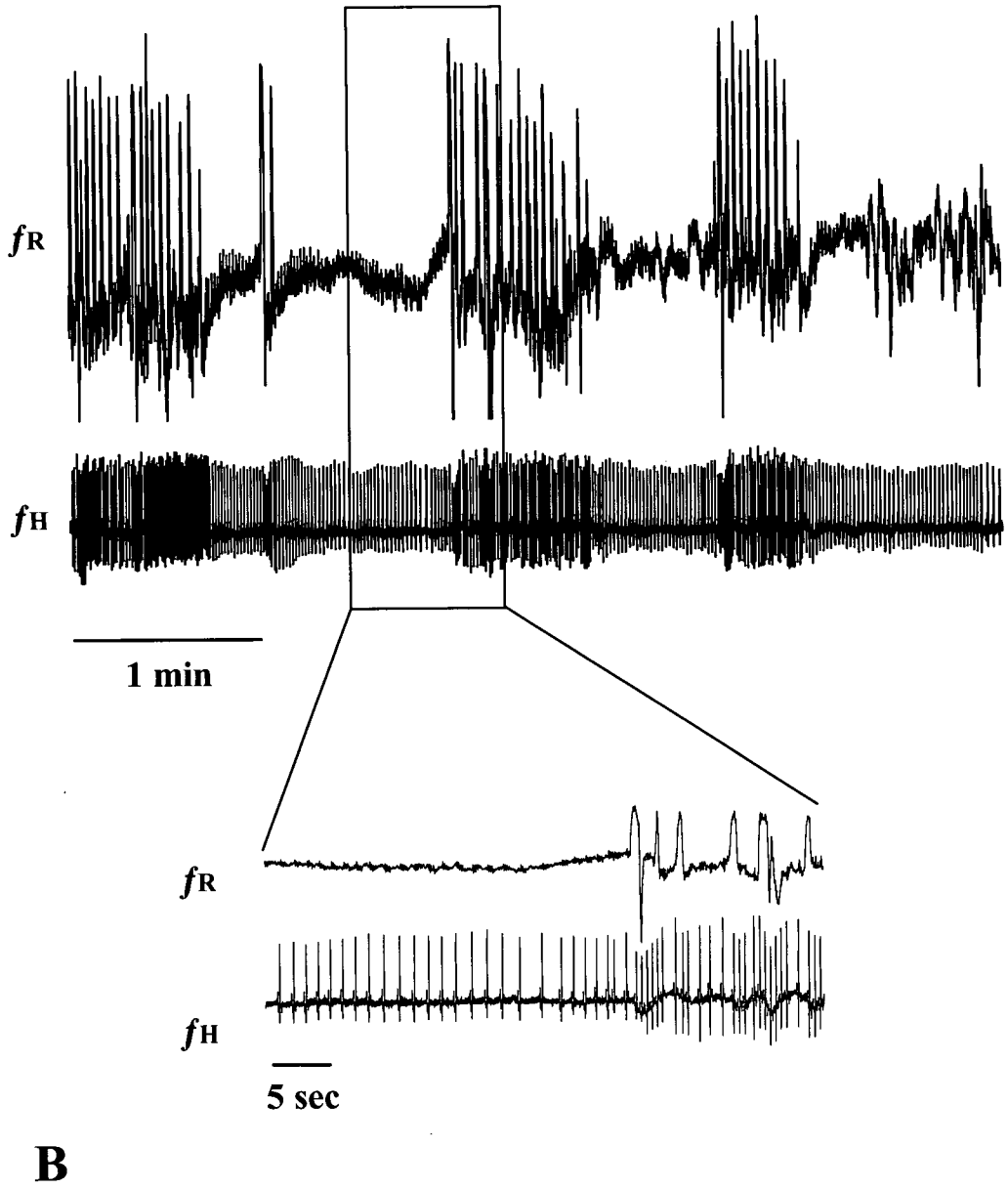


Figure 2.11. Breathing patterns of harbour seal pups in air during (A) WAKE (continuous and irregular and (B) SWS (episodic).

adult Caspian seals (Mukhametov et al. 1984), harp seal pups (Lyamin et al. 1993), and northern elephant seal pups (Castellini et al. 1994a). The study on northern elephant seal pups is the most in-depth study, focusing exclusively on the changes that occur in respiratory and cardiac frequency, as well as breathing patterns during the different states of arousal. In the present study, I have extended these studies to harbour seal pups and have also quantified the accompanying changes that occur in V_T, $\dot{V}_E$, and $\dot{V}_{O2}$.

In terrestrial mammals, decreases in state of arousal from WAKE to SWS are accompanied by decreases in both breathing frequency and metabolic rate, and the transition to REM sleep is accompanied by irregular breathing and metabolic rates that are increased above SWS levels, but still decreased compared to WAKE (Lydic 1987; McGinty and Szymusiak 1994). Castellini et al. (1994a) determined that these state-dependent changes in breathing pattern were also present, although to a greater degree in northern elephant seal pups, with continuous and irregular breathing during wakefulness, giving way to episodic breathing during SWS, and a complete cessation of breathing during REM sleep. I found similar, but less profound changes in harbour seal pups. During WAKE, breathing was continuous and irregular with periodic apneas of short-duration. During SWS breathing was more episodic with longer apneas, and in REM sleep breathing was rare.

It has been suggested that the decrease in f_R seen during sleep, a common attribute of all vertebrates studied to date, most likely reflects the 10-20% decrease commonly seen in $\dot{V}_{O2}$ (Orem et al. 1978; Phillipson 1978; White et al. 1995, Milsom et al. 1997). My results are consistent with this hypothesis; f_R, $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ were all significantly lower in SWS than WAKE in the harbour seal pups (44.3%, 12.4%, and

22.0% respectively) and were accompanied by an increase in both the frequency and length of periods of apnea.

Given life history strategies, it is difficult to obtain reliable measurements of basal metabolic rate (BMR) (post-absorptive, mature, non-pregnant, and quiescent) in marine mammals (notably phocid seals) since at the times of the year during which most are accessible, they usually violate at least one of the criteria required for establishing basal metabolism (Huntley 1987; Rosen and Renouf 1995). Most studies on marine mammals tend to report resting metabolic rates (RMR) where animals are resting quietly while one or more of the variables defining BMR are not met. In the present study, the seals were both immature and had been fed prior to the experiments, violating two of the criteria required for recording BMR. Despite this, the values for WAKE $\dot{V}_{O_2}$ that I collected were similar to the RMR reported by Miller and Irving (1975), and Rosen and Renouf (1995) for young harbour seals.

Although there was a strong correlation between the fall in f_R and the fall in $\dot{V}_{O_2}$ during SWS in harbour seal pups, the fall in f_R was not accompanied by an equal fall in $\dot{V}_E$ as it is in terrestrial mammals (Lydic 1987). In terrestrial mammals, V_T can either increase or decrease during SWS compared to WAKE, however the decrease in f_R is dramatic enough to produce a significant decrease in $\dot{V}_E$. In harbour seal pups, V_T increased during SWS, offsetting the fall in f_R such that the fall in $\dot{V}_E$ between WAKE and SWS was not significant. Consequently, the change in $\dot{V}_{O_2}$ was not accompanied by an equal change in $\dot{V}_E$, leading to a small, but insignificant rise in the air convection requirement ($\dot{V}_E/\dot{V}_{O_2}$). In the harbour seal pups of this study, the air convection requirement in WAKE (136.7 ml air/ml O_2) was more than double that reported for small

terrestrial mammals (Frappell et al., 1992) (typically between 40 and 60 ml air/ml O_2). This would appear to be partly due to the relatively low $\dot{V}_{O2}$ of the harbour seals compared to other terrestrial mammals and the relatively high $\dot{V}_E$. There are few studies that examine the ACR of terrestrial mammals, and even fewer that compare the changes in ACR during different arousal states. Given the decreases that occur in both $\dot{V}_{O2}$ and $\dot{V}_E$, during sleep in terrestrial mammals (Phillipson et al. 1976; White et al. 1985), it would seem reasonable that the ACR remain relatively constant, as both Phillipson et al. (1976) and White et al. (1985) demonstrated in their studies on dogs and humans, respectively. This is similar to my results. The net effect of the respiratory changes in the harbour seal pups was that SWS was primarily accompanied by a slowing and deepening of breathing with little effect on $\dot{V}_E$.

The changes in respiration that accompany REM sleep differ between species of terrestrial mammal. In most cases, REM sleep leads to an irregular breathing pattern with increases in f_R and $\dot{V}_{O2}$ over levels seen in SWS (White 1985; Lydic 1987). Breathing in phocid seals during REM sleep, however, is rare. Castellini et al. (1994a) suggested that the absence of breathing during REM sleep in northern elephant seal pups might be due to an extension of the muscle atonia that occurs in REM sleep to the respiratory muscles. While this may be the case for northern elephant seals, my data for harbour seal pups, along with data for yearling gray seals, adult Caspian seals, and harp seal pups (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin, 1993) indicate that these species of phocid seal can breathe during REM sleep, even though most typically don't, with the majority of REM sleep episodes occurring within one respiratory pause. Further evidence that phocid seals can breathe during REM sleep comes from the report that

yearling gray seals breathe continuously during REM sleep (Ridgway et al. 1975). The reason for the diversity that is seen in breathing patterns during REM sleep between species is not clear.

2.4.2 Hypoxic Ventilatory Response

During wakefulness in harbour seal pups, hypoxia led to increases in f_R and $\dot{V}_E$, due primarily to significant decreases in the length and frequency of periods of apnea. In most (but not all) terrestrial mammals, under hypoxic conditions, increases in $\dot{V}_E$ are due primarily to increases in f_R with either small increases, no change, or decreases in V_T (Gothe et al. 1982; Garland et al. 1994; Webb and Milsom 1994; Bisgard and Neubauer 1995). In other marine mammals, however, increases in $\dot{V}_E$ during hypoxia have also been reported to stem from increases in breathing frequency alone (hooded seal, Påsche 1976a; Amazonian manatee (*Trichechus inunguis*), Gallivan 1980; newborn gray seals, Mortola and Lanthier 1989). In the case of the hooded seals, the increase in f_R during hypoxia was due primarily to decreases in the length and frequency of periods of apnea (Påsche 1976a), similar to the harbour seal pups of the present study.

Because of the variability in the relative contributions of changes in f_R and V_T to changes in total ventilation in response to hypoxia seen in different species, the sensitivity of animals to changes in respiratory drive is generally expressed as the slope of the ventilatory response curve ($\bullet \dot{V}_E / \bullet F_{IO_2}$). My data indicate that, like other marine mammals, harbour seal pups exhibit a strong hypoxic ventilatory response and are just as sensitive to hypoxia as terrestrial mammals.

In general, the ventilatory response to hypoxia is blunted during SWS in terrestrial mammals compared to the response seen during WAKE (Henderson-Smart 1995; Corfield et al. 1999). The threshold for medullary respiratory neuronal activation is increased during SWS, and it requires lower levels of blood P_{O_2} and higher levels of blood P_{CO_2} to stimulate increases in breathing (Daly 1997). Given their tolerance for prolonged apnea during diving, the presence of episodic breathing during SWS, and the large fluctuations in blood P_{O_2} and P_{CO_2} this should produce, one might expect an even greater decrease in hypoxic sensitivity during SWS in phocid seals. In harbour seal pups, however, this was not the case. Hypoxic chemosensitivity was not reduced in SWS. Milsom et al. (1996), using changes in f_R alone as an indication of respiratory chemosensitivity, found that there was an increase in hypoxic sensitivity in northern elephant seal pups. In the present study, I also found a greater increase in f_R during hypoxia in SWS compared to that seen while the animals were awake, however, when the changes in V_T and $\dot{V}_E$ during hypoxia in the two states were taken into account, there was no difference in chemosensitivity. Given this, indications of changes in chemosensitivity based on measure of respiratory frequency alone should be viewed cautiously.

As the amount of oxygen available in ambient air decreases under hypoxic conditions, animals must compensate by either increasing the supply of oxygen to the blood or by decreasing the demand for oxygen. The former is done by increasing $\dot{V}_E$, increasing the efficiency with which oxygen is extracted from each breath, or both (Chappell and Roverud 1990), while the latter is done by reducing metabolic rate (Frappell et al. 1992). In the harbour seal pups, I saw increases in f_R and $\dot{V}_E$, as well as

reductions in $\dot{V}_{O_2}$ at more extreme levels of hypoxia. I saw no change in the relative amount of oxygen extracted from each breath during hypoxia, consistent with reports for terrestrial mammals (Chappell and Roverud 1990). Due to the large increase in $\dot{V}_E$ and small reduction in $\dot{V}_{O_2}$, the air convection requirement ($\dot{V}_E/\dot{V}_{O_2}$) increased significantly during hypoxia in both WAKE and SWS, as has also been reported for other mammals (Frappell et al. 1992).

2.4.3 Hypercarbic Ventilatory Response

Many studies on phocid seals have now shown that these, and other marine mammals are as sensitive to hypercarbia as terrestrial mammals, although their stimulus threshold is elevated (Bainton et al. 1973; Påsche 1976b; Gallivan 1980; Parkos and Wahrenbrock 1987). The increased threshold for hypercarbia appears to be due to an increased buffering capacity of the blood for CO_2 (Gallivan 1980), which is felt to be an adaptation that allows these animals to tolerate the high levels of blood P_{CO_2} that occur due to the accumulation of the by-products of cellular metabolism during periods of apnea (either diving or voluntary). Retention of hypercarbic sensitivity is believed to be an adaptation that produces shorter surface intervals during diving (Gallivan 1980; Daly 1984). From the present study, it is apparent that harbour seal pups also express sensitivity to hypercarbia similar to that of other phocid seals and terrestrial mammals, but they did not exhibit the increased response threshold to hypercarbia that is seen in other phocid seals. Significant increases in f_R, V_T, and $\dot{V}_E$ were evident at the lowest level of hypercarbia tested.

In harbour seal pups, hypercarbia led to increases in $\dot{V}_E$, due to increases in both f_R and V_T, at all levels tested. At the higher levels of CO_2 tested, however, V_T and $\dot{V}_E$ did not increase as much as they did on lower levels of CO_2, although f_R increased more. The reasons for this are not clear. Påsche (1976b) found similar trends in female hooded seals breathing 6% CO_2. These data may suggest that these animals had quickly reached the limit to which they could increase V_T, and that for f_R to increase any further as the levels of CO_2 continued to climb, the time available for inspiration had to be reduced leading to the reduction in V_T.

Because of the large increase in total ventilation, the percentage of time spent breathing, rather than apneic, increased significantly. Despite this, there was no increase in $\dot{V}_{O2}$ in the harbour seal pups in WAKE. This is in contrast to reports for northern elephant seal pups where there was a significant increase in $\dot{V}_{O2}$ during wakefulness at inspired CO_2 levels of 7% (Kohin et al. 1999). Because of the large increase in ventilation and constant metabolic rate, the air convection requirement increased dramatically while the amount of oxygen extracted from each breath fell. Similar results have been reported for terrestrial mammals (Barros et al. 2001).

Along with the decrease in $\dot{V}_E$ that accompanies SWS in terrestrial mammals, there is typically a decrease in sensitivity to hypercarbia, accompanied by slightly elevated blood P_{CO_2} levels (Orem et al. 1977; White et al. 1985; Daly 1997). Just as with the hypoxic sensitivity, while one might expect the hypercarbic sensitivity of the harbour seals during SWS to be reduced as much as, or more than, is typically seen in terrestrial mammals, there was no difference in sensitivity between the two states. Although f_R was significantly lower in SWS compared to WAKE at air% and 2% CO_2, there were no

differences in V_T, and hence, no changes in $\dot{V}_E$ as arousal state decreased. The stable $\dot{V}_{O2}$ that I recorded in both WAKE and SWS during hypercarbia in harbour seal pups is similar to what has been reported for terrestrial mammals (Mortola and Gautier 1995).

2.4.4 Heart Rate

Respiratory-induced cardiac sinus arrhythmias are common in mammals, leading to an increase in heart rate during inspiration and a decrease in heart rate during expiration or during periods of apnea. This tends to match ventilation and perfusion and minimizes the work of the heart. In the present study, harbour seal pups showed a decrease in heart rate during apnea by as much as 31% in wakefulness and 68% in SWS under normoxic and normocarbic conditions.

When seals breath-hold, they typically due so on expiration (Elsner et al. 1977). The act of lung deflation decreases pulmonary stretch receptor activity, increasing cardiac vagal tone, and thus allows a bradycardia to develop (Daly et al. 1977). Once an apnea has begun, blood P_{O_2} levels begin to decrease, stimulating the peripheral carotid body chemoreceptors. This should cause hyperventilation, but it is overridden by trigeminal and superior laryngeal nerve stimulation, by water in submerged animals, and by central mechanisms during apnea on land. In seals (see below), it also contributes to the bradycardia, and vasoconstriction (Elsner et al. 1977; Daly et al. 1977; Daly 1984). When breathing resumes, the low blood P_{O_2} and high blood P_{CO_2} continue to stimulate the carotid bodies transiently, which would act to maintain the bradycardia. However, the decrease in vagal tone brought about by increases in excitatory input from the pulmonary stretch receptors causes f_H to increase, overriding the carotid body induced bradycardia

and vasoconstriction (Elsner et al. 1977; Daly 1984). For a representative trace illustrating the degree of the hypoxic bradycardia in harbour seal pups, refer to Figure 2.12.

In terrestrial mammals, hypoxia causes hyperventilation and tachycardia. Hypoxia in harbour seal pups however, caused hyperventilation and a significant bradycardia (up to a 50% reduction in heart rate) during all states of arousal, while at the same time causing a significant increase in f_R and $\dot{V}_E$ (by up to 50%). Elsner et al. (1977) have previously shown that stimulation of carotid body chemoreceptors in anesthetized harbour seals by hypoxia leads to decreases in f_H and increases in f_R. This was also the case in the harbour seal pups in the present study. It is interesting to note that while lung inflation appears to be able to suppress the cardio-inhibitory response arising from the carotid body stimulation during the resumption of breathing following diving and breath holding in seals (Elsner et al. 1977), it does not do so under conditions of chronic hypoxia.

Previous studies performed on northern elephant seal pups (Milsom et al. 1996; Kohin et al. 1999) did not find a bradycardia present under hypoxic conditions (13% O_2, and 14 % and 11% O_2 respectively in the two studies). In the study by Milsom et al. (1996) hypoxia had no effect on f_H or f_R during any state of arousal, while in the study by Kohin et al. (1999) there was a trend for f_H to increase as hypoxia ensued, although this appeared to be primarily due to an increase in the amount of time spent breathing. Perhaps the levels of hypoxia used in the studies on northern elephant seal pups (Milsom et al. 1996; Kohin et al. 1999) were at the threshold of hypoxic sensitivity in these animals, and were not great enough to induce the hypoxic bradycardia seen in this study.

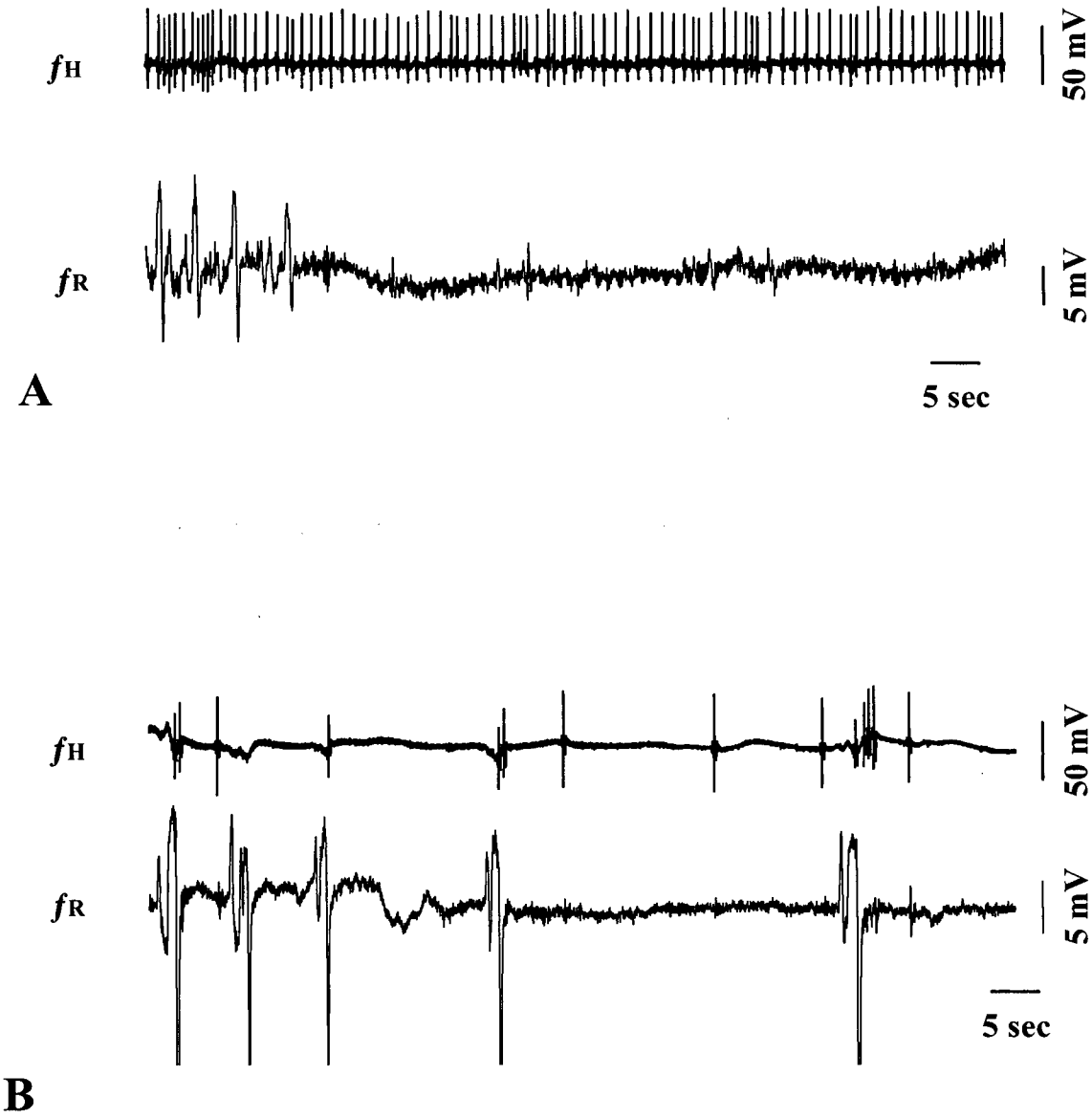


Figure 2.12. Hypoxic bradycardia. In harbour seal pups, during WAKE, SWS (shown here) and REM sleep, there was a dramatic hypoxic bradycardia with heart rate (f_H) decreasing up to 50% on 8% O_2 (B) when compared to air (A), while breathing frequency (f_R) increased.

Currently there are no other studies in which the effects of hypoxia on f_H are examined in phocid seals.

Hypercarbia in most terrestrial mammals stimulates central chemoreceptors, causing increases in both f_R and f_H. Milsom et al. (1996) found that the effects of hypercarbia on f_H were state dependent in northern elephant seals with increases in f_H only being observed during wakefulness. In the harbour seal pups in the present study, there were no increases in f_H during wakefulness, however in SWS f_H was slightly elevated over levels obtained in air. Thus while these data support the idea that changes in f_H during hypercarbia are state dependent in phocid seals, they also suggest that the pattern of change is species dependent, with some species increasing f_H during wakefulness only, while others increase f_H during SWS. More likely, however, the results from both studies reflect low sample size.

2.5 Conclusions

My data indicate that the breathing pattern of harbour seal pups is state dependent with animals exhibiting irregular breathing during wakefulness, episodic breathing during SWS, and sporadic, but rare incidents of breathing during REM sleep. These data are in agreement with the data presented for other phocid seals (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1994; and Milsom et al. 1996).

Harbour seals responded vigorously to large increases in respiratory drive, both in WAKE and SWS, and were just as sensitive to these changes in drive as terrestrial mammals. The hypercarbic ventilatory response in harbour seals was similar to that seen in terrestrial mammals, as was the hypoxic ventilatory response. Unlike the reductions

seen in hypoxic and hypercarbic sensitivity in many terrestrial mammals during sleep, the sensitivity to hypoxia and hypercarbia was unchanged during the transition from WAKE to sleep in the harbour seal pups. The changes in heart rate seen in harbour seal pups during both hypercarbia and hypoxia also did not follow the pattern seen in terrestrial mammals. In hypercarbia, heart rate remained relatively constant, while in hypoxia heart rate decreased significantly. Both changes occurred concurrent with slight increases in total ventilation. The retention of normal ventilatory sensitivity during sleep, despite the increased occurrence of periods of prolonged apnea, is reminiscent of the relatively normal ventilatory sensitivity seen in these animals (relative to their terrestrial counterparts) during WAKE despite their ability to dive for prolonged periods. Both may reflect an adaptation for rapidly and efficiently renewing lung gas stores following prolonged periods of breath holding, during which time this sensitivity must be suppressed.

Chapter 3

General Discussion and Conclusions

3.1 Critique of Methodology

Although the data appear to be overwhelmingly convincing that arousal state and respiratory drive significantly affect the cardio-respiratory patterns of harbour seal pups in a manner similar to terrestrial mammals, the low N-values of this study should be acknowledged. Due to logistical constraints, only three male harbour seal pups were used as study subjects. The variability of the data collected from the three animals however, was minimal, suggesting that my data are true reflections of breathing patterns and cardio-respiratory responses to increased respiratory drive in harbour seals.

Each animal underwent four experimental sessions per gas level, with each session lasting 2 to 4 hours in length, with 6 to 10 WAKE-sleep episodes being analyzed per seal, per gas level.

Both the sex and age of the seals used in this study were kept constant (i.e. male harbour seal pups), thus eliminating the possibility of sex and age influencing results. Male harbour seals were used as opposed to females due to their relatively docile and friendly demeanor (personal observation). The pattern of response to changes in arousal state and increased respiratory drive in female mammals is similar to that seen in males, although the level of response tends to be somewhat lower (White et al. 1985; Mortola and Gautier 1995). Our data on male harbour seal pups, therefore, is likely representative of the responses of both male and female animals.

It is well known that age influences both the hypoxic and hypercarbic ventilatory responses of mammals (Mortola and Lanthier 1996; Mortola 1999). Castellini et al. (1994b) and Blackwell and Le Boeuf (1993) suggested that in phocid seals there are also age related responses of the breathing pattern to changes in arousal state, due in part to

the development of the dive response. It was essential then, that all seals were at similar developmental stages and of similar age. In my study, harbour seal pups (6 to 12 months) were used as study subjects. The data from these animals can be compared with older phocid seals, regardless of age, as the dive response in precocial harbour seal pups should be fully developed by this stage.

3.2 State of Arousal

The first objective of my thesis was to describe the breathing and cardio-respiratory patterns of a phocid seal, the harbour seal, during different states of arousal while on land. As mentioned in Chapter 1, as arousal state decreases from WAKE to SWS to REM sleep in terrestrial mammals, many changes occur in both breathing and cardio-respiratory patterns. These include decreases in breathing frequency, tidal volume, metabolic rate, total ventilation, and heart rate (for a review see McGinty and Szymusiak 1994). In phocid seals, the effect of arousal state on breathing pattern has been studied in four species (gray seals (Ridgway et al. 1975), Caspian seals (Mukhametov et al. 1984), harp seals (Lyamin 1993), and northern elephant seals (Castellini et al. 1994a)), while the effect of arousal state on heart rate and cardio-respiratory patterns (total ventilation, metabolic rate, etc.) has never been examined.

Comparing data from my study on harbour seal pups with that from other studies, it is apparent that arousal state does affect the cardio-respiratory system in the same manner in phocid seals as it does in terrestrial mammals. As arousal state decreases from WAKE to SWS, breathing pattern changes from continuous and irregular, to episodic, possibly as a result of decreases in $\dot{V}_{O_2}$. Although the sleep state pattern of the gray seal

is different from that of other phocid seals (i.e. WAKE - REM sleep - SWS), the breathing patterns during both WAKE and SWS are similar to those reported for other seals (Ridgway et al. 1975). During SWS, there are decreases in overall f_R (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; Castellini et al. 1994a; current study) and $\dot{V}_{O_2}$, as well as increases in V_T, with no apparent change in $\dot{V}_E$ (current study), when compared to WAKE state values.

During REM sleep, where muscle atonia is present, it is plausible that life history strategies and/or the environment of the phocid seal in question are key in determining the pattern of breathing observed during this arousal state. Northern elephant seals (subgroup Monachinae) spend eight to nine months of the year at sea, with 80-95% of that time spent submerged (Andrews et al. 1997). Because of its importance physiologically, REM sleep must occur during this time at sea. The muscle atonia that is prominent during REM sleep prevents submerged individuals from surfacing without waking. The absence of breathing during REM sleep in northern elephant seals (Castellini et al. 1994a; Castellini et al. 1994b; Milsom et al. 1996) therefore, may be an adaptation of the animal to its environment, allowing complete sleep cycles to occur while the animal is out at sea.

The gray seal, harp seal, Caspian seal, and harbour seal are phylogenetically closely related members of the sub-group Phocinae, and all have similar life history strategies in that they haul out on a regular, almost daily basis, either on the mainland, islands, or ice flows, and therefore have almost constant access to land (Riedman 1990). Because of their similar life history strategies, one might expect similarities in their adaptations to sleep in the water. It is possible, therefore, that the breathing pattern of all

species is similar, during all states of arousal. This however, is not the case. Although all species exhibit an episodic breathing pattern during SWS, the pattern of breathing in REM sleep is variable. During REM sleep, the harp, Caspian, and harbour seals maintain a breathing pattern that is sporadic and rare, while the gray seal breathes continuously (Ridgway et al. 1975; Mukhametov et al. 1984; Lyamin 1993; current study). The reasons for the difference in breathing pattern during REM sleep of seals with similar life history strategies are unknown and require further study and speculation.

In terrestrial mammals, as state of arousal decreases, there are decreases in both $\dot{V}_{O_2}$ and $\dot{V}_E$, with variable changes in V_T. Although my study is the first to examine the effect of physiologically determined arousal state on V_T, $\dot{V}_{O_2}$, and $\dot{V}_E$ in phocid seals, the effects of behaviourally defined arousal states on $\dot{V}_{O_2}$ have been previously measured in freely diving Weddell seals (Castellini et al. 1992), captive harp and gray seals (Worthy 1987), as well as captive harbour seals (Matsuura and Whittow 1973; Miller et al. 1976) and yield similar conclusions. These data, along with my data on harbour seal pups indicate that, similar to terrestrial mammals, $\dot{V}_{O_2}$ does decrease with arousal state.

Changes in V_T during different arousal states in terrestrial mammals are highly variable, with some species, such as humans and dogs, showing an increase in V_T (Phillipson et al. 1976; White et al. 1985), while other species exhibit no change in V_T, such as golden-mantled ground squirrels (*Spermophilus lateralis*) (Hunter and Milsom 1998). As mentioned in Chapter 1, and confirmed by my study, while on land, in an awake state, phocid seals have a large V_T (40-90% of TLC), especially when compared to terrestrial mammals (Kooyman 1973). In harbour seal pups, V_T increases as the seal

enters SWS by as much as 61% (current study) suggesting that although the V_T may be larger than in terrestrial mammals, it is probably closer to 40% than 90% of the TLC.

Values of $\dot{V}_E$ in phocid seals have not been reported during behaviourally or physiologically defined periods of sleep. In the harbour seal pups of my study, it appears that $\dot{V}_E$ remains unchanged as arousal state decreases from WAKE to SWS, a pattern different from terrestrial mammals where $\dot{V}_E$ typically decreases during SWS. The similarity in $\dot{V}_E$ values for WAKE and SWS in the harbour seal are due, in part, to the significant increase in V_T during SWS, offsetting the decrease in f_R that is present as arousal state decreases. Because of the state-dependent differences in breathing pattern among other species of phocid seal, it is difficult to suggest what the changes in their $\dot{V}_E$ during decreased arousal states might be. However, due to the similarities in the changes in f_R in response to SWS, in all species of phocid seals studied to date, it is probable that as arousal state decreases, most seals maintain a constant $\dot{V}_E$.

Due to the decrease in $\dot{V}_{O_2}$ and the constant $\dot{V}_E$ as arousal state decreases in harbour seal pups, one might expect the $\%E_{O_2}$, or the relative efficiency of oxygen extracted per breath, to decrease as well. In most terrestrial mammals, however, there is a relatively constant $\%E_{O_2}$ as $\dot{V}_{O_2}$ values change (Chappell and Roverud 1990). In the current study, there was also no change in $\%E_{O_2}$ as arousal state decreased from WAKE to SWS, suggesting that the relative efficiency of oxygen extraction was maintained throughout the different states of arousal, regardless of the levels of $\dot{V}_{O_2}$ or $\dot{V}_E$.

Most of the data collected and information discussed in my thesis refer to changes in arousal state from WAKE to SWS. In phocid seals, REM sleep episodes can be long, however most are relatively short (>20seconds) and therefore, due to equipment

constraints, it is difficult to obtain accurate, trustworthy measurements of most variables during this short window of time.

3.3 Increased Respiratory Drive

The second objective of my thesis was to determine the effects of increased respiratory drive in phocid seals during both WAKE and SWS. Because of their prolonged breath-holding abilities, during which blood P_{O_2} levels decrease and P_{CO_2} levels increase, it was initially thought that WAKE phocid seals were less sensitive to hypoxia and hypercarbia than terrestrial mammals (Irving et al. 1935; Robin et al. 1963). Later studies revealed, however, that most species of seal are in fact, just as sensitive to increases in respiratory drive as terrestrial mammals, however the threshold level at which they respond to changes in blood gases is increased (Bainton et al. 1973; Påsche 1976a; Påsche 1976b; Milsom et al. 1996; Kohin et al. 1999). Almost no data exists, however, describing the interplay of arousal state and level of respiratory drive on the various cardio-respiratory and breathing patterns (i.e. full stimulus response curve showing changes in $\dot{V}_E$, $\dot{V}_{O_2}$, f_R, V_T, and f_H).

3.3.1 Hypoxic Ventilatory Response

As mentioned in Chapter 1, freely diving juvenile Weddell seals have a decreased hypoxia sensitivity compared to terrestrial mammals, illustrated by little or no change in $\dot{V}_E$ during exposure to hypoxia. Parkos and Wahrenbrock (1987) hypothesized that the decreased hypoxic sensitivity of juvenile Weddell seals could be due to the exposure of

the pups to hypoxia during diving early in life, and therefore could be an adaptation for prolonged dives. Data from more precocial species of seal that begin diving earlier in life, disagree with this hypothesis. My data on hypoxia sensitivity in harbour seal pups agree with the data of Påsche (1976a) on diving hooded seals, of Craig and Påsche (1980) in diving juvenile harbour seals, and of Mortola and Lanthier (1989) in newborn gray seals, all precocial species. In these studies, hypoxia elicited increases in either f_R, V_T, or both, resulting in increases in $\dot{V}_E$.

In harbour seal pups (current study), newborn gray seals (Mortola and Lanthier 1989), and yearling hooded seals (Påsche 1976a), hypoxia produced increases in f_R and $\dot{V}_E$, with no change in V_T. The increases in f_R of all three studies appear to be primarily caused by increases in time spent breathing and decreases in time spent apneic. Craig and Påsche (1980) also noted that the primary cause of increases in $\dot{V}_E$ in hypoxia was an increase in time spent breathing and a decrease in time spent apneic. Opposite to my study on harbour seal pups however, Craig and Påsche (1980) noted that in freely diving, captive, juvenile harbour seals, f_R did not increase during hypoxia, but that V_T and $\dot{V}_E$ did. Just prior to diving, Craig and Påsche (1980) observed an increase in V_T, possibly a final effort to remove CO_2 and to "top-up" oxygen stores. It should be noted that there were also small increases in V_T before and after prolonged voluntary apneas in my study, although this did not appear to affect overall changes in V_T.

In studies of hypoxia sensitivity of northern elephant seals by Milsom et al. (1996), values of $\dot{V}_E$ were not reported, and changes in f_R alone were used as a measure of respiratory sensitivity. In terrestrial mammals, as mentioned in Chapter 2, and phocid seals, as already noted, the relative contributions of f_R and V_T to changes in $\dot{V}_E$ are

species and arousal state specific. To accurately compare the hypoxic sensitivity of different species, values of $\dot{V}_E$ should be used instead of values of its individual components. Because my data on harbour seal pups indicate a significant rise in f_R as well as a significant rise in $\dot{V}_E$ as hypoxia levels increase, it is possible that the results from the northern elephant seals, where f_R also increased with hypoxia levels, are an accurate reflection of changes in $\dot{V}_E$ in phocid seals, however further measurements should be made.

Under conditions of hypoxia, mammals can increase the supply of oxygen to the blood (increase $\dot{V}_E$ / hyperventilation), decrease the demand for oxygen (decrease $\dot{V}_{O_2}$ / hypometabolism), or both (Mortola et al. 1989; Frappell et al. 1992; Kohin et al. 1999). The metabolic response to increasing levels of hypoxia (hypometabolism) is affected by numerous factors including size, age, and relative amount of exposure to hypoxia (Frappell et al. 1992; Kohin et al 1999; Mortola 1999). In normoxia, metabolic rate in both terrestrial and marine mammals is related to body size through the exponent 0.75 ($\dot{V}_{O_2}=aM^{0.75}$ where M is body mass) (Weibel 1984; Lavigne et al. 1986b; Huntley 1987). This suggests that as body mass and size decrease, there is an increase in $\dot{V}_{O_2}$ and oxygen consumption rates such that smaller mammals have a higher mass specific $\dot{V}_{O_2}$ than larger mammals. Under conditions of hypoxia, due to their higher mass specific $\dot{V}_{O_2}$ and oxygen consumption rates, smaller mammals typically show a greater degree of hypometabolism than larger mammals (Frappell et al. 1992). During hypoxic exposure in very large mammals (above 50kg), metabolic down-regulation (hypometabolism) is absent, and is suggested to be an event limited to small mammals (Frappell et al. 1992; Kohin et al. 1999).

Newborn mammals also typically show a greater metabolic response to hypoxia than older animals, usually with a greater decline in $\dot{V}_{O_2}$ and a decreased ventilatory response, possibly allowing for a greater tolerance to asphyxia during the transition from placental respiration to lung respiration (Kohin et al. 1999; Mortola 1999). Whether or not the hypometabolic response to hypoxia in newborn animals is related to size or age is still unknown, however it is most likely a combination of the two factors (Frappell et al. 1992; Mortola 1999).

Harbour seal pups show small decreases in $\dot{V}_{O_2}$ (9.9%) during hypoxia when compared to normoxia, however these are never significant (current study), while newborn gray seals show significant decreases in $\dot{V}_{O_2}$ (23%) at F_{IO_2} levels of 0.10 (Mortola et al. 1989). The difference in magnitude of the metabolic response to hypoxia between the gray and harbour seals may be due to the age at which the animals were tested, or the mass of the animals, as the harbour seal pups of the current study were twice the mass of the newborn gray seals (Mortola and Lanthier 1989). In phocid seals, this difference in magnitude of hypoxic response could also represent the inexperiences in diving between a newborn seal and a more experienced pup (i.e. newborn animals are not exposed to hypoxic conditions on a frequent basis while 6-12 month old pups frequently dive for prolonged periods) (Mortola and Lanthier 1989; Mortola et al. 1989).

When I compare my results to those obtained in WAKE northern elephant seal pups exposed to 14% and 11% O_2, the $\dot{V}_{O_2}$ response is quite different. As F_{IO_2} levels decreased from normoxic levels to 0.11, $\dot{V}_{O_2}$ increased by 38% in northern elephant seal pups (Kohin et al. 1999). The differences in response to hypoxia in phocid seals (increased $\dot{V}_{O_2}$ in northern elephant seals, and decreased $\dot{V}_{O_2}$ in harbour seals and gray

seals) may be a result of age, size, or both, as the northern elephant seal is, on average, four times the weight of the harbour and gray seals. Regardless, these data confirm that phocid seals do respond to hypoxia in a manner similar to terrestrial mammals, with different magnitudes of response dependent upon the size and age of the animals tested.

During sleep in most terrestrial mammals, there is a decrease in both the sensitivity and threshold for the ventilatory response to hypoxia (Corfield 1999). In some terrestrial mammals however, such as ground squirrels and rats, there is an increase in hypoxic sensitivity during SWS (Milsom et al. 1996). The sensitivity to increased hypoxia during different arousal states in marine mammals has previously only been examined in the northern elephant seal (Milsom et al. 1996). Again, using changes in f_R as a determinant of respiratory sensitivity, Milsom et al. (1996) concluded that as arousal state decreased from WAKE to SWS, there was a corresponding increase in sensitivity to hypoxia (13.3% $O_2 \pm 0.15$ SEM), illustrated by the greater magnitude of increase in f_R during SWS as hypoxia ensued. In my study on harbour seal pups, similar to the northern elephant seal pups, as arousal state decreased, there was a greater magnitude of increase in f_R during SWS than WAKE. However when $\dot{V}_E$ values were compared, there was no difference between the two states of arousal, suggesting no change in hypoxic sensitivity as arousal state decreased. Because there are only two species of phocid seal studied with respect to hypoxic sensitivity in the different arousal states, it is impossible to make generalizations about the hypoxic sensitivity of phocid seals, and therefore more studies are needed.

3.3.2 Hypercarbic Ventilatory Response

The primary stimulus to breathe in most mammals is the level of CO_2 present in the blood (P_{CO_2}). As mentioned in Chapter 1, it is not the CO_2 per se that causes the increases in f_R and V_T, rather it is the increase in $[H^+]$, due to the bicarbonate reaction, that the central chemoreceptors are responding to (Phillipson 1978; Milsom 1992). By increasing f_R and V_T in response to increased CO_2, the body removes the excess CO_2 at a faster rate, and the bicarbonate equation shifts to the left, removing [H+] and preventing acidosis of the blood.

In phocid seals, the response to hypercarbia is similar to that seen in terrestrial mammals, with CO_2 once again considered as the primary stimulus for breathing (Bainton et al. 1973; Påsche 1976b; Gallivan 1980; Milsom et al. 1996; Kohin et al. 1999). While my study on harbour seal pups demonstrated a similar sensitivity to hypercarbia, the threshold level of change in CO_2 required to produce a change in $\dot{V}_E$ was different from that observed in other marine mammals. Påsche (1976b), Gallivan (1980), and Parkos and Wahrenbrock (1987) found that changes in $\dot{V}_E$ of unrestrained, freely diving phocid seals and manatees occurred at a higher threshold level than was observed in terrestrial mammals. In the harbour seal pups of my study, however, the threshold level of $\dot{V}_E$ was similar to that of terrestrial mammals, with strong hypercarbic ventilatory responses occurring at the lowest level of CO_2 tested.

Similar results were found during SWS. No other study to date has quantified the changes in $\dot{V}_E$ of phocid seals during different arousal states in response to changes in respiratory drive. Milsom et al. (1996) showed that there was no change in the magnitude of the change in f_R in response to hypercarbia in northern elephant seal pups

during SWS, when compared to WAKE state values. In my study, there were no differences in the magnitude of the changes in V_T and f_R, or $\dot{V}_E$ during SWS. This suggests that, similar to the northern elephant seal pups, there was no decrease in sensitivity to hypercarbia as arousal state decreased in harbour seal pups.

3.4 Heart Rate

At times when lung function is not needed and there is no new intake of O_2, such as seen in dives and respiratory apneas, the work done by the heart (i.e. pumping blood) is minimized through a decrease in heart rate, thereby keeping ventilation and perfusion matched. In this study, harbour seal pups, under normoxic and normocarbic conditions (i.e. air), showed a decrease in the heart rate during apnea by as much as 31% of the eupneic values in wakefulness and 68% of the eupneic values in SWS. When phocid seals breath-hold, either on land or in water, their nostrils close, stimulating the trigeminal and superior laryngeal nerves, as well as receptors in the upper respiratory tract (nasal mucosa and larynx). This causes a decrease in heart rate and re-enforces the inhibition of breathing (Daly 1984).

When seals breath-hold, they do so either on expiration (during a dive apnea) or on inspiration (during a resting apnea) (Kooyman 1973; Elsner et al. 1977). The act of lung deflation (expiration) decreases pulmonary stretch receptor activity, increasing cardiac vagal tone, and allowing a bradycardia to develop (Daly et al. 1977). Once an apnea has begun, blood P_{O_2} levels begin to decrease, stimulating the carotid body peripheral chemoreceptors, causing hyperventilation (overridden by trigeminal and superior laryngeal nerve stimulation to sustain apnea), bradycardia, and vasoconstriction

(Elsner et al. 1977; Daly et al. 1977; Daly 1984). When breathing resumes, the low blood P_{O_2} and high blood P_{CO_2} are initially still present. These stimuli should continue to stimulate the carotid bodies and maintain the bradycardia, however the decrease in vagal tone brought about by increases in excitatory input from the pulmonary stretch receptors causes f_H to increase, overriding the carotid body induced bradycardia and vasoconstriction (Daly 1984).

In terrestrial mammals, hypoxia causes hyperventilation and tachycardia. In northern elephant seal pups, hypoxia either has no effect on f_H or f_R (Milsom et al. 1996) or causes increases in f_H, although this appears to be primarily due to an increase in eupneic time (Kohin et al. 1999). Hypoxia in harbour seal pups however, caused a significant bradycardia (up to 50%) during all states of arousal, while at the same time, f_R and $\dot{V}_E$ increased (up to 50%). Elsner et al. (1977) revealed that stimulation of carotid body chemoreceptors in anesthetized harbour seals by stimuli such as hypoxia led to decreases in f_H, and increases in f_R. In most terrestrial mammals and post-dive marine mammals, when f_R and $\dot{V}_E$ increase, lung inflation suppresses the carotid body's cardio-inhibitory response through removal of vagal input, leading to an increase in f_H regardless of the carotid body input (Elsner et al. 1977). In harbour seal pups, the removal of vagal input, caused by the substantial increase in f_R and $\dot{V}_E$ in hypoxia, does not appear to be enough to override the carotid body induced bradycardia, leading to the pronounced hypoxic bradycardia.

Although the efficiency of oxygen removal remained unchanged during hypoxia, (~5% at each level) the total amount of oxygen being removed from the air at 8.63% $O_2 \pm$ 0.22 SEM was less than half the total amount of oxygen being removed when

atmospheric air was breathed. Perhaps the vasoconstriction brought about by the hypoxic environment, allowing vital organs to maintain normal $\dot{V}_{O2}$ levels, is so profound in these seals that the f_H must be kept at a low level to maintain constant cardiac output levels. Or, perhaps, as a mechanism to allow constant blood flow to the brain and other vital organs, there is some other centrally controlled neural mechanism preventing removal of the vagal input as the pulmonary stretch receptors are stimulated.

Hypercarbia in most terrestrial mammals stimulates central chemoreceptors, causing increases in both f_R and f_H. Milsom et al. (1996) suggest that the effects of hypercarbia on f_H are state dependent in phocid seals, with increases in f_H observed only during wakefulness. In hypercarbic conditions, harbour seal pups show no increase in f_H during wakefulness or in SWS. REM sleep shows a significant increase in heart rate at 2.04% CO_2 ± 0.06 SEM, which disappears at higher levels of CO_2. These data support the idea of state dependent changes in f_H during hypercarbia in phocid seals. These data also suggest, however, that the pattern of change is species dependent, with some species increasing f_H during wakefulness only, while others increase f_H during SWS and/or during REM sleep. Low n-values in both studies, however, could also explain the differences.

3.5 Conclusions

Little is known about the respiratory physiology of phocid seals, and even less is known about the relationship between arousal state and respiratory drive. My data add support to the data of previous studies, and raise new questions regarding the cardio-respiratory sensitivity of harbour seals and other phocid seals to changes in both arousal

state and respiratory drive. It is apparent that the state-dependent changes in breathing pattern are universal to all species of phocid seal, however the exact pattern that is present is, as of yet, unpredictable. The differences in breathing pattern in REM sleep may be associated with life history strategies and/or behaviour, however more studies are needed to predict possible respiratory patterns. The maximum observed sleep associated apneas are similar in length to average dive times (~3 minutes in harbour seal pups) possibly suggesting oxygen stores and central regulation play some role in determining breathing pattern and apnea length.

Respiratory sensitivity in phocid seals is similar to that reported in terrestrial mammals, with increases in both f_R and V_T leading to increases in $\dot{V}_E$. Hypoxia is associated with a decrease in $\dot{V}_{O_2}$, while hypercarbia is associated with minimal changes in $\dot{V}_{O_2}$. Although phocid seals are primarily aquatic, there are strong similarities between their respiratory physiologies and those of terrestrial mammals. One of the most intriguing pieces of data to come out of my study is the inverse relationship between changes in heart rate and breathing frequency in hypoxic conditions. In terrestrial mammals, as breathing frequency increases, so too does heart rate, a way of maintaining a match between ventilation and perfusion. In the harbour seals of my study, the profound bradycardia observed during hypoxia at all levels of arousal, suggests a strong ventilation-perfusion mismatch. The reasons behind this are unknown at this time and further studies should be carried out, possibly on other species of phocid seal.

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