DAY-NIGHT DIFFERENCES IN VENTILATION, METABOLISM, AND BODY TEMPERATURE DURING NORMOXIA, HYPOXIA AND HYPERCAPNIA IN THE AWAKE ADULT RAT

BY

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A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Zoology, University of Toronto

This thesis is dedicated in memory of my grandfather, Howard H Black.

ABSTRACT

Day-Night Differences in Ventilation, Metabolism, and Body Temperature During Normoxia, Hypoxia, and Hypercapnia in the Awake Adult Rat.
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During normoxia, metabolism and body temperature were significantly ($P < 0.05$) higher at 10 pm than at 10 am, and ventilation and tidal volume remained unchanged while respiratory frequency was significantly higher at 10 pm than at 10 am.

At 10 am, metabolism decreased in response to hypoxia, but body temperature did not suggesting that the thermic and metabolic responses to hypoxia are independently controlled.

Both ventilation and mean inspiratory airflow are elevated at 10 am compared with 10 pm suggesting that the ventilatory response to hypoxia may follow a circadian rhythm.

The increase in ventilation in response to hypercapnia was significantly greater at 10 pm than at 10 am. Mean inspiratory airflow was also elevated at 10 pm compared with 10 am. These observations suggest that the hypercapnic ventilatory response may follow a circadian rhythm.

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GLOSSARY AND SYMBOLS

Minute Ventilation ($\dot{V}_I$): The volume of gas inspired per unit of time.

Gaseous Metabolism: The volume of oxygen consumed ($\dot{V}_{O2}$) and the volume of carbon dioxide produced ($\dot{V}_{CO2}$) per unit of time.

Rate of oxygen consumption ($\dot{V}_{O2}$): The volume of oxygen removed from the inspired gas per unit of time.

Rate of carbon dioxide production ($\dot{V}_{CO2}$): The volume of carbon dioxide expired per unit of time.

Respiratory Exchange Ratio (R.E.): The ratio of $\dot{V}_{O2}$ to $\dot{V}_{CO2}$. The value of this ratio is generally 0.85.

O_2: Oxygen

CO_2: Carbon Dioxide

P_{O2}: Partial Pressure of oxygen.

P_{CO2}: Partial Pressure of carbon dioxide.

Pa_{O2}: Aterial partial pressure of oxygen.

Pa_{CO2}: Arterial partial pressure of carbon dioxide.

Tb: Deep-core body temperature

Tidal Volume (V_T): The volume of gas entering the lungs in each breath.

Respiratory frequency (f_R): The number of breaths occurring per unit of time.

Total breath duration (Ttot): The time required to complete an inspiration and an expiration of a single breath.

Inspiratory Interval (Ti): The time required to complete the inspiratory phase of one breath.

Expiratory Interval (Te): The time required to complete the expiratory phase of one breath.

Mean inspiratory airflow rate (V_T/Ti): The volume of gas inspired per unit of time.

$\dot{V}_I/\dot{V}_{CO2}$: The ratio of minute ventilation to carbon dioxide production.

$[H^+]$: Hydrogen ion concentration

STPD: Standard temperature and pressure, dry (zero Celcius, at 760 mmHg atmospheric pressure, and free of water vapour).

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CHAPTER ONE

INTRODUCTION

There are many behavioural and physiological variables which oscillate rhythmically over twenty-four hours. These rhythmic oscillations are known as circadian rhythms. Behavioural variables such as activity levels and arousal state follow characteristic circadian oscillations, as do several physiological variables, such as body temperature, metabolic rate, heart rate, blood pressure, and hormonal secretion (Rusak and Zucker 1979).

It is well established that mammalian basal ventilation is influenced by both body temperature (Gautier and Bonora 1992; Maskrey 1990) and metabolic rate (Mortola and Gautier 1995; Saiki and Mortola 1995). Therefore, if both body temperature and metabolic rate follow a circadian cycle and ventilation is influenced by these two variables, it raises the question of whether ventilation itself follows a circadian cycle which corresponds with these two variables.

Ventilation can be stimulated by both hypoxia and hypercapnia. Since the ventilatory responses to hypoxia and hypercapnia vary with changes in body temperature and metabolic rate (Saiki and Mortola 1996; Mortola and Gautier 1995; Maskrey 1990), it is reasonable to predict that these ventilatory responses will follow the circadian oscillations in body

temperature and metabolic rate.

The two primary questions addressed in this thesis are: (1) Does basal ventilation closely follow the circadian changes in metabolism and body temperature? (2) Do the ventilatory responses to hypoxia and hypercapnia follow the circadian changes in metabolism and body temperature?

The introduction to this thesis is divided into three sections. The first and second sections are intended to provide a general overview of both the respiratory and circadian control systems. The third section is an explanation of the possible physiological links between the respiratory and circadian systems.

PART I:

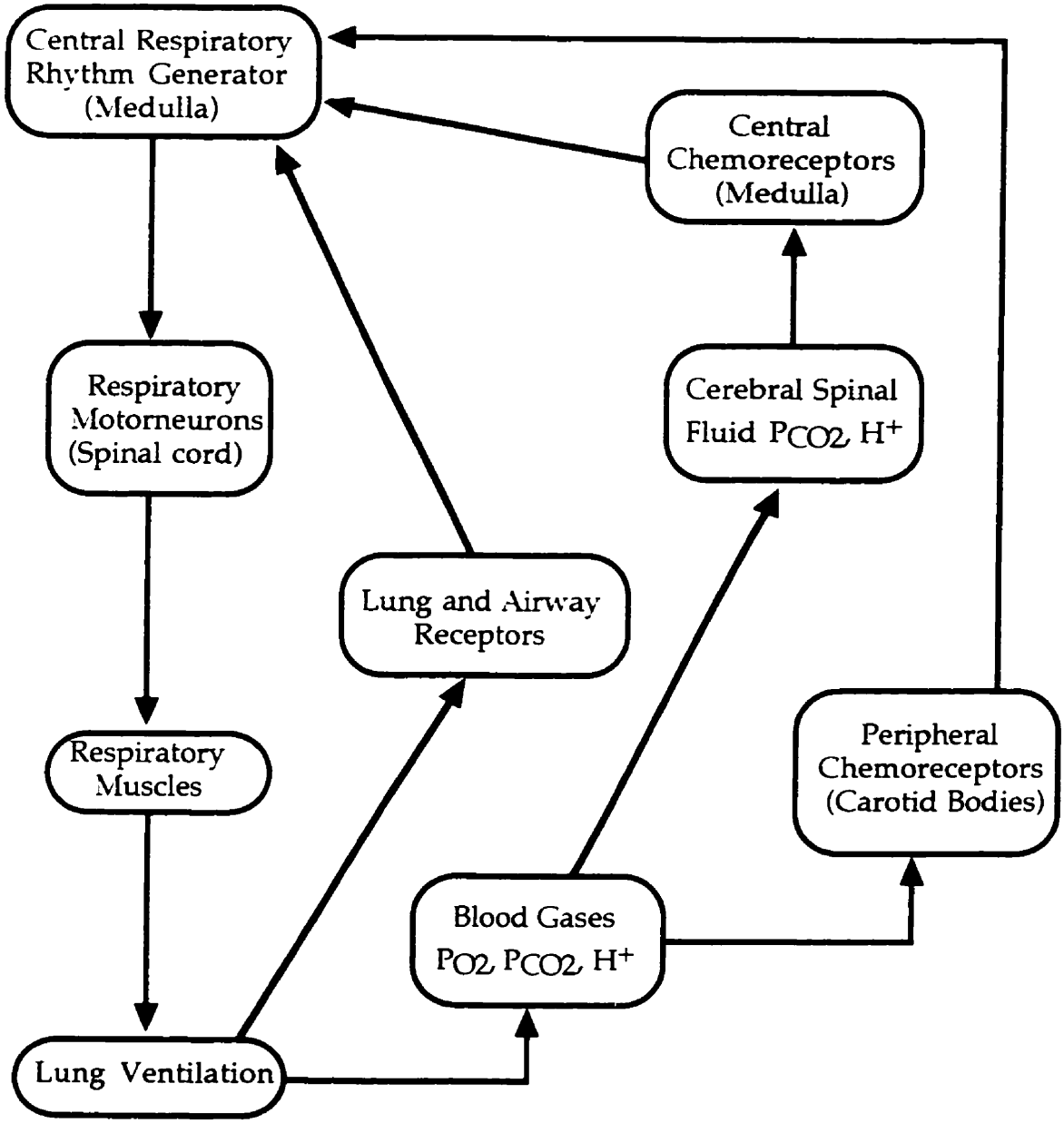
COMPONENTS OF THE RESPIRATORY CONTROL SYSTEM

The primary goal of lung ventilation is to meet the metabolic demands of the body. This is achieved by actively inspiring air into the lungs thus acquiring oxygen and then passively (or actively) expiring air out of the lungs thus eliminating carbon dioxide. Unlike the heart, which has its own intrinsic rhythm generator, the muscles driving respiration do not "beat" on their own, rather they are driven by a rhythm generator located in the medulla. Because an organism's environment is subject to

change (for example, hypoxic conditions, as at altitude) and the metabolic demands of the organism itself are subject to change (increased physical activity), there is clearly a need to modify the output to the respiratory muscles driving ventilation so that the metabolic needs of the organism are met. Therefore, the respiratory control system is designed such that it has receptors which sample parameters such as partial pressures of oxygen and carbon dioxide, hydrogen ion concentration, degree of lung inflation, upper airway tone, and muscle activity (Figure 1.1).

Only the receptors which govern the partial pressures of oxygen and carbon dioxide and hydrogen ion are pertinent to this thesis. The carotid body chemoreceptors monitor arterial oxygen partial pressure and hydrogen ion concentration. These peripheral chemoreceptors relay their information to the central rhythm generator, as do the central chemoreceptors which monitor brain tissue hydrogen ion concentration. Information from the peripheral and central chemoreceptors is fed back to the central respiratory rhythm generator to drive the respiratory muscles at a level that satisfies the metabolic needs of the organism. In order to understand the intricate dynamics of how the peripheral and central chemoreceptors respond to varied partial pressures of oxygen and carbon dioxide, it will be necessary to examine the physiology of the ventilatory responses to acute hypoxia and hypercapnia.

Figure 1.1. A schematic of the components of the respiratory control system.



EFFERENT **AFFERENT**

THE VENTILATORY AND METABOLIC RESPONSES TO ACUTE HYPOXIA

Physiological responses and adaptations to environmental hypoxia have been well studied because the hypoxic stimulus affects the respiratory system in a complex manner. The ventilatory response to hypoxia is dramatically modified depending on the strength and duration of the hypoxic stimulus. The initial ventilatory response is a rapid increase in minute ventilation (Mortola and Gautier 1995; Bisgard and Neubauer 1995; Howard and Robbins 1995). This initial response in humans and most mammals is not sustained and ventilation declines over the first 20-30 minutes of hypoxic exposure (Howard and Robbins 1995). More prolonged exposure (several hours to days) to hypoxia results in a secondary increase in ventilation and this latter response has been termed ventilatory acclimatization to hypoxia (Weil 1986; Howard and Robbins 1995; Mortola and Gautier 1995; Olson and Dempsey 1978). If life-long exposure to hypoxia occurs the hypoxic ventilatory response is "blunted" and the amount of hyperventilation found during acclimatization is reduced (Weil 1986).

These observations indicate that hypoxia has both stimulatory and depressant effects on the ventilatory system, and that there are time-dependent components of the control system governing ventilatory output. There are also indications that hypoxia has different effects on discrete central nervous system (CNS) structures, and these may change

during different states such as sleep and anaesthesia (Poncet *et al.* 1994; Nolan *et al.* 1995; Ashkenazi *et al.* 1982; Phillipson and Bowes 1986; Bisgard and Neubauer 1995; Tamaki and Nakayama 1987).

In order to fully understand the ventilatory and metabolic responses to hypoxia it will be necessary to briefly overview the components and mechanisms of the hypoxic sensors, the peripheral chemoreceptors.

PERIPHERAL CHEMORECEPTORS:

In mammals, the peripheral chemoreceptors, carotid and aortic bodies, are considered the only sources for reflex ventilatory stimulation via hypoxia. In rats and humans, the aortic bodies do mediate some of the ventilatory response to hypoxia, but mostly they respond to changes in O_2 content and play a relatively insignificant role in the overall ventilatory response to hypoxia (Sapru and Krieger 1977). Currently, it is thought that the chemoreceptors of the carotid body are the primary sensory receptors which are able to detect blood gas (P_{O2}) and [H^+] levels and transduce these stimuli into appropriate ventilatory responses.

The carotid bodies are paired organs located in the vicinity of the bifurcations of the common carotid arteries. Vascularly, they are supplied by one or more small arteries and drained by a small vein that originates from the dense vascular plexus on the surface of the structure. The carotid bodies are innervated by the carotid sinus nerve (CSN). This

branch of the IXth cranial nerve (glossopharyngeal) provides the sensory innervation to the carotid body, as well as components of the organ's sympathetic and parasympathetic innervation. The carotid bodies are also innervated by the ganglioglomerular nerve which comes from the superior cervical ganglion. The ganglioglomerular nerve provides sympathetic innervation to the carotid body vasculature (Gonzalez *et al.* 1995) (Figure 1.2).

VENTILATORY RESPONSES TO ACUTE HYPOXIA:

The carotid bodies respond to both arterial hypoxia (low oxygen partial pressure, Pa_{O2}) and hypercapnia (high carbon dioxide partial pressure, Pa_{CO2}). The carotid bodies are highly sensitive to changes in Pa_{O2}, but do not respond directly to high Pa_{CO2}. Rather, they respond to changes in arterial hydrogen concentrations (Duffin 1990; Cunningham *et al.* 1986) which are directly related to Pa_{CO2} in the following manner:

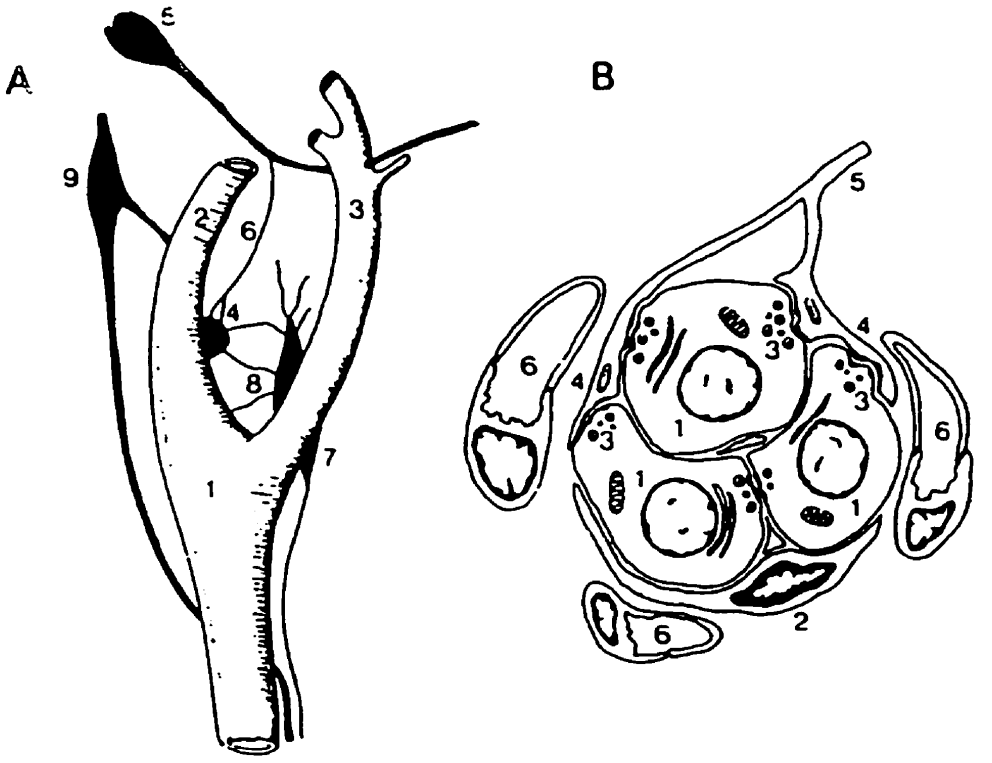
$$CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+.$$

This relation can be expressed as the linear form of the Henderson-Hasselbalch equation:

$$[H^+] = 24\ P_{CO2} / [HCO_3^-],$$

where $[H^+]$ is the hydrogen ion concentration in nanomoles per litre, P_{CO2} is the partial pressure of CO_2 in mmHg, and $[HCO_3^-]$ is the bicarbonate ion concentration in millimoles per litre (Duffin 1990). Changes in P_{CO2} result

Figure 1.2. Carotid artery bifurcation and cellular lobule in the carotid body. (A) Frontal view of the right carotid artery bifurcation in the rabbit. The common carotid artery (1) gives rise to the internal (2) and external (3) carotid arteries. The carotid body (4) is located on the internal carotid artery close to the bifurcation. Sensory fibres from the petrosal ganglion (5) reach the carotid body via the carotid sinus nerve (6). The superior cervical ganglion (7) also innervates the bifurcation area, including the carotid body, via the ganglioglomerular nerves (8). The nodose ganglion (9) is situated externally to the internal carotid artery. (B) Lobule of parenchymal cells of the carotid body, comprised of chemoreceptor cells (1) partly surrounded by sustentacular cells (2). The proportion of chemoreceptor to sustentacular cells is approximately 3-5 to one. Chemoreceptor cells have in their cytoplasm a heterogenous population of synaptic vesicles (3), some of which are located near the contacts with the sensory nerve endings (4) of the carotid sinus nerve (5). The lobules are surrounded by a dense network of capillaries (6). (Reproduced, with permission, from Gonzalez *et al.* (1995). Regulation of Breathing. pp. 391-471.)

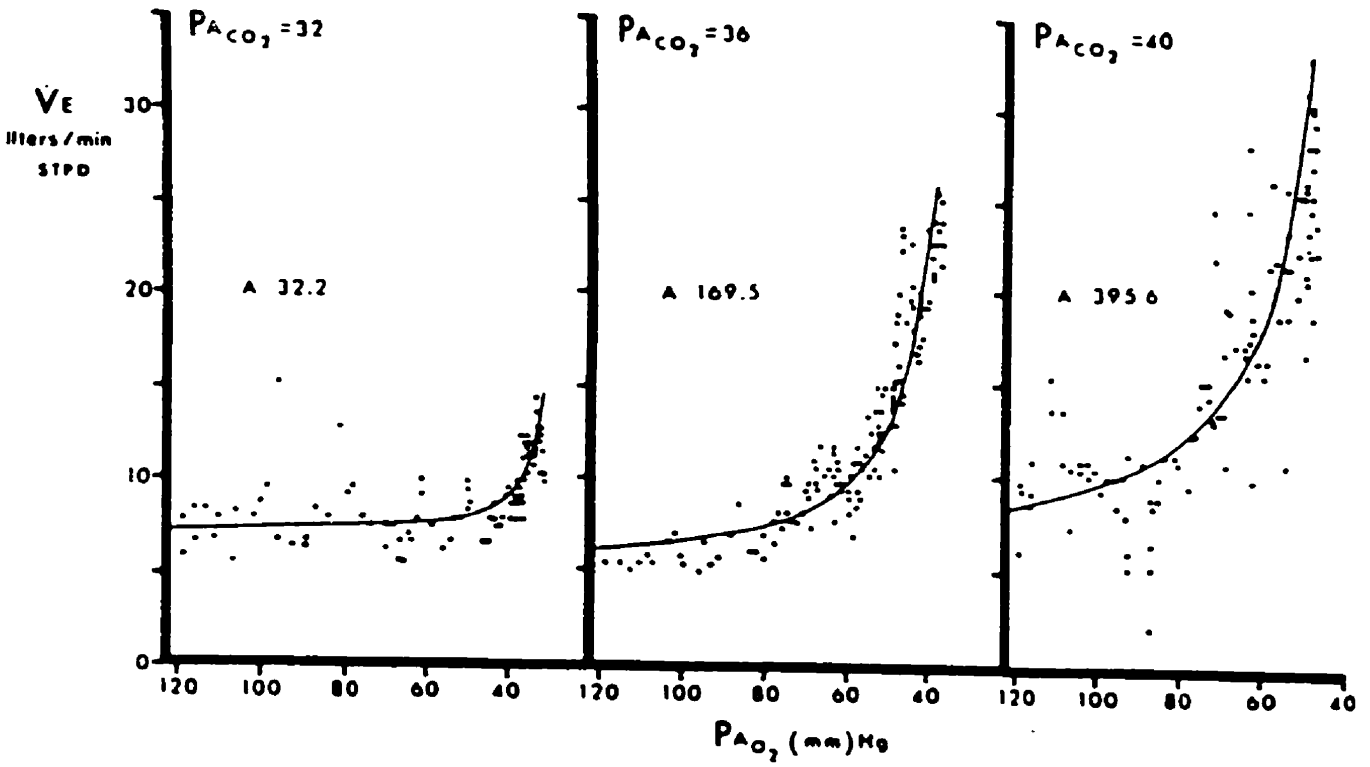

A
B
5
9
2
6
3
4
8
7
1
6
4
3
5
4
3
3
6
1
1
1
2
6

in changes in [H$^+$] with little change in [HCO_3^-] because there are such large stores of [HCO_3^-] in the body (mmol/L) relative to [H$^+$] stores (nmol/L).

In animal studies, progressive isocapnic hypoxic stimulation of the carotid bodies results in an hyperbolic afferent discharge pattern (Neilsen *et al.* 1988). This hyperbolic increase in carotid chemoreceptor afferent activity induced by progressive isocapnic hypoxia evokes a corresponding hyperbolic increase in ventilation (Figure 1.3). The increase in ventilation is due to significant increases in both tidal volume and respiratory frequency (Pappenheimer 1977; Sapru and Krieger 1977; Cardenas and Zapata 1983).

In rats and humans the carotid body chemoreceptor responses to hypoxia and hypercapnia [H$^+$] are not simply additive, rather they have a multiplicative effect on ventilation (Cunningham *et al.* 1986; Duffin 1990; Fitzgerald and Dehghani 1982; Walker et al. 1985; Bisgard and Neubauer 1995). In the absence of maintained arterial isocapnia, the ventilatory response to hypoxia is markedly attenuated by the hypocapnia associated with hyperventilation (Fitzgerald and Dehghani 1982; Duffin 1990; Bisgard and Neubauer 1995) (Figure 1.3). Therefore, poikilocapnic hypoxia results in a markedly attenuated ventilatory response as compared with that observed during isocapnic hypoxia (Howard and Robbins 1995; Bisgard and Neubauer 1995). Another factor which results in the attenuation of

Figure 1.3. Acute ventilatory response to hypoxia in a single subject with ventilation plotted against alveolar P_{O2} at three levels of maintained isocapnia. "A" Values determine the shape of the curve and are an index of the strength of the hypoxic response. (Reproduced, with permission, from Bisgard and Neubauer (1995). Regulation of Breathing. pp. 617-669).


$P_{A_{CO_2}}$ =32
$P_{A_{CO_2}}$ =36
$P_{A_{CO_2}}$ =40
$\dot{V}_E$
liters / min
STPD
A 32.2
A 169.5
A 395.6
30
20
10
0
120
100
80
60
40
$P_{A_{O_2}}$ (mm)Hg

the ventilatory reponse to acute hypoxic exposure is the reduction in gaseous metabolism (Mortola 1993; Mortola and Gautier 1995).

METABOLIC RESPONSES TO HYPOXIA:

Exposure to acute hypoxia results in hyperpnea[1] which is immediate, and tends to characterize the physiological response to the stimulus. However, a decrease in metabolic rate (hypometabolism) and deep-core body temperature has also been observed in small and medium sized mammals (Saiki *et al.* 1994; Pappenheimer 1977). Both the magnitude and threshold of the hypometabolic response to hypoxia can differ between species due to numerous factors, probably including habitat, such as for diving mammals (Tenny and Boggs 1986), or species living in burrows or high altitude (Milsom 1992; Mortola 1993) and the circadian time at which it occurs (Kwarecki *et al.* 1977). Frappell *et al.* (1992) studied 27 mammals ranging in size from 8 g to 47 kg to determine the influence of basal metabolic rate and body mass to the hypometabolic response to hypoxia. It was observed that the basal normoxic $\dot{V}_{O2}$ of the animals expressed per unit of body mass ($\dot{V}_{O2}$/kg) and the magnitude of the decrease in $\dot{V}_{O2}$ during hypoxia (10% O_2) were related. The decrease in

1 The term 'hyperpnea' is strictly applied to indicate an increase in the absolute value of ventilation, the term hyperventilation is used to indicate an increase in ventilation relative to CO_2 production ($\dot{V}_{CO2}$). The terms 'gaseous metabolism' and 'metabolic rate' are used interchangeably, and are represented by either $\dot{V}_{O2}$ or $\dot{V}_{CO2}$. Unless stated otherwise, they are assumed to be related in the following manner: $0.85=\dot{V}_{CO2}/\dot{V}_{O2}$. The term 'hypometabolism' is used to indicate a decrease in metabolic rate with respect to the corresponding normoxic condition.

$\dot{V}_{O2}$ was larger in small animals which have high $\dot{V}_{O2}$/kg (Figure 1.4).

It has been proposed that the decrease in metabolic rate is related to the concommitant decrease in body temperature via the Q_{10} effect[2]. This does not appear to be the case. First, the decrease in $\dot{V}_{CO2}$ occurred very rapidly in kittens, generally within two minutes from the onset of hypoxia, while the decrease in body temperature was slow and gradual (Frappell *et al.* 1991). Second, the decrease in body temperature was small compared with the decrease in $\dot{V}_{CO2}$ in kittens. Mortola and Rezzonico (1988) found that, in rats, exposure to 10% O_2 resulted in a 50% reduction in $\dot{V}_{O2}$ while body temperature decreased by only 1-1.5°C. This yielded a Q_{10} several orders of a magnitude higher than expected from the passive effect of temperature on biological reactions. Third, Pedraz and Mortola (1991) found that maintaining body temperature at normoxic levels in kittens that were exposed to moderate hypoxia did not abolish the hypometabolic effect of hypoxia. In hypoxia, the decrease in $\dot{V}_{CO2}$ is minimal or absent at thermoneutrality[3] and is larger in small species with high $\dot{V}_{O2}$/kg. Together these factors suggest that a large component of the hypometabolic response is related to thermogenesis, and not to the Q_{10} effect (Mortola and Gautier 1995). Therefore, it seems reasonable to suggest

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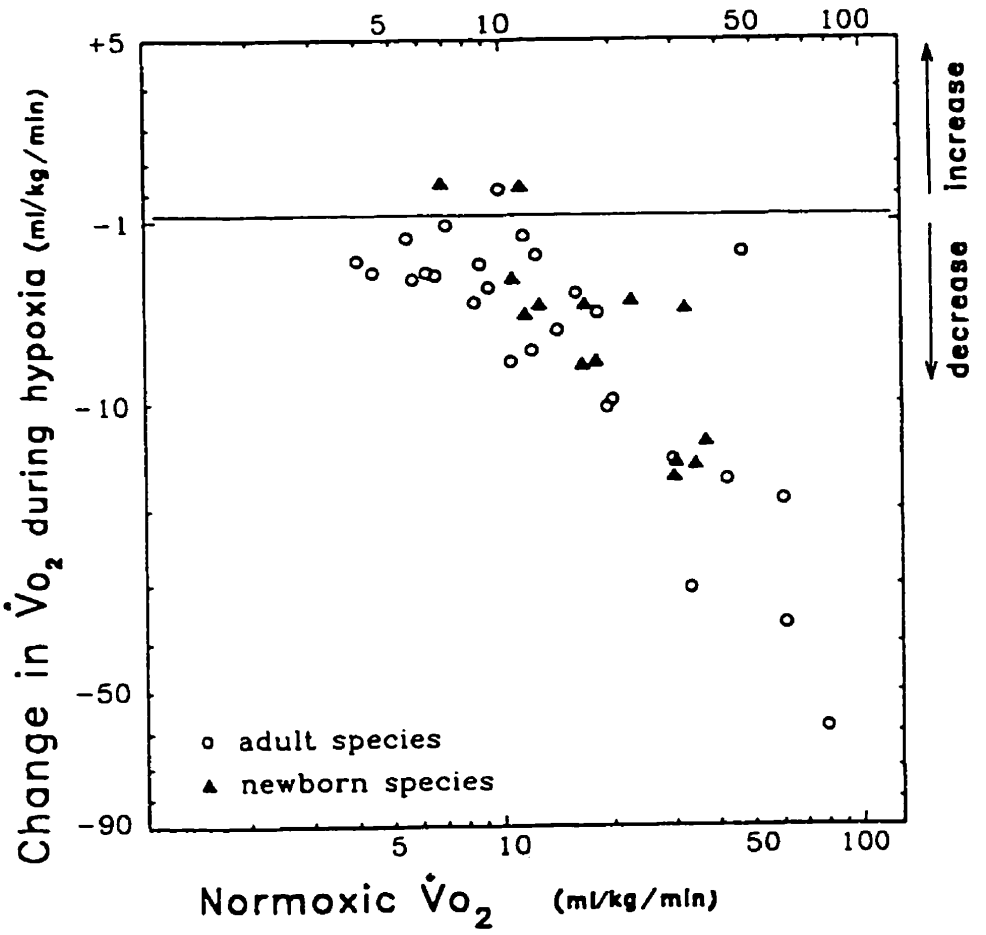
2 The Q_{10} effect is the change in metabolic rate for a 10°C change in temperature.

3 Thermoneutrality is defined as the range in ambient temperature where body temperature is maintained with minimal normoxic $\dot{V}_{O2}$.

Figure 1.4. Double logarithmic representation of the drop in oxygen consumption ($\dot{V}_{O2}$/kg) during hypoxia versus the normoxic value for several newborn and adult mammals. Each data point refers to a different species. The magnitude of the $\dot{V}_{O2}$ drop seems to increase in the smaller animals, which have large resting values, whether newborns or adults. (Reproduced, with permission, from Mortola and Gautier (1995) Regulation of Breathing. pp. 1011-1064.


5
10
50
100
+5
-1
-10
-50
-90
increase
decrease
Change in $\dot{V}O_2$ during hypoxia (ml/kg/min)
adult species
newborn species
Normoxic $\dot{V}O_2$ (ml/kg/min)

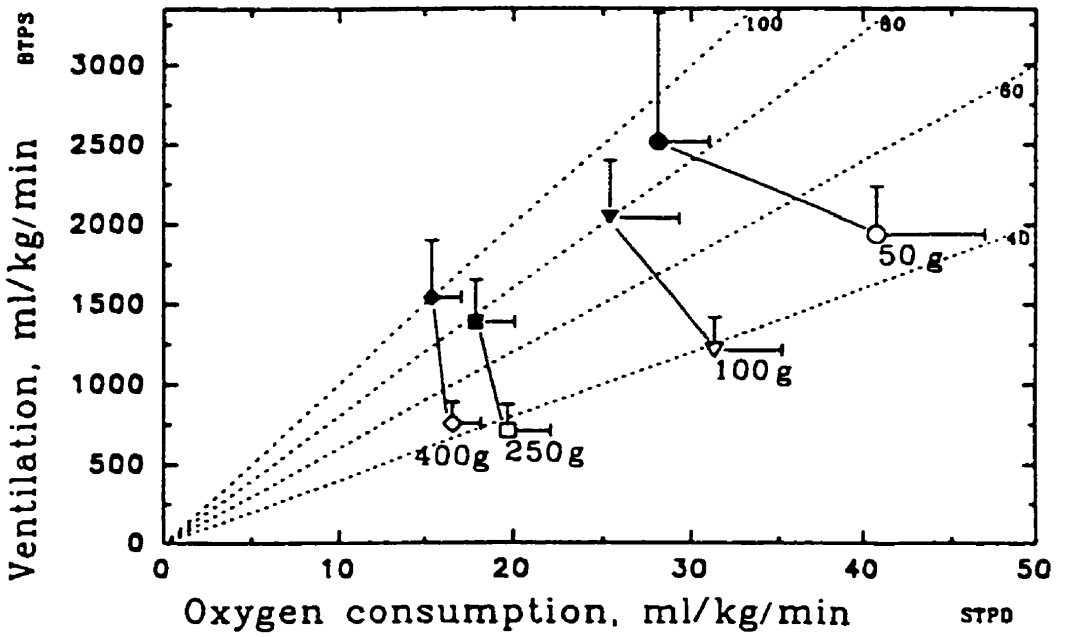
that a decrease in thermogenesis represents a significant mechanism contributing to hypoxic hypometabolism.

The carotid bodies do not appear to be involved in the hypoxic hypometabolic response. Gautier and Bonora (1992) found that $\dot{V}_{O2}$ was reduced in response to hypoxia both before and after carotid body denervation. Although the carotid bodies are known to project to the hypothalamic thermoregulatory regions (Dillon and Waldrop 1993; Evans 1976) their resection does not affect the hypometabolic response to hypoxia. Hence, chemosensory afferent information is not directly involved in the hypoxic hypometabolic response (Gautier *et al.* 1992). Therefore, the decrease in O_2 may be directly sensed by the hypothalamic thermosensory areas, or it may act on smooth muscle cells which can influence the distribution of the microcirculation and thus the relative contribution of tissues to the $\dot{V}_{CO2}$ of the whole body (Mortola and Gautier 1995).

As mentioned earlier, in rats and humans, exposure to hypoxia produces a sudden increase in ventilation which declines over time and eventually returns toward basal levels. This has been called the "biphasic reponse" or "hypoxic ventilatory decline" (Olson and Dempsey 1978; Easton *et al.* 1986) . The respiratory drive during hypoxia reflects a balance between the stimulation of respiration via the carotid bodies and the inhibition of respiration via the hypoxic depression of the CNS (Berkenbosch and DeGoede 1988; Bisgard and Neubauer 1995). The former

generally predominates to yield hyperventilation and associated hypocapnia. Hypocapnia and changes in metabolic rate, body temperature, and blood pressure are some of the factors which affect respiratory drive during hypoxia (McQueen and Eyzaguirre 1974; Paterson and Nye 1994; Ohtake and Jennings 1992). Therefore, it is not surprising that ventilation does not immediately reach a steady state in response to a sudden exposure to hypoxia. Ventilation responds first to events with the shortest time constant, which are certainly the peripheral chemostimuli (Mortola and Gautier 1995; Paterson and Nye 1994). As both metabolic rate and body temperature begin to fall, ventilation follows these variables, and this can be expressed via the ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$). It has been documented that those animals that decrease metabolic rate the most also have the smallest hyperpnea (Frappell *et al.* 1992; Mortola *et al.* 1994). Mortola *et al.* (1994) found that the reciprocal relation between the degree of hypoxic hypometabolism and the degree of hyperpnea was also observed in the same species at different stages of development (Figure 1.5). During hypoxia, the youngest rats had the most marked hypometabolic response with minimal hyperpnea, while the converse occurred in the oldest animals. This evidence strongly suggests that respiratory output appears to follow the metabolic state of the animals.

Figure 1.5. Oxygen consumption ($\dot{V}_{O2}$)-ventilation ($\dot{V}_E$) relationship during normoxia (open symbols) or hypoxia (10 % O_2, filled symbols) in rats of different body weights. Each data point is the average of 10 rats, five females and five males; bars are standard deviations. Oblique isopleths are $\dot{V}_E$-$\dot{V}_{O2}$ ratios. (Reproduced, with permission, from Mortola and Gautier (1995). Regulation of Breathing. pp. 1011-1064.


Ventilation, ml/kg/min BTPS
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Oxygen consumption, ml/kg/min STPD
100
80
60
40
50 g
100 g
400g
250g

MODULATORS OF THE VENTILATORY RESPONSE TO ACUTE HYPOXIA:

There are many factors which modulate the ventilatory response to hypoxia. As mentioned previously, inspired P_{CO2} results in an increased sensitivity to hypoxic stimuli (Figure 1.3). Arousal state is another important modulator of the ventilatory response to hypoxia. As animals move across different plains of consciousness the ventilatory response to hypoxia changes. Phillipson and Sullivan (1978) found that, in humans and dogs, the hypoxic ventilatory response was significantly different during different sleep states. During slow-wave sleep and rapid-eye-movement sleep the ventilatory response to hypoxia is reduced relative to that observed during the awake state. The response is more reduced during rapid-eye-movement sleep than it is during slow-wave sleep (Figure 1.6). During anaesthesia the peripheral chemoreceptor response to hypoxia is greatly depressed (Phillipson and Bowes 1986; Bisgard and Neubauer 1995).

Although it was shown in the previous section that temperature (that is, Q_{10}) is not the proximate cause of the hypoxic hypometabolism and associated reduced hyperpnea, changes in body temperature can nevertheless have marked effects on the ventilatory response to hypoxia. Maskrey (1990) found that in rats with lowered body temperatures (34.5-35.5°C) the ventilatory response to hypoxia (15-7% O_2) was significantly reduced compared to that at normal body temperatures (Figure 1.7).

Figure 1.6. Breath-by-breath response of minute ventilation ($\dot{V}_I$) to decreasing arterial O_2 saturation (Sa_{O2}) and to increasing alveolar partial pressure of CO_2 (P_{ACO2}) in sleeping dog. •, Slow-wave sleep; o, rapid-eye-movement sleep. Note scatter of data points around calculated linear regression lines during rapid-eye-movement sleep and marked decrease in $\dot{V}_I$ response to increasing P_{ACO2}. (Reproduced, with permission, from Phillipson and Bowes (1986) Handbook of Physiology. pp. 649-689.

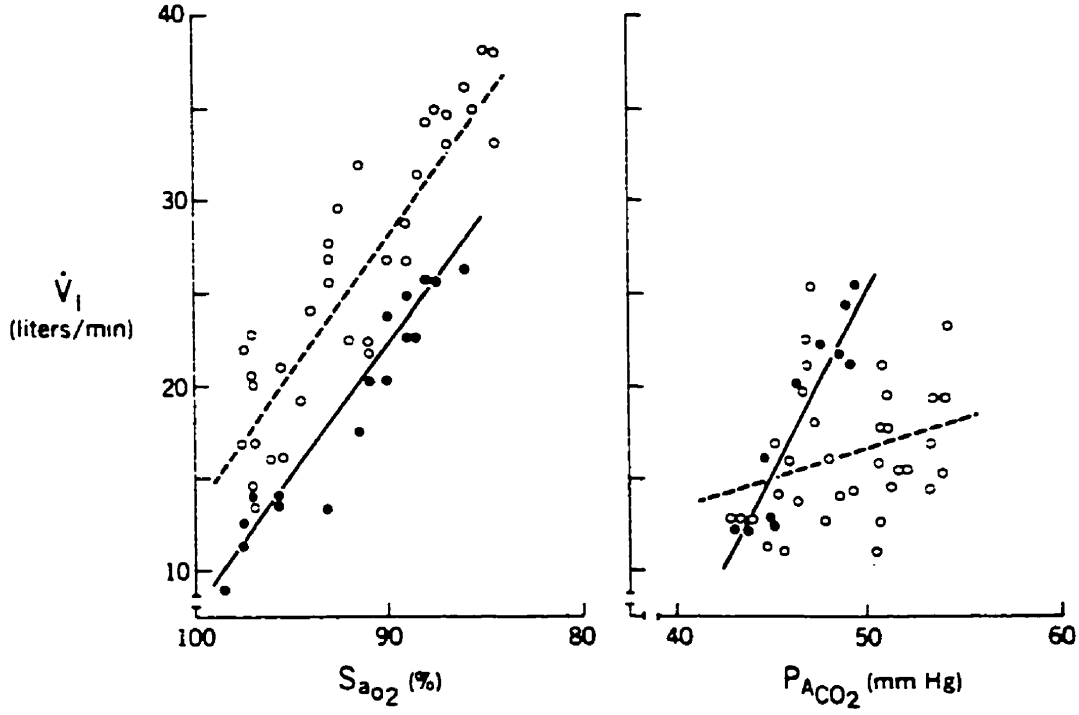
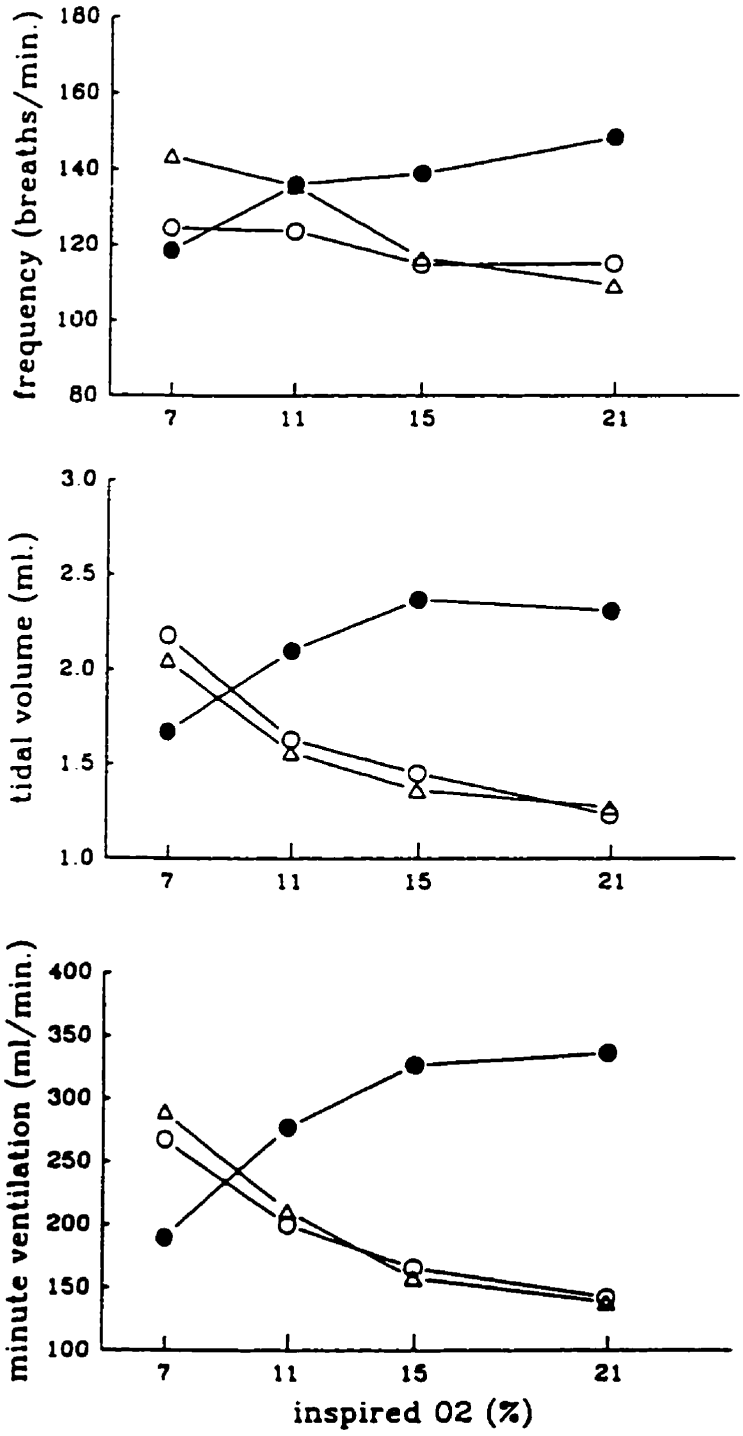

40
30
20
10
$\dot{V}_I$
(liters/min)
100
90
80
$S_{a_{O2}}$ (%)
40
50
60
$P_{A_{CO_2}}$ (mm Hg)

Figure 1.7. Effects of level of inspired O_2 on respiratory responses of rats at normal body core temperature (37.5-38.5°C; o), lowered core temperature (34.5-35.5°C; •), and raised core temperature (40.5-41.5°C; Δ). All values are means (n =14). (Reproduced, with permission, from Maskrey (1990). *Am. J. Physiol.* 259:R492-R498).


frequency (breaths/min.)
180
160
140
120
100
80
7
11
15
21
tidal volume (ml.)
3.0
2.5
2.0
1.5
1.0
7
11
15
21
minute ventilation (ml/min.)
400
350
300
250
200
150
100
7
11
15
21
inspired 02 (%)

Increases in body temperature had no effect on the ventilatory response to the hypoxic stimuli (Figure 1.7). However, for reasons given in the previous section, these results are difficult to interpret without simultaneous measurements of metabolic rate.

McQueen and Eyzaguirre (1974) found that the in vivo carotid body of the cat exposed to varying temperatures had afferent discharge frequencies which were significantly correlated with temperature. At higher temperatures the discharge frequency was higher than at lower temperatures. These results indicate that the carotid body and the ventilatory reponse to hypoxia are temperature sensitive.

THE VENTILATORY AND METABOLIC RESPONSES TO HYPERCAPNIA

Raising alveolar and thus arterial P_{CO2} provides a strong chemical stimulus to breathe. Hypercapnia is a very potent respiratory stimulant because is acts on both the peripheral and central chemoreceptors in the same direction (to increase ventilation) (Mines 1994; Duffin 1990; Cunningham *et al.* 1986). Ventilation is increased by significant increases in both respiratory frequency and tidal volume (Phillipson and Bowes 1986; Walker *et al.* 1985). Unlike the ventilatory response to acute exposures (hours) to hypoxia, the rat and human ventilatory responses to hypercapnia are sustained. However, chronic exposure to hypercapnia results in a decline in the ventilatory response to the stimulus in the rat

(Lai *et al.* 1981). In order to fully understand the ventilatory response to hypercapnia it will be necessary to briefly examine the receptor groups which mediate the response.

CENTRAL CHEMORECEPTORS:

As mentioned above (see *PERIPHERAL CHEMORECEPTORS*), the carotid bodies respond to changes in arterial [H^+] which are directly proportional to the arterial P_{CO2}. However, the main effect of altering P_{CO2} levels in the inspired air are mediated via the central chemoreceptors. These receptors are located somewhere in the ventrolateral medullary region (Bruce and Cherniack 1987; Pilowsky *et al.* 1993; Sato *et al.* 1992; Nattie 1995), however, the specific neurons and their precise location within the medullary shell are not known. It is not clear whether there are chemo-sensitive neurons which provide input to the central respiratory rhythm generator neurons, or if the central respiratory rhythm generator neurons themselves act as the chemosensors to the hypercapnic stimulus (Pilowsky *et al.* 1993; Nattie 1995). It is clear, however, that some neurons in the medullary shell respond to changes in ionic H^+ concentrations. As mentioned above (see *THE VENTILATORY RESPONSE TO ACUTE HYPOXIA)* H^+ is related to P_{CO2} in a linear fashion such that increases in Pa_{CO2} result in increases in H^+. The central chemoreceptors do not equilibrate with arterial H^+ because of the blood-

brain barrier. The blood-brain barrier does not allow polar solutes to readily pass, however, CO_2 does easily permeate this barrier (Duffin 1990). Therefore, the H^+ sensed by the central chemoreceptors is related to the Pa_{CO2} and not to arterial H^+.

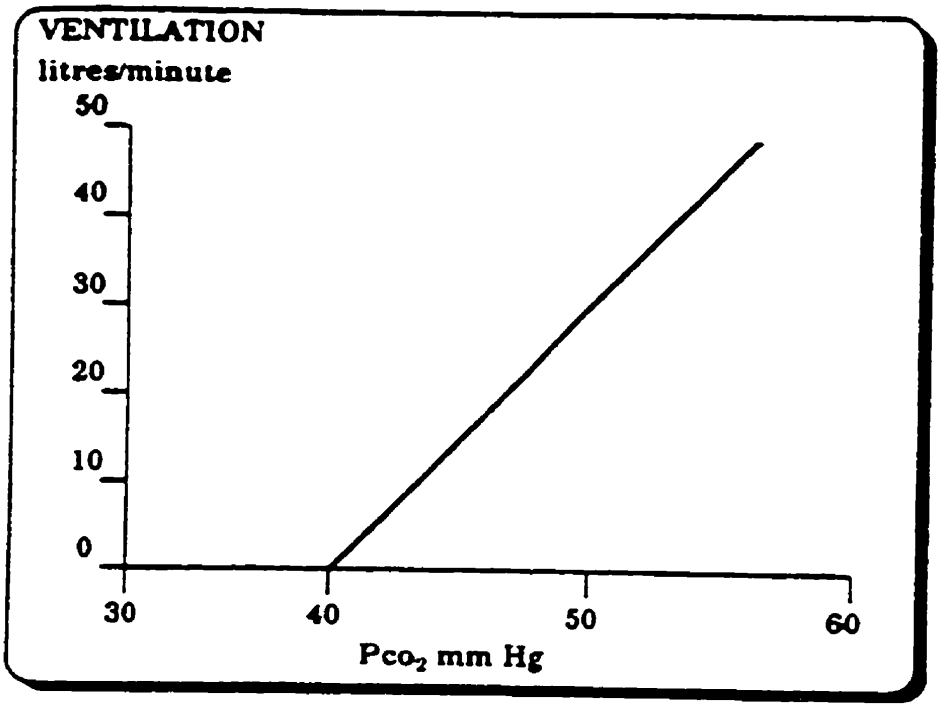
THE VENTILATORY RESPONSE TO HYPERCAPNIA:

The drive related to P_{CO2} at the chemoreceptors is the main source of the tonic input into the neurons that comprise the kernel of the respiratory rhythm generator (Bruce and Cherniack 1986; Phillipson *et al.* 1981). Some of this drive comes from the peripheral chemoreceptors, however, their denervation does not abolish the ventilatory response to hypercapnia (Phillipson *et al.* 1981), thus indicating that the receptors in the ventrolateral medulla are the major sensors of altered CO_2 levels.

When only the central chemoreceptors are functioning, the relationship between the arterial P_{CO2} and ventilation is linear (Figure 1.8) (Duffin 1990; Cunningham *et al.* 1986). The same is true of that portion of the hypercapnic response which is mediated via the peripheral chemoreceptors. The total ventilatory response to hypercapnia can be determined by adding the peripheral and the central responses (Duffin 1990; Cunningham *et al.* 1986).

The sensitivity of the ventilatory response (that is, the slope of the ventilation versus Pa_{CO2} line) can be changed by several factors. Changes

Figure 1.8. The alveolar ventilation response to arterial carbon dioxide mediated by the central chemoreceptors. (Reproduced, with permission, from Duffin (1990). *Can. J. Anaesth.* 37(8):933-941.


VENTILATION
litres/minute
50
40
30
20
10
0
30
40
50
60
Pco2 mm Hg

in sleep state are known to alter the ventilatory response to hypercapnia. In dogs and humans, Phillipson and Sullivan (1978) found that the ventilatory response to hypercapnia is more depressed in rapid-eye-movement sleep than in slow-wave sleep compared with quiet resting states. Anaesthesia, like sleep, reduces the sensitivity of the ventilatory response to hypercapnia in humans (Duffin 1990). Changes in body temperature are also known to alter the sensitivity of the ventilatory response to hypercapnia. Maskrey (1990) found that rats with raised body temperature (40.5-41.5°C) had a greater response to hypercapnia, but lowering body temperature to 34.5-35.5°C had no significant effect on the ventilatory response to hypercapnia. These results suggest that increases in body temperature result in an increased sensitivity to CO_2.

METABOLIC RESPONSES TO HYPERCAPNIA:

There have been very few studies which specifically examine the interaction between hypercapnia and the metabolic response to it. Those that have examined the effects of hypercapnia on metabolic rate have found differing results. The most recent and conclusive study was done by Saiki and Mortola (1996). They measured the ventilatory and metabolic responses of adult rats to 2% and 5% CO_2 at two different ambient temperatures (10 and 25°C). Normocapnic metabolic rate ($\dot{V}_{O2}$) was higher at an ambient temperature of 10°C than at 25°C reflecting increased

thermogenesis at the lower temperature. At both ambient temperatures, inspired CO_2 did not significantly change $\dot{V}_{O2}$, but did significantly increase ventilation. At 10°C ventilation increased less than at 25°C in response to inspired CO_2 despite a significantly higher $\dot{V}_{O2}$. These results indicate that hypercapnia unlike hypoxia, does not elicite a metabolic response; and that ventilation does not closely follow metabolic rate during the hypercapnic stimulus.

PART II:

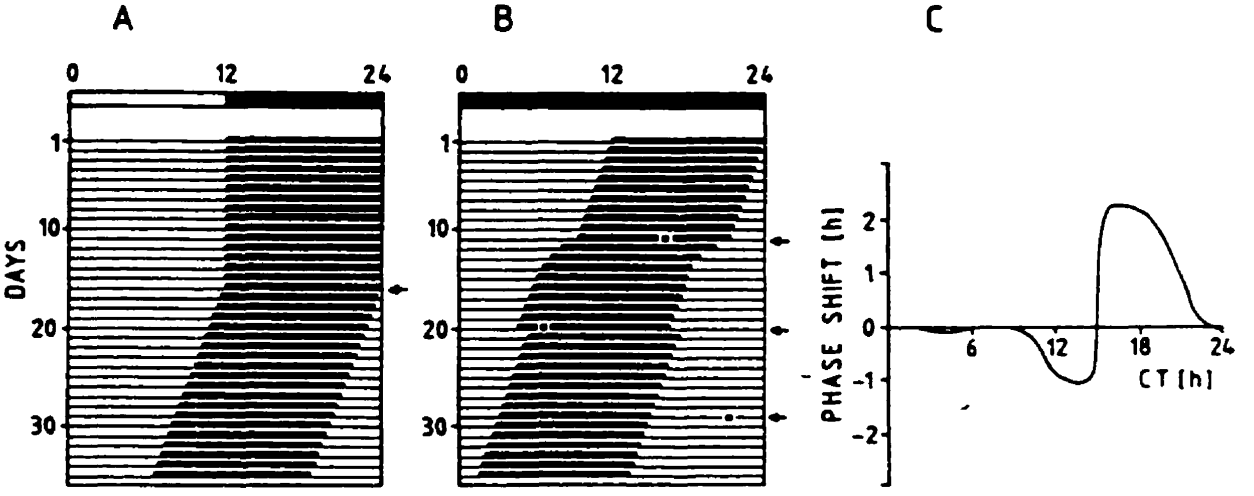
THE CIRCADIAN SYSTEM

The existence of twenty-four hour rhythms in many behavioural and physiological variables is a well known phenomenon. Historically, it was thought that the 24 hour rhythms which both plants and animals exhibit were due to passive responses to the environmental day-night cycle. However, it is now known that when an organism is isolated from all environmental time cues, many of its rhythms continue (Moore-Ede *et al.* 1982; Rusak and Zucker 1979). During the 18th century, de Mairan noticed that the leaves of the *Mimosa* plant opened and closed with the day-night cycles. He remarked that when the plant was subjected to constant darkness the leaves continued to open and close periodically. This was perhaps the first documented experiment which indicated the

existence of endogenous behavioural activity (Meijer and Rietveld 1989; Moore-Ede *et al.* 1982; Rusak and Zucker 1979). However, de Mairan concluded that some other environmental cues must have resulted in this sustained periodicity. In 1939, Johnson showed that mice kept in constant darkness maintained an activity rhythm, and that the period of the rhythm was not precisely 24 hours. These 24 hour rhythms are called circadian rhythms, and under constant conditions are said to be "free-running", thus indicating that they are not driven by an environmental cue, and are therefore endogenous (Moore-Ede *et al.* 1982; Meijer and Rietveld 1989; Pittendrigh 1974).

Although circadian rhythms are endogenous, they can be manipulated by both photic and non-photic stimuli (Moore-Ede *et al.* 1982; Mrosovsky 1995; Rusak and Zucker 1979; Amir and Stewart 1996). A circadian rhythm can be advanced or delayed, depending on the time of the cycle (phase) at which the stimulus is presented. Figure 1.9 illustrates: (1) that when placed into constant conditions a hamster's wheel running activity will free-run; (2) that when a light pulse is presented to the hamster, its activity rhythm will shift in response to the stimulus; and (3) when the light pulse is presented at different times the shift in the activity rhythm is different. The process of maintaining a constant phase relation with an external stimulus such as light is known as entrainment. The process of changing the phase relation in response to a stimulus is referred

Figure 1.9 A: activity rhythm of a hamster is schematically drawn. Each horizontal line represents 1 day. Consecutive days are plotted beneath one another. Active period of animal is indicated in black. This nocturnal animal is entrained to a light-dark regime (indicated above record) until it is released in constant darkness (indicated by arrow) at *day 17*. Activity rhythm is now free running. B: schematic representation of effects of light pulses on free-running activity rhythm of a hamster. Day of light pulse is indicated by an arrow and hour by an asterisk. First pulse is applied at end of animal's activity and induces a phase advance. A phase delay is induced by a light pulse presentation at beginning of activity. No shift is observed after a light pulse during inactive period. C: phase-response curve for circadian activity rhythm of hamster. Phase advances are plotted in positive direction along vertical axis, and phase delays are plotted downward. Horizontal axis represents 1 circadian day. Circadian time (CT) 12 corresponds with onset of activity. (Reproduced, with permission, from Meijer and Rietveld (1989). *Physiol. Rev.* 69(3):671-707).


A
B
C
0
12
24
1
10
20
30
DAYS
PHASE SHIFT [h]
2
1
0
-1
-2
6
12
18
24
CT [h]

to as phase shifting (Meijer and Rietveld 1989; Moore-Ede *et al.* 1982; Rusak and Zucker 1979).

The idea of an endogenous circadian clock (pacemaker) that was first postulated by Johnson remained largely hypothetical for another 30 years (Meijer and Rietveld 1989). In 1972, two independent research teams identified a small, bilateral pair of nuclei in the anterior hypothalmus, the suprachiasmatic nuclei (SCN), as possible circadian pacemakers (Moore and Eichler 1972; Stephan and Zucker 1972). Lesions of the SCN revealed that these structures are essential for circadian rhythmicity in the sleep-wake cycle, wheel running, drinking, body temperature, and the synthesis and secretion of several hormones such as pineal melatonin (Moore-Ede *et al.* 1982; Meijer and Rietveld 1989). These findings strongly suggest that the SCN are instrumental in coordinating circadian variables.

The SCN receive many afferent inputs. The two main afferent inputs to the SCN which appear to coordinate photic information from the retina are the retinohypothalamic tract (RHT) and the geniculohypothalamic tract (GHT) (Meijer and Rietveld 1989; Rusak and Zucker 1979). There are other afferent inputs to the SCN. Afferent systems originate in the anterior hypothalamic area, the retrochiasmatic area the paraventricular thalamic nucleus, the paraventricular nucleus (PVN) and the raphe nucleus (RN) (Meijer and Rietveld 1989).

The precise role of these inputs are not well understood. It is

interesting that both the PVN of the anterior hypothalamus and the RN are both part of the neural circuitry of the cardiorespiratory system. Stimulation of the PVN is known to increase both heart and respiratory rates (Waldrop and Porter 1995). The PVN is also known to project to the nucleus of the tractus solitarius (NTS) (Waldrop and Porter 1995). The NTS is the dorsal-medial medullary nucleus which receives input from the carotid body chemoreceptors and relays their input to the respiratory rhythm generating neurons. The raphe nucleus has been shown to modulate respiratory activity during both the awake and sleep states (Pack 1995).

This evidence clearly indicates that there are neural connections between both the circadian and cardiorespiratory control systems. This leads to the question as to whether the circadian and respiratory systems communicate with one another.

PART III:

RATIONALE

There is an overwhelming amount of indirect evidence which suggests that the respiratory system output should vary in a circadian fashion, however, there have been no studies which have specifically addressed this idea. In an attempt to explain the phenomenon of

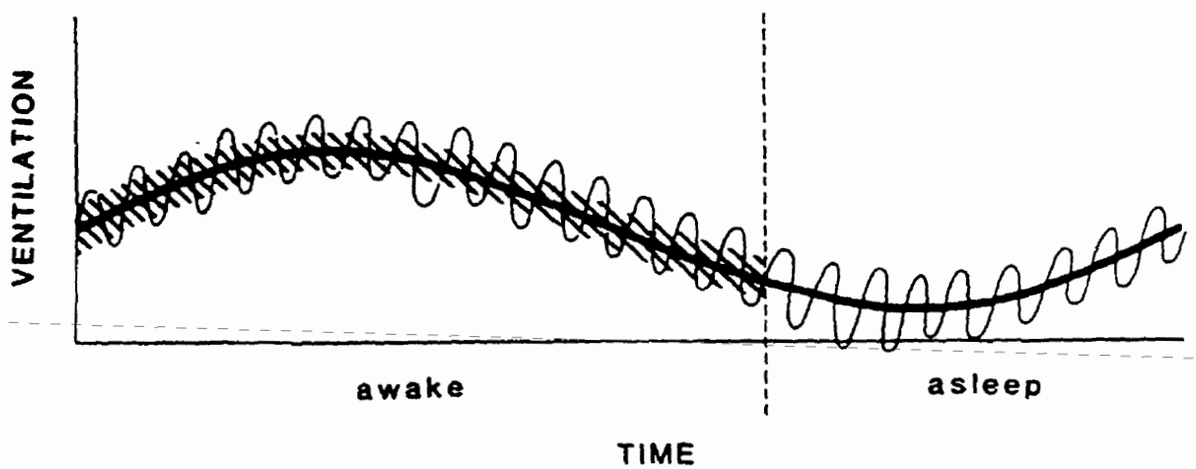
respiratory sleep apnea it was proposed by Cherniack (1984) that perhaps the respiratory output fell close to zero during sleep and that the small oscillations in ventilation ultimately resulted in zero ventilation or central sleep apnea (Figure 1.10). This idea contradicts the commonly held idea that decreases in ventilation during sleep are due to the lack of the "awake" or "arousal" stimulus (Phillipson and Bowes 1986).

The sleep-wake cycle has profound effects on the respiratory control system (Orem 1995), however, some of the reduction in ventilation during the sleep cycle may well be the result of an independent circadian rhythm in the ventilatory output itself. Cherniack's idea begged the question: does respiratory output occur in a circadian fashion independent of arousal state?

There are many physiological variables which are known to affect ventilation which themselves occur in well defined circadian cycles. Strupfel *et al.* (1987, 1989) and Aschoff and Pohl (1970) have shown that small rodents exhibit circadian rhythms in metabolic rate. There are numerous studies which have demonstrated that there are circadian oscillations in deep-core body temperature (Aschoff and Pohl 1970; Honma and Hiroshige 1978; Satinoff and Prosser 1988; Fioretti *et al.* 1974), and that the oscillations in metabolic rate and body temperature occur in parallel.

Ventilation is known to be affected by both changes in body temperature (Maskrey 1990; Jennings and Laupais 1982) and changes in

Figure 1.10. Oscillatory changes in ventilation over a day. Horizontal line represents zero ventilation. Heavy line shows a possible circadian change in ventilation level, while thin line sine waves show shorter spontaneously occurring breathing oscillations. When awake, short-term periodic oscillations in ventilation occur that are obsured by nonspecific environmental stimuli (----). These oscillations become more apparent during sleep when the effects of environmental stimuli are minimized. Also, with the sleeping decrease in ventilation, the shorter fluctuations in ventilation are sufficient to cause apnea or near apnea. (Reproduced, with permission, from Cherniack (1984). *J. Clin. Invest.* 73:1501-1506).


VENTILATION
awake
asleep
TIME

metabolic rate (Mortola 1995; Saiki and Mortola 1996). Ohtake and Jennings (1992) have demonstrated that small reductions in mean arterial blood pressure stimulate ventilation, and Su *et al.* (1987) have shown that rats exhibit circadian rhythms in mean arterial blood pressure, and that the baroreflex itself is different at different times of the day. These data suggest that ventilation should follow the modest changes in mean arterial blood pressure. From these observations, it is reasonable to predict that the circadian oscillations in metabolic rate, body temperature and mean arterial blood pressure should result in oscillations in ventilation.

Whether the ventilatory control system passively tracks these variables or whether it receives actual input from the circadian system itself will not be specifically addressed in the current preliminary study. However, there is evidence which suggests that the SCN controls the circadian rhythm of heart rate via the sympathetic nervous system (Warren *et al.* 1994; Warren and Cassone 1995), and Daly (1986) has shown that the respiratory system and the cardiovascular system share many common sympathetic pathways. Therefore, it seems likely that the respiratory system may be indirectly affected via the sympathetic nervous system, and will thus exhibit circadian oscillations in its overall motor output.

OBJECTIVES

There were two primary objectives of this study. The first was to design and implement an experimental apparatus which could be used to simultaneously monitor ventilation, metabolic rate, and deep-core body temperature. The second was to determine: (a) whether there was a difference in ventilation and metabolism at two different times of the day (10 am and 10 pm); and (b) whether the ventilatory response to hypoxia and hypercapnia varied between 10 am and 10 pm.

CHAPTER TWO

MATERIALS AND METHODS

THEORY AND DESIGN OF APPARATUS

The apparatus illustrated in Figure 2.1 serves three primary purposes: (a) measurement of ventilation, (b) measurement of gaseous metabolism, and (c) measurement of deep-core body temperature.

MEASUREMENT OF VENTILATION:

THEORY

Ventilation was measured using whole-body plethysmography. It is simple in its principle, however, a very difficult technique to use properly. The principle behind the technique is as follows: when a subject respires in an environment cooler than its body temperature, inspired air expands because it is warmed and humidified as it is drawn into the lungs. If an experimental animal is placed into a chamber of a fixed volume, pressure in the chamber increases by an amount proportional to the animal's tidal volume because expansion of the tidal gas compresses the gas in the chamber. With this technique ventilation is therefore represented by oscillations in pressure due to cyclic variations of temperature and water vapour pressure. Inspiration results in warming and humidification of

FIGURE 2.1. Photograph of experimental apparatus.

the inspired gas which results in a pressure rise, whereas, expiration results in the cooling and condensation of expired air, and therefore results in a pressure fall. Drorbaugh and Fenn (1955) derived a mathematical equation for calculating tidal volumes (V_T) from these observed pressure oscillations (Pm):

$$V_T = G_A * Vcal * Pm / Pcal$$

where Pcal is the pressure deflection measured when a known volume of gas, Vcal, is injected into the chamber, and G_A is the dimensionless constant:

$$G_A = Tb * (Pb - P_{CH2O}) / [Tb * (Pb - P_{CH2O}) - Ta * (Pb - P_{AH2O})]$$

where Tb is alveolar temperature (°K), Ta is chamber temperature (°K), Pb is barometric pressure in the chamber (mmHg), and P_{AH2O} and P_{CH2O} are the water vapour pressures (mmHg) of the gases in the alveoli and the chamber, respectively.

This equation is based on the assumption that expired gas returns to chamber conditions before the next inspiration commences. This assumption was challenged by Epstein and Epstein (1978). They asserted that during expiration only the transition from alveolar to nasal conditions is rapid enough to contribute to the phasic pressure change. Jacky (1980) demonstrated that exhaled gas does not return to ambient conditions before the subsequent inspiration, rather it remains at nasal temperatures during the expiratory phase. Jacky (1980) also predicted that

the error in the Drorbaugh and Fenn (1955) equation increases as the temperature of the expired air leaving the nares (Tn) approaches alveolar temperatures as the ratio of the inspiratory interval (Ti) to total breath length (Ttot) increases. If these factors are ignored, Jacky (1980) found that the Drorbaugh and Fenn (1955) equation underestimates true tidal volumes by approximately 30 per cent. Jacky (1980) derived a formula which corrects for this potential underestimation in tidal volume. He defined a new constant, G_N analogous to G_A but representing the volume change from alveolar to nasal volume,

$$G_N = Tb^* (Pb - P_{NH2O}) / [Tb^* (Pb - P_{NH2O}) - Tn^* (Pb - P_{AH2O})]$$

where P_{NH2O} is the partial pressure (mmHg) of the gas at the nares. Jacky's (1980) corrected formula becomes:

$$V_T / V_{Tcor} = 1-(Ti/Ttot)(1-G_A/G_N)$$

This formula requires the measurement of both deep-core body temperature and nasal temperature. It is relatively simple to measure core body temperature in the rat by use of chronically implanted radiotransmitters. However, obtaining nasal temperatures in awake and unrestrained animals is very difficult, therefore, an experiment was conducted to determine whether nasal temperature could be correlated with deep-core body temperature, ambient temperature and respiratory frequency.

The methods and results of this experiment are explained in

Appendix I. The results of this preliminary study indicated that nasal temperature (Tn) can be predicted from the measurement of deep-core body temperature (Tb), ambient temperature (Ta), and respiratory frequency (f_R, breath/minute) in the following manner:

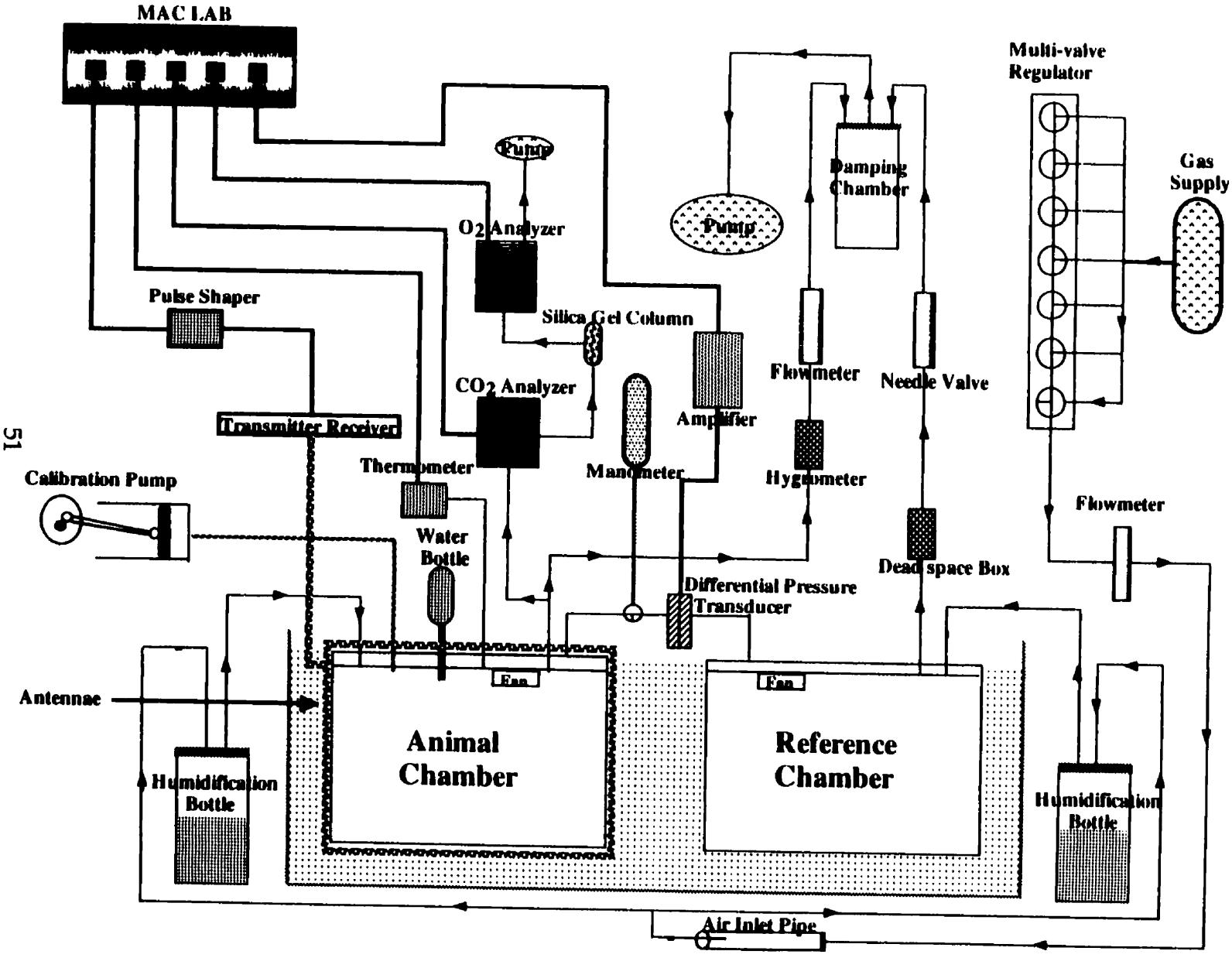
$$Tn = -15.9 + 0.29\ (Ta) + 1.2\ (Tb) - 0.04\ (f_R)$$

DESIGN

A schematic of the system is presented in Figure 2.2. The apparatus consisted of two identical Plexiglas chambers, one contained the experimental animal, the other chamber acted as a thermobarometer. The two chambers had removeable lids which allowed easy access to the animal chamber, and could be vacuum sealed using rubber gaskets. The two 12.5 litre chambers were also enclosed by a water jacket which helped maintain a constant animal chamber temperature. A water circulator (Model K-2, Brinkman Instruments) was used to ensure a constant temperature of 25-26°C, which was assumed to be within the thermoneutral range for these animals (Gordon 1990).

A differential pressure transducer (Model DP45-14, Validyne Engineering Corp.) measured the pressure differences between the two chambers. It was connected to an amplifier (Model CD15, Validyne Engineering Corp.) whose output was fed into the data acquistion system (MacLab/8, Analog Digital Instruments Ltd).

FIGURE 2.2. Schematic of the experimental apparatus.


MAC LAB
Pump
O_2 Analyzer
Pump
Damping Chamber
Multi-valve Regulator
Gas Supply
Pulse Shaper
Silica Gel Column
CO_2 Analyzer
Amplifier
Flowmeter
Needle Valve
Transmitter Receiver
Thermometer
Manometer
Hygrometer
Calibration Pump
Water Bottle
Flowmeter
Dead space Box
Differential Pressure Transducer
Antennae
Fan
Fan
Animal Chamber
Reference Chamber
Humidification Bottle
Humidification Bottle
Air Inlet Pipe

Flow through the chambers was produced by a vacuum pump ('Air Cadet', Model 7530-60, Cole Parmer Instruments Co.); the vacuum was applied to the chambers through a 2 litre flask. This bottle acted as a compliance to damp any pressure oscillations created by the pump. High-resistance needle-valves (18 gauge needles) acted as critical orifices to further dampen any pressure fluctuations from the vacuum pump. Both the damping bottle and the needle-valves helped ensure that flow through the chambers was maintained constant.

The inlet orifice to the animal chamber was comprised of a 200 cm piece of 1/4inch inner diameter plastic tubing. The tubing was connected to a 1 litre bottle filled with water. The water filled bottle was connected to the animal chamber by a 250 cm length of tubing. Therefore, air being drawn into the chamber was nearly saturated with water vapour. The inlet orifice of the reference chamber was constructed in precisely the same manner as that of the animal chamber except that the length of tubing leading to the water filled bottle measured 225 cm in length. [1] The inlets into the reference and animal chambers were mutually connected via a Y-

[1] The inlet pathways were designed such that there was a significant resistance to flow so that more energy was required to accelerate the gas in the pathway, that is, to change flow rate, than was required to maintain a constant flow (Jacky 1978). Therefore, the small pressure changes due to respiratory movements did not generate enough energy to alter flow through the inlet and outlet orifices. In essence, this system functioned as if the inlet and outlet orifices were closed. The inlet pathway to the reference chamber was made longer because it was necessary to match the inertance of the two chambers. Inertances were considered matched when swinging of the laboratory door did not result in any pressure deflection between the two chambers (Jacky 1978). This indicated that the two inlet impedances were equal, and therefore, the pressure change in the room due to the swinging of the door were equally propagated through both chambers. The inlet pathways had equal inertances when the inlet tubing to the reference chamber was 25 cm longer than that to the animal chamber.

piece which in turn was connected to a 30 cm length of 1/4 inch inner diameter tubing. This piece of tubing was inserted into a large 4 cm diameter cylinder into which the test gases were delivered. This design ensured that any pressure change which occurred while test gas mixtures were changed during the experimental protocol (see PROTOCOL below) were not propagated into the experimental and reference chambers.

The outlet orifice from the animal chamber was connected to a 500 ml sealed Plexiglas box which housed a digital hygrometer (Model 57550-21, Canadawide Scientific Co.). This allowed for constant monitoring of ambient humidity in the animal chamber. The reference chamber was also connected to a 500 ml Plexiglas box, however, it did not contain a hygrometer. Temperature of the animal chamber (Ta) was monitored via a digital thermometer (Bat-10, Physiotemp Inc.). The thermocouple tip was inserted into a rubber stop cock which was tightly sealed into the lid of the chamber. The thermometer signal was fed to the data acquisition system and then to the computer to produce a continuous record of Ta.

Flow through the animal chamber was adjusted via a needle-valve on the flowmeter (Model N102-05ST, Cole Parmer Co.) until the desired flow of 2595 ml/minute was attained. In order to match the flow between the two chambers , the needle-valve leading from the reference chamber to the pump was adjusted until the pressure reading between the chambers read zero according to the differential pressure transducer.

Calibration of the differential pressure transducer was performed dynamically with the animal present in the experimental chamber. An animal ventilator (Columbus Instruments International Corp.) was connected to the animal chamber. It was used to pump a known volume of air (20 ml) into and out of the animal chamber in a sinsusoidal fashion at a known frequency (40 cycles/minute). The calibration volume produced pressure deflections which were approximately 5 times that produced by the respiratory movements of the animal. This ensured that the breathing pattern of the animal did not impinge upon the calibration pressure signal (Jacky 1978, 1980; Epstein and Epstein 1979).

MEASUREMENT OF GASEOUS METABOLISM:

THEORY

The technique of open-flow respirometry was used to determine the metabolic rate of the animal. The animal was placed into the experimental chamber (as above). The chamber had a fixed volume (12.5 litre) and a known flow rate (2895 ml/minute) passing through it, therefore, according to the Law of Conservation of Mass, the number of molecules entering the chamber must equal the number leaving it. From this simple principle, the calculation of carbon dioxide production ($\dot{V}_{CO2}$) can be determined.

Flows into and out of the chamber can be described by the following

material balance equation:

$$\dot{V}e^{*} (Feco_2 + Feo_2) = \dot{V}i^{*} (Fico_2 + Fio_2) - \dot{V}_{O2} + \dot{V}_{CO2} \quad (1)$$

where $\dot{V}e$ represents flow out of the chamber (STPD); $\dot{V}i$ represents flow into the chamber (STPD); $Feco_2$ and Feo_2 represent the fractional concentration of carbon dioxide and oxygen leaving the chamber, respectively; $Fico_2$ and Fio_2 represent the fractional concentration of carbon dioxide and oxygen entering the chamber, respectively. $\dot{V}_{O2}$ represents the rate of oxygen consumption of the animal (STPD), and is equal to:

$$\dot{V}_{O2} = \dot{V}e^{*} (Feo_2) - \dot{V}i^{*} (Fio_2) \quad (2);$$

and $\dot{V}_{CO2}$ represents the rate of carbon dioxide production of the animal (STPD), and is equal to:

$$\dot{V}_{CO2} = \dot{V}e^{*} (Feco_2) - \dot{V}i^{*} (Fico_2) \quad (3).$$

The $\dot{V}_{CO2}/\dot{V}_{O2}$ ratio *(4)* is termed the respiratory exchange ratio (RE), and under most circumstances is equal to 0.85. It should be noted that the contribution of ambient water vapour pressure and evaporative water loss from the animal play no significant role in altering flows through the chamber (Withers 1977). They have therefore been omitted from the material balance equation. Equation *(1)* can be rearranged in order to solve for either $\dot{V}_{O2}$ or $\dot{V}_{CO2}$. In this case the equation for $\dot{V}_{CO2}$ will be derived. Substituting equations *(2)*, *(3)*, and *(4)* into *(1)* and rearranging, $\dot{V}_{CO2}$ can be written as:

$$\dot{V}_{CO2} = \dot{V}e^{\bullet} (Feco_2 - Fico_2) / (1 - Fico_2 * (1 - 1/RE)),$$

where $RE = \dot{V}_{CO2}/\dot{V}_{O2} = 0.85$. In order for this equation to hold true, flow into ($\dot{V}i$) and out of ($\dot{V}e$) the system must be equal and must remain constant. Flow out of the system was continuously monitored and was known to remain constant.

DESIGN

Figure 2.2 is a schematic of the whole system and illustrates the organization of the open-flow respirometry system. As mentioned earlier, the flow through the system was maintained constant at 2895 ml/minute by adjustment of the flowmeter needle-valve. Air, hypoxic gas mixtures, or hypercapnic gas mixtures were delivered into the inlet pipe at a flow rate of approximately 6000 ml/minute. This was determined by a flowmeter (Model 044-40G, Cole Parmer Co.) which was connected to the multi-valve regulator. The latter allowed the rapid switching of test gas mixtures. The test gas was drawn into both the animal and reference chambers where it was mixed using small electric fans (Model FS1243, Comair Rotron, Electrosonic Inc.) installed on the removeable lids.

Airflow from the animal chamber was sampled just before the flowmeter leading to the vacuum pump. The sample gas was drawn through a dessicating column of silica gel (Sigma Corp.) and then through both the oxygen and carbon dioxide analyzers (Model S-3A/1 and Model

3D-3A, Ametek Corp.). The pump which drew the sample past the gas analyzers was adjusted to a constant flow of 300 ml/minute (total flow = 2595 ml/minute + 300 ml/minute = 2895 ml/minute). The signals from the gas analyzers were connected to the data acquisition system, and then to the computer allowing continuous recording of chamber gas concentrations.

MEASUREMENT OF BODY TEMPERATURE:

THEORY AND DESIGN

Small disc shaped radiotransmitters (Mini Mitter, Model VM-FH-Disc) were placed into the peritoneal cavity (see SURGICAL PROTOCOL) of the animals. The transmitter therefore monitored the animals deep-core body temperature and were capable of sensing temperatures from 10-45°C with a sensitivity of 0.1°C. The transmitter signal is a series of short bursts of radio frequency energy, coding temperature by pulse interval modulation; the shorter the time between pulses, the higher the temperature, and the longer the duration between pulses, the lower the temperature. The signal from the transmitters was received with a loop antenna (Mini Mitter Co.) coiled around the animal chamber. The apparatus is illustrated in Figure 2.2. The antenna was part of a receiver system (Mini Mitter Co., RTA-500 Receiver) specifically designed to receive the radio signal from these transmitters. The radio signal was

amplified and converted to square wave voltage pulses using a custom-built signal conditioner (Zoological Electronic Shops).

Four transmitters were individually calibrated over a physiological temperature range. The experimental chamber was half-filled with water and a water circulator (Model K-2, Brinkman Instruments) was used to regulate the temperature of the water in the chamber to six specific (and constant) temperatures: 34.5°C, 35.5°C, 36.5°C, 37.5°C, 38.5°C, and 39.5°C. Each transmitter was placed in the water bath (experimental chamber) for 5 minutes and the output from the receiver was measured for 30 seconds at the end of the 5 minute period. A regression equation describing the relationship between temperature and pulse frequency for each specific transmitter was generated so that the pulse frequency displayed by the data acquisition system could be expressed as a specific temperature.

SURGICAL PROCEDURES:

Temperature transmitters were surgically implanted into the peritoneal cavity at least two weeks prior to the commencement of the experiments. Transmitters were calibrated (see above) after the experimental protocols were complete.

For implantation of the temperature sensors, the rats were anaesthetized with halothane (2%) (Halocarbon Laboratories). In order to ensure postoperative pain was reduced to a minimum nitrous oxide was

concurrently supplied to the animals during anaesthesia. Once deep reflexes were abolished, a small patch of abdominal fur was shaved and the skin cleansed with iodine. A small 2-2.5 cm incision was made in the skin on the medial aspect of the abdomen, and an opening was made in the abdominal muscle and peritoneal lining. The sterlized transmitter was carefully inserted into the abdominal cavity. The peritoneal lining and muscle were then sutured together using chronic catgut absorbable suture. The outer incision was then closed using surgical staples (Mik Ron Precision Inc.), and the area was then disinfected with iodine. The animal was removed from the anaesthesia and analgesic. A long-lasting (3 days) broad-spectrum antibiotic (Penlong XL [Pfizer Canada Inc.], 0.5ml/kg, intramuscularly) and long-lasting (12 hours) analgesic (Temgesic [Reckitt & Colman Ltd], 0.012 mg/kg, subcutaneously) were given. The surgical incision was examined daily for signs of irritation and/or infection. After the incision was healed the surgical staples were removed.

EXPERIMENTAL PROTOCOL:

Adult, male Sprague-Dawley rats (N=7; mass=565.6±27.4g) were studied. All animals were individually housed, fed a standard lab chow diet (Purina Laboratory Rodent Diet #5001) and provided with water at all times. This diet was supplemented on a daily basis with sunflower seeds

and apples. Animals were housed under a 12 hour light: 12 hour dark photoperiod with lights on at 8 am and off at 8 pm. All experiments were conducted in an environmentally controlled room at an ambient temperature of 25.7±0.5°C. In order to conduct experiments during the dark phase, three dim red lights were installed to provide the experimenter with working visibility. The dim red lighting was left on at all times, and all animals were exposed to these conditions, even when not being used in experiments.

All animals were introduced to the experimental chamber a minimum of three times for at least four hours each time before the actual experimental protocol was completed.

The primary objective of this experiment was to determine whether the ventilatory and metabolic responses to either a hypoxic or a hypercapnic stimulus was different at two different times of the day. Experiments were conducted at 10 am when the ambient lighting was illuminated and the animals were normally inactive (sleeping), and at 10 pm when the ambient lighting was off and the animals were normally awake and active.

Animals were placed into the experimental chamber at least 10 hours before a particular experiment commenced. During this period the animals had free access to both rat chow and water. This time allowed the animal the opportunity to familiarize itself with its relatively novel

environment. During this period the experimental apparatus was functioning in exactly the same manner as during the actual experimental protocols. There were two experimental protocols: (1) the hypoxic protocol; and (2) the hypercapnic protocol.

HYPOXIC PROTOCOL:

Seven male rats were tested at both 10 am and 10 pm (Appendix II explains why these times were chosen). The protocols at both times were identical. Experiments began after the 10 hour familiarization period. Resting ventilation, fractional expired carbon dioxide levels, deep-core body temperature, ambient temperature, barometric pressure, relative humidity, and flow through the experimental chamber were recorded for five minutes while the animals remained quiet and awake in the experimental chamber. If the animals were "alseep" (only during the 10 am recordings) they were awakened by the experimenter saying in a loud, clear manner "wake-up". If the animals became active, recording was stopped and the experimenter waited until the animals became quiet yet remained awake. When the animal remained in a resting condition for at least five minutes recording recommenced.

After a recording of resting ventilation and metabolic rate was obtained in air, the hypoxic stimulus (12% O_2 /balance N_2) was presented. In order to increase the rate at which the experimental chamber

equilibrated with 12% O_2, pure N_2 was first introduced into the system until the digital readout on the oxygen analyzer read 15% O_2, at this point, N_2 flow was stopped and the 12% O_2 flow was started. The system was allowed 25 minutes to come to equilibrium[2]. At the end of the 25 minute period, five minutes of recording was taken while the animal was resting quietly and awake. After this recording, room air was reintroduced into the system.

In order to determine if the animal could detect the change from one gas to another, sham changes were performed. The experimenter changed the multi-valve regulator from air to air to determine whether the animal was alerted to this change. This sham gas change was performed before or after the hypoxic stimulus was presented into the system.

The system was calibrated while the animal was present in the chamber. The animal ventilator was turned-on at 40 cycles/minute with each cycle having a volume of 20 ml. The ventilator was connected to the experimental chamber and pressure was recorded for 1 minute. Flow through the system remained constant (2595 ml/min) during this

---

[2] In order to determine the amount of time it took the experimental chamber to become fully equilibrated with a gas mixture a simple experiment was performed. Pure N_2 was flowed into the system at exactly the same flow rate as seen during the experiments. The time required for the digital signal on the O_2 analyzer to reach zero was considered the time it took for the system to reach equilibrium. It took the system 18-20 minutes to equilibrate with N_2. Therefore, a 25 minute period was considered an appropriate time for both the system and animal to reach equilibrium with the gas mixture.

procedure.

After the experiment and pressure calibration the animals were removed from the experimental chamber, weighed, and returned to their respective home cages.

The system was resealed and both chamber flow and pressure were determined. The oxygen and carbon dioxide analyzers were calibrated by allowing room air to equilibrate in the system for 25 minutes. At the end of this period a 15 second recording was taken. The gas mixture flowing into the system was then changed to a mixture containing 12.10% O_2 with a balance of N_2. This mixture was also allowed to equilibrate for >25 minutes, and then a 15 second recording was taken. The ambient thermometer was calibrated by placing the thermocouple tip into two beakers of water at two known temperatures for 15 seconds. The barometric pressure in the experimental chamber was determined by connecting a water-filled manometer to the chamber, and comparing the pressure difference relative to barometric pressure in the room.

HYPERCAPNIC PROTOCOL:

The seven rats used for the hypoxic protocol were also used for this protocol. The hypercapnic trials were carried out in precisely the same manner as that described in the *HYPOXIC PROTOCOL* except that the gaseous stimulus used was a 3.5% CO_2 (balance air) mixture. The gas used

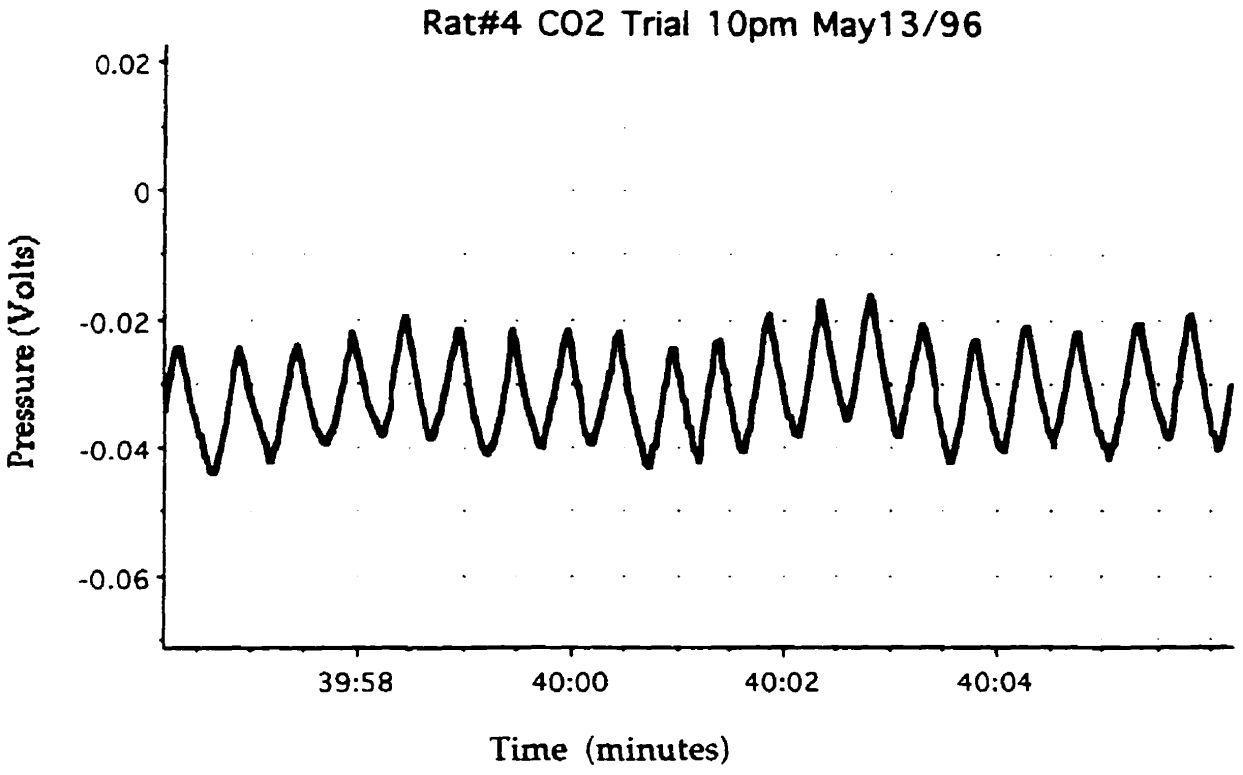
to calibrate the carbon dioxide analyzer was a 5% CO_2/20.75% O_2 mixture instead of the 12.10% O_2 mixture used in the hypoxic protocol.

DATA ANALYSIS:

Each experimental trial for each animal was assessed in the following manner. Five minutes of noise-free (animal completely immobile) recording was chosen for data analysis. Figure 2.3 is a representative example of the breathing traces used for data analysis. Twenty consecutive breaths were analyzed. Breathing frequency (f_R) was determined by dividing 20 breaths by the time it took for them to occur and multiplying by 60 seconds to give breaths per minute. Each breath was divided into three components: the inspiratory interval (Ti, s), the expiratory interval (Te, s), and the total breath duration (Ttot, s), determined by adding Ti and Te. Ti, Te, and Ttot were averaged for each 20 breath period. The magnitude (in volts) of the upward pressure deflection (Pm) for each of the 20 breaths was calculated and then averaged for the consecutive 20 breath period. Inspired volume (V_T, ml) was calculated using the following equation (Jacky (1980):

$$V_T = (Pm/Pcal) * Vcal * \{[Tb*(Pb-P_{CH2O})]/[Tb*(Pb-P_{CH2O}) - Ta*(Pb-P_{AH2O})]\}/$$
$$1 - (Ti/Ttot) * \{1-[(Tb*(Pb-P_{CH2O})/Tb*(Pb-P_{CH2O})-Ta*(Pb-$$
$$P_{AH2O}))]/[(Tb*(Pb-P_{NH2O})/Tb*(Pb-P_{NH2O})-Tn*(Pb-P_{AH2O}))]\},$$

FIGURE 2.3. A representative breathing trace from a rat breathing the hypercapnic gas mixture.


Rat#4 CO2 Trial 10pm May13/96
0.02
0
-0.02
-0.04
-0.06
Pressure (Volts)
39:58
40:00
40:02
40:04
Time (minutes)

where Pm is the observed pressure deflection due to a ventilatory inspiration, Pcal is the calibration pressure deflection, Vcal is the calibration volume (ml), Tb is deep-core body temperature (°K), and is assumed to equal alveolar temperature, Ta is chamber temperature (°K), Tn is the estimated nasal temperature (°K), Pb is barometric pressure in the chamber (mmHg), P_{AH2O} is the water vapour pressure (mmHg) at Tb, P_{CH2O} is the water vapour pressure (mmHg) at Ta, T_{NH2O} is the water vapour pressure (mmHg) at Tn, Ti is inspiratory interval (s), and Ttot is total breath duration (s). Minute ventilation ($\dot{V}_I$, ml/min) was calculated by multiplying f_R by V_T. $\dot{V}_{CO2}$ was calculated as described above (see *MEASUREMENT OF GASEOUS METABOLISM*). For the calculation of $\dot{V}_{CO2}$, RE was assumed to be 0.85.

STATISTICAL ANALYSIS:

Data for each experimental trial were averaged for all animals, and were expressed as means ± standard error. Significant differences between averaged data of the sequential measurements performed on the same animals were assessed by paired two-tailed t-test comparisons with Bonferroni limitation where appropriate. In all cases, the overall level of significance was considered at $P < 0.05$.

CHAPTER THREE

RESULTS

NORMOXIC GROUP:

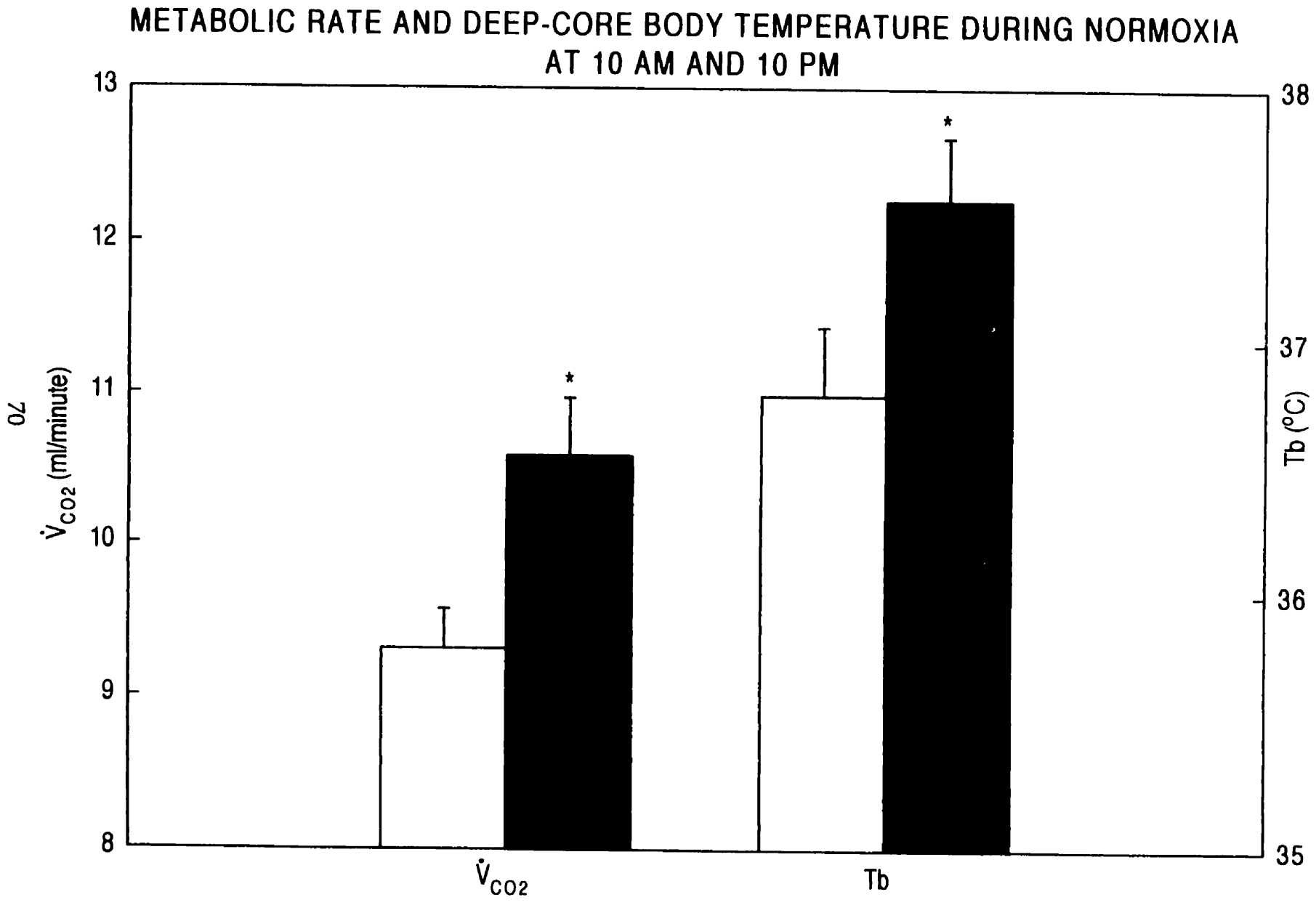
Judging from their general behaviour, rats appearred to be unaware of the experimenter's presence during this protocol period. All animals followed their usual circadian activity rhythm, and behaved as if they were in their respective home cages. During the 10 am recording rats slept, and therefore needed to be aroused to the apparent waking state. This was not the case during the 10 pm recording time when rats were always awake.

Normoxic ventilation, metabolism, and body temperature were obtained before the hypoxic and hypercapnic stimuli, thus two independent normoxic records were obtained for each rat (N=7) at both 10 am and 10 pm, and therefore all normoxic values are expressed as means.

Figure 3.1 graphically summarizes the day-night differences in carbon dioxide production ($\dot{V}_{CO2}$), and deep-core body temperature (Tb) in the normoxic rats (N=7). During the 10 am (day) recording $\dot{V}_{CO2}$ was 9.3±0.3 ml/min. $\dot{V}_{CO2}$ during the 10 pm recording was significantly elevated to 10.6±0.4 ml/min. Deep-core body temperature was 36.8±0.3 °C at 10 am and was significantly elevated to 37.6±0.3 °C at 10 pm.

The day-night differences in resting, normoxic ventilation are

FIGURE 3.1. Mean (± 1 SE) values of metabolic rate (carbon dioxide production, $\dot{V}_{CO2}$) and deep-core body temperature (Tb) in normoxia. Measurements were taken from 7 awake adult male rats at 10 am (open bars) and at 10 pm (filled bars). • Indicates a significant difference from the corresponding 10 am value.


METABOLIC RATE AND DEEP-CORE BODY TEMPERATURE DURING NORMOXIA
AT 10 AM AND 10 PM
13
12
11
10
9
8
$\dot{V}_{CO2}$ (ml/minute)
38
37
36
35
Tb (°C)
*
*
$\dot{V}_{CO2}$
Tb

summarized in Figure 3.2. Respiratory frequency (f_R) and tidal volume (V_T) were 92±5 breaths/min, and 4.3±0.2 ml, respectively during the 10 am recording time. There was no significant change in V_T during the 10 pm recording; however, there was a significant increase in f_R to 104±3 breaths/minute at 10 pm . Minute ventilation ($\dot{V}_I$) was higher during the night (10 pm) compared with that observed during the day (10 am), but the difference was not statistically significant.

During the 10 am recording the $\dot{V}_I/\dot{V}_{CO2}$ ratio was 42.5±1.1, and at 10 pm was 41.7±2.4. These day-night values are not statistically different from one another.

Breathing pattern is expressed by three fundamental variables: Ti, inspiratory interval (s); Te, expiratory interval (s); and Ttot, total breath duration (Ttot = Ti + Te) (s). The inspiratory interval relative to the total breath duration (Ti/Ttot) expresses the proportion of the total respiratory cycle devoted to inspiration. The tidal volume to inspiratory interval ratio (V_T/Ti, ml/s) represents mean inspiratory airflow rate per unit of time (inspiratory interval). This ratio may be interpretated as an approximate measure of the central drive to breathe (Lydic and Baghdoyan, 1989; Milsom 1988).

The day-night variability in breathing pattern and V_T/Ti are graphically illustrated in Figure 3.3. There was no significant difference in either Ti or Te between 10 am and 10 pm (Table 3.1). Therefore, there was

FIGURE 3.2. Mean (± 1 SE) values of tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_I$), and ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$) in normoxia. Measurements were taken from awake adult male rats at 10 am (open bars) and at 10 pm (filled bars). * Indicates a significant difference from the corresponding 10 am value.

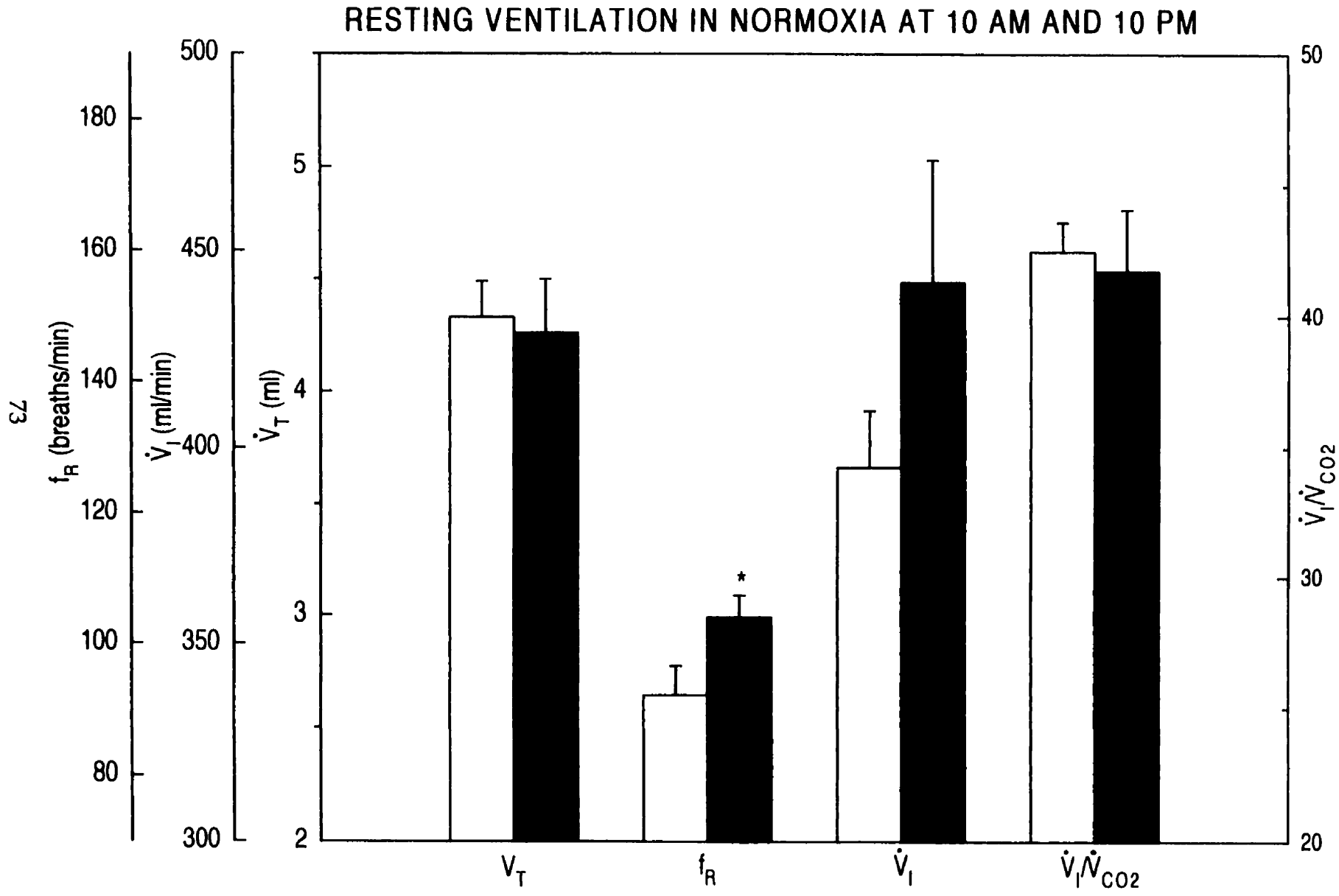

RESTING VENTILATION IN NORMOXIA AT 10 AM AND 10 PM
f_R (breaths/min)
$\dot{V}_I$ (ml/min)
$\dot{V}_T$ (ml)
$\dot{V}_I/\dot{V}_{CO2}$
180
160
140
120
100
80
500
450
400
350
300
5
4
3
2
50
40
30
20
*
V_T
f_R
$\dot{V}_I$
$\dot{V}_I/\dot{V}_{CO2}$

FIGURE 3.3. Mean (± 1 SE) values of inspiratory interval (Ti), expiratory interval (Te), total breath duration (Ttot), the inspiratory interval to total breath duration ratio (Ti/Ttot), and mean inspiratory airflow (V_T/Ti) in normoxia. Measurements were taken from 7 awake adult male rats at 10 am (open bars) and at 10 pm (filled bars). * Indicates a significant difference from the corresponding 10 am value.

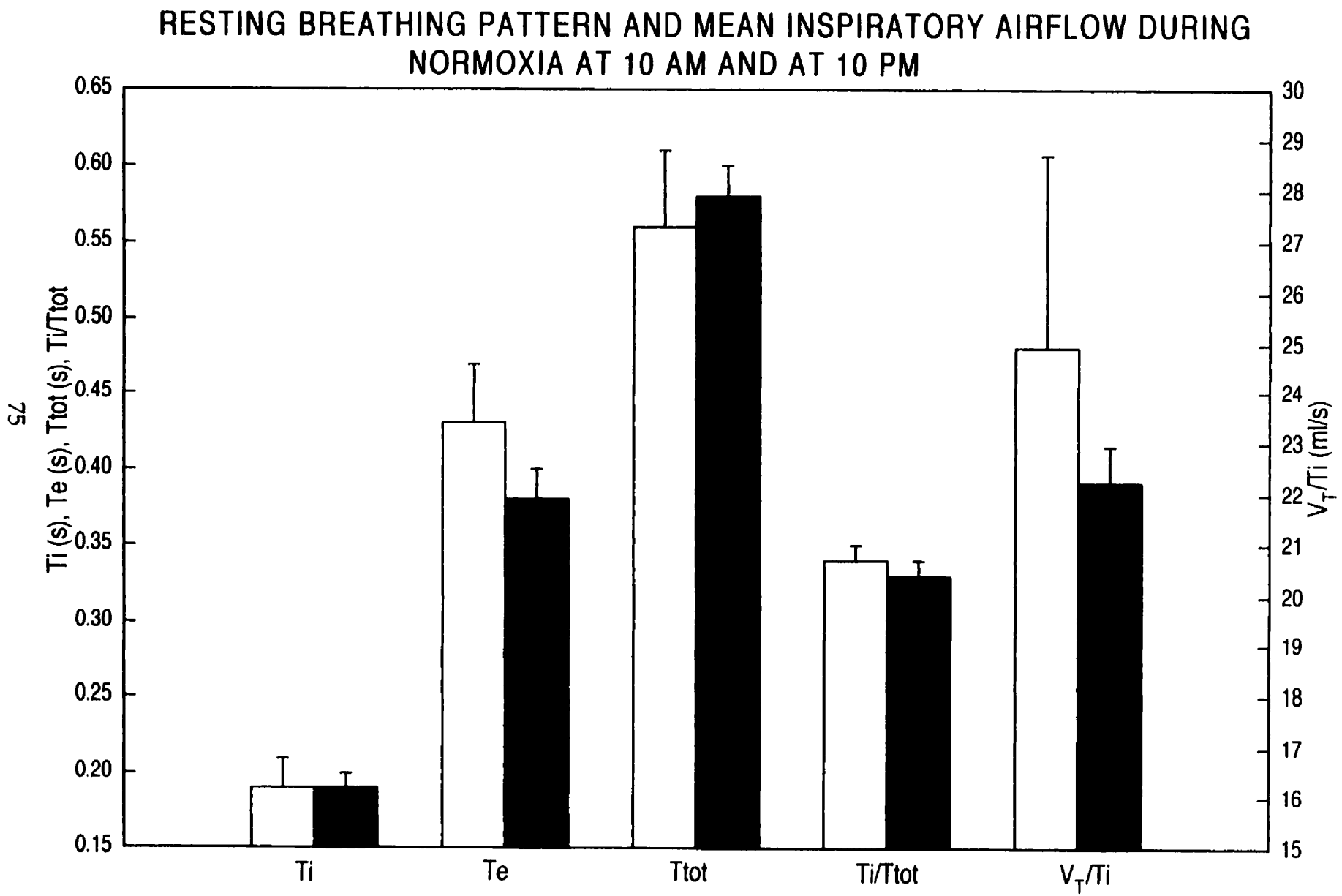

RESTING BREATHING PATTERN AND MEAN INSPIRATORY AIRFLOW DURING NORMOXIA AT 10 AM AND AT 10 PM
Ti (s), Te (s), Ttot (s), Ti/Ttot
V_T/Ti (ml/s)
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
Ti
Te
Ttot
Ti/Ttot
V_T/Ti

TABLE 3.1. Mean (± 1 SE) values of metabolic rate (carbon dioxide production, $\dot{V}_{CO2}$), deep-core body temperature (Tb), tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_I$), and ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$) in normoxia and in hypoxia. Measurements were taken from 7 awake adult male rats at 10 am and at 10 pm. * Indicates a significant difference from the corresponding resting normoxic value. ◊ Indicates a significant difference from the corresponding 10 am value.

MEAN VALUES OF METABOLIC AND VENTILATORY VARIABLES BEFORE AND DURING EXPOSURE TO HYPOXIA AT 10 AM AND 10 PM

	HYPOXIC RESPONSES					
	AM			PM		
	Air (a)	12% O_2 (b)	(b)-(a)	Air (c)	12% O_2 (d)	(d)-(c)
$\dot{V}_{CO2}$ (ml/min)	9.1±0.3	7.6±0.4 *	-1.5±0.4	10.7±0.4 ◊	8.3±0.4 *	-2.3±0.5
Tb (°C)	37.0±0.2	36.7±0.3 *	-0.3±0.2	37.8±0.3 ◊	37.1±0.3 *	-0.7±0.2 ◊
V_T (ml)	4.2±0.2	4.0±0.2	-0.2±0.1	4.1±0.3	3.7±0.2	-0.4±0.3
f_R (breaths/min)	94±6	140±9 *	46±8	105±6 ◊	146±9 *	41±7
$\dot{V}_I$ (ml/min)	386.6±19.7	551.5±27.5 *	164.9±28.5	429.4±40.4	528.7±36.2 *	99.3±29.6
$\dot{V}_I/\dot{V}_{CO2}$	42.7±2.2	73.2±4.5 *	30.5±5.4	39.9±2.7	63.2±2.7 *	23.3±1.7

no subsequent difference in Ttot or Ti/Ttot between 10 am and 10 pm. At 10 am V_T/Ti was 18.69±0.60 ml/s and at 10 pm was 21.49±1.22 ml/s (Table 3.1). There is no significant difference between these values.

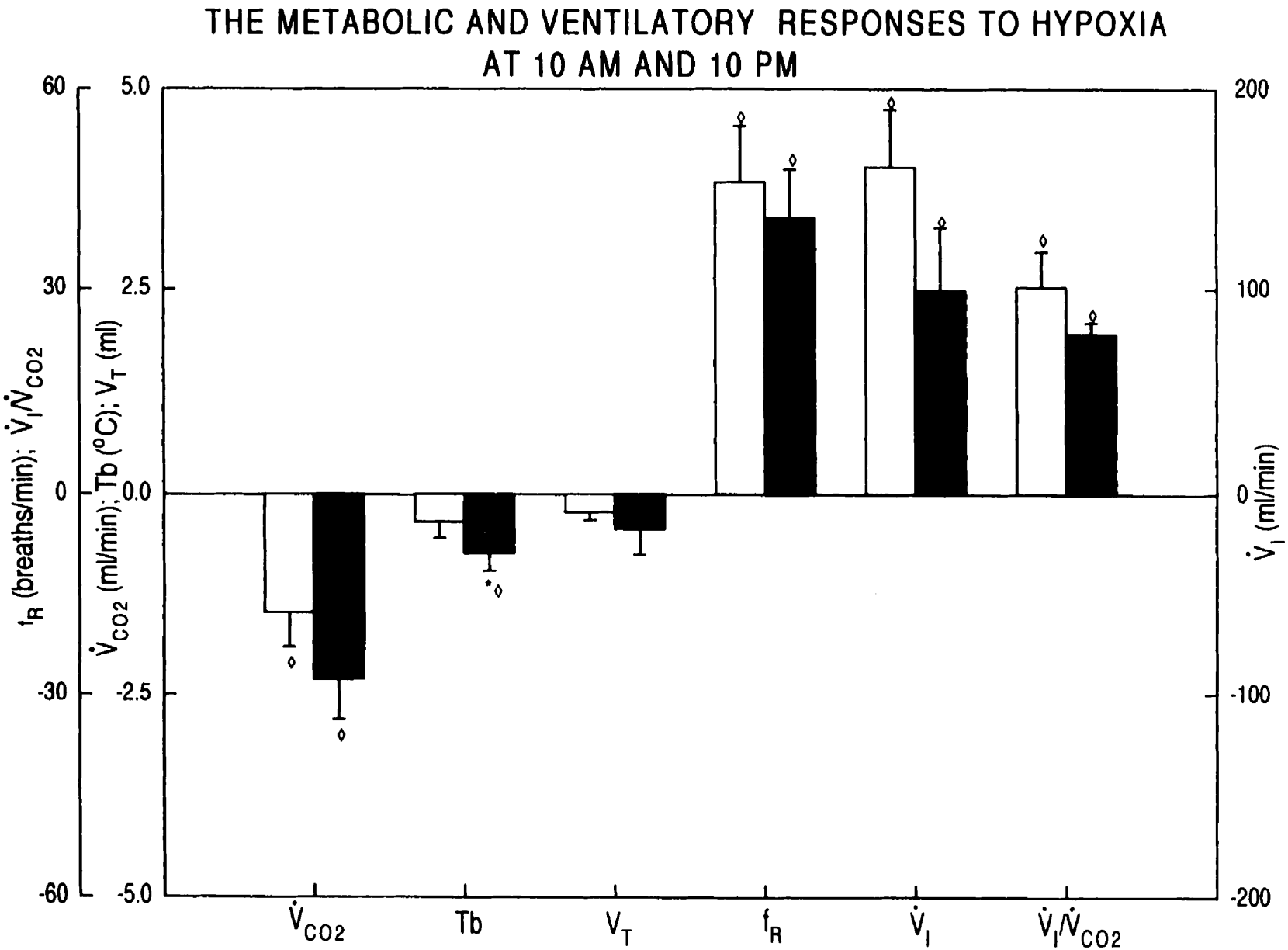
HYPOXIC GROUP:

Behavioural observations indicated that in most cases the rats (N=7) were alerted to the hypoxic stimulus. Within 30 seconds of the switch from air to hypoxia most rats were observed smelling the inlet orifice or they became more active. Often they were observed turning around in the animal chamber or they would commence with grooming themselves. Arousal to the stimulus was only transient. After several minutes animals were observed returning to their previously quiet state, and during the 10 am recording would often attempt to return to the sleeping state. Rats which fell into the sleeping state were vocally aroused after the 25 minute recording (10 am), so that they returned to the apparent awake state.

The hypoxic stimulus resulted in a significant decrease in $\dot{V}_{CO2}$ (Figure 3.4). There was no statistical difference in the observed hypometabolic response at 10 am compared with that at 10 pm (Table 3.1). $\dot{V}_{CO2}$ was reduced to statistically similar absolute values both at 10 am and 10 pm, despite starting out at significantly different values.

At 10 pm Tb decreased significantly in response to the hypoxic stimulus, but there was no change in Tb at 10 am. Unlike the metabolic

FIGURE 3.4. Mean (± 1 SE) values of metabolic rate (carbon dioxide production, $\dot{V}_{CO2}$), deep-core body temperature (Tb), tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_I$), and ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$) in response to hypoxia. Data are expressed as the absolute change from the corresponding normoxic value. Measurements were taken from 7 awake adult male rats at 10 am (open bars) and at 10 pm (filled bars). * Indicates a significant difference froms the corresponding 10 am value. ◊ Indicates a significant difference from the resting value.


THE METABOLIC AND VENTILATORY RESPONSES TO HYPOXIA
AT 10 AM AND 10 PM
60
30
0
-30
-60
5.0
2.5
0.0
-2.5
-5.0
200
100
0
-100
-200
f_R (breaths/min); $\dot{V}_I/\dot{V}_{CO2}$
$\dot{V}_{CO2}$ (ml/min); Tb (°C); V_T (ml)
$\dot{V}_I$ (ml/min)
$\dot{V}_{CO2}$
Tb
V_T
f_R
$\dot{V}_I$
$\dot{V}_I/\dot{V}_{CO2}$

response to hypoxia, there was a significant difference between the decrease in Tb at 10 am compared with that at 10 pm (Figure 3.4 and Table 3.1).

Figure 3.4 graphically summarizes the day-night differences in the ventilatory response to the hypoxic stimulus. $\dot{V}_I$ increased significantly in response to hypoxia (Table 3.1). There was no statistical difference between the 10 am and 10 pm hypoxic ventilatory responses. The increase in $\dot{V}_I$ was the result of a significant increase in f_R with no significant change in V_T. There was no difference in the increase in f_R in response to the hypoxic stimulus at 10 am compared with that at 10 pm.

There was a significant increase in the $\dot{V}_I/\dot{V}_{CO2}$ ratio in response to the hypoxic stimulus compared with that observed during air breathing (Table 3.1). There was no significant difference between the 10 am increase in $\dot{V}_I/\dot{V}_{CO2}$ compared with the increase at 10 pm (Figure 3.4).

Figure 3.5 presents the results for changes in breathing pattern and mean inspiratory airflow in response to hypoxia. There was a significant reduction in Ttot in response to hypoxia both at 10 am and 10 pm (Table 3.2). There was no significant difference between Ttot at 10 am compared with 10 pm. At 10 am Ttot was reduced by significant decreases in both Ti and Te. However, at 10 pm Ttot was shortened by significant reductions in Te only (Table 3.2).

The Ti/Ttot ratio was significantly increased in response to hypoxia at both 10 am and 10 pm; however, there was no difference between the

FIGURE 3.5. Mean (± 1 SE) values of inspiratory interval (Ti), expiratory interval (Te), total breath duration (Ttot), inspiratory interval to total breath duration ratio (Ti/Ttot), and mean inspiratory airflow (V_T/Ti) in response to hypoxia. Data are expressed as the absolute change from the corresponding normoxic value. Measurements were taken at 10 am (open bars) and at 10 pm (filled bars) from 7 awake adult male rats. * Indicates a significant difference from the corresponding 10 am value. ◊ Indicates a significant difference from the resting normoxic value.

CHANGES IN BREATHING PATTERN AND MEAN INSPIRATORY AIRFLOW IN RESPONSE TO HYPOXIA AT 10 AM AND 10 PM

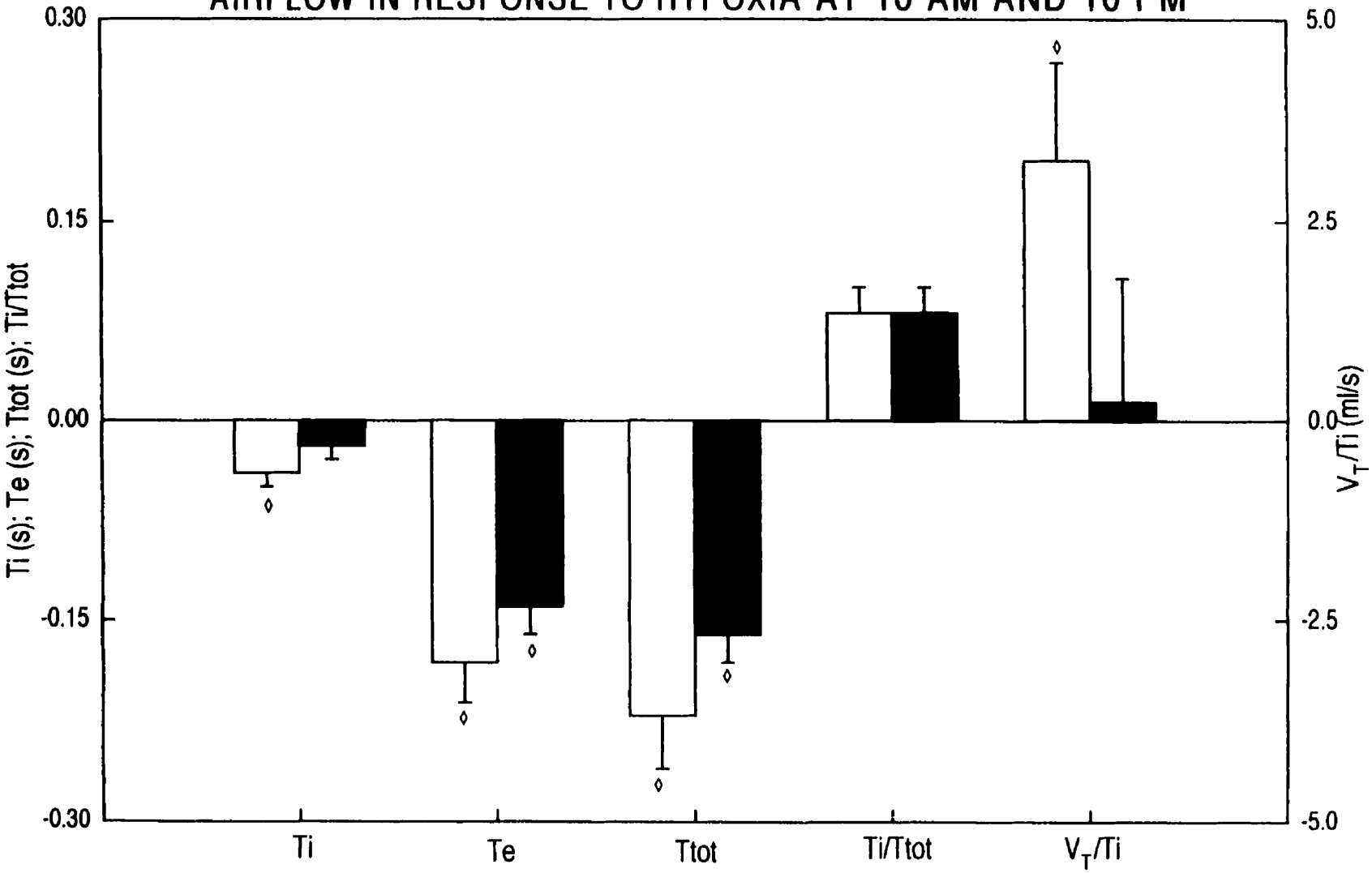


TABLE 3.2. Mean (± 1 SE) values of inspiratory interval (Ti), expiratory interval (Te), total breath duration (Ttot), inspiratory interval to total breath duration ratio (Ti/Ttot), and mean inspiratory airflow (V_T/Ti) in normoxia and hypoxia. * Indicates a significant difference from the corresponding resting normoxic value. ◊ Indicates a significant difference from the corresponding 10 am value.

MEAN VALUES OF BREATHING PATTERN AND MEAN INSPIRATORY AIRFLOW BEFORE AND DURING EXPOSURE TO HYPOXIA AT 10 AM AND 10 PM

	HYPOXIC RESPONSES					
	AM			PM		
	Air (a)	12% O_2 (b)	(b)-(a)	Air (c)	12% O_2 (d)	(d)-(c)
Ti (s)	0.23±0.02	0.19±0.01 *	-0.04±0.01	0.19±0.01	0.17±0.01	-0.02±0.01
Te (s)	0.43±0.03	0.25±0.02 *	-0.18±0.03	0.38±0.02	0.24±0.01 *	-0.14±0.02
Ttot (s)	0.66±0.04	0.43±0.03 *	-0.22±0.04	0.57±0.03	0.42±0.03 *	-0.16±0.02
Ti/Ttot	0.35±0.02	0.43±0.02 *	0.08±0.02	0.33±0.01	0.42±0.02 *	0.08±0.02
V_T/Ti (ml/s)	18.69±0.60	21.95±1.26 *	3.26±1.22	21.49±1.22	21.73±1.77	0.24±1.54

night and day values. The V_T/Ti ratio was significantly increased by hypoxia at 10 am, but not at 10 pm (Table 3.2).

HYPERCAPNIC GROUP:

Behavioural observations indicated that all rats (N=7) were alerted by the hypercapnic stimulus. Unlike the behaviour seen during the hypoxic stimulus, rats were not initially alerted to the switch from air to the hypercapnic gas mixture. Gradually the rats became less active and by the end of the 25 minute stimulus they were immobile. They appeared frightened and "froze" in a crouched and stiff position. During the 10 am recording rats were awake after the stimulus had been introduced, in all cases, contrary to the behaviour observed during the hypoxic stimulus.

Figure 3.6 graphically summarizes the day-night differences in the gaseous metabolic and body temperature responses to the hypercapnic stimulus. $\dot{V}_{CO2}$ decreased in response to hypercapnia. At 10 am $\dot{V}_{CO2}$ was significantly decreased in response to hypercapnia, but at 10 pm it was not (Table 3.3). However, there was no statistical significance between the 10 am and 10 pm changes in metabolic rate.

Unlike Tb observed in the hypoxic trials, Tb did not follow the changes in gaseous metabolism. There was no significant change in Tb at either 10 am or 10 pm in response to the hypercapnic stimulus (Figure 3.6).

The day-night differences in the ventilatory response to hypercapnia

FIGURE 3.6. Mean (± 1 SE) values of metabolic rate (carbon dioxide production, $\dot{V}_{CO2}$), deep-core body temperature (Tb), tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_I$), and ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$) in response to hypercapnia. Data are expressed as the absolute change from the corresponding normoxic values. Measurements were taken from 7 awake adult male rats at 10 am (open bars) and 10 pm (filled bars). * Indicates a significant difference from the corresponding 10 am value. ◊ Indicates a significant difference from the resting normoxic value.

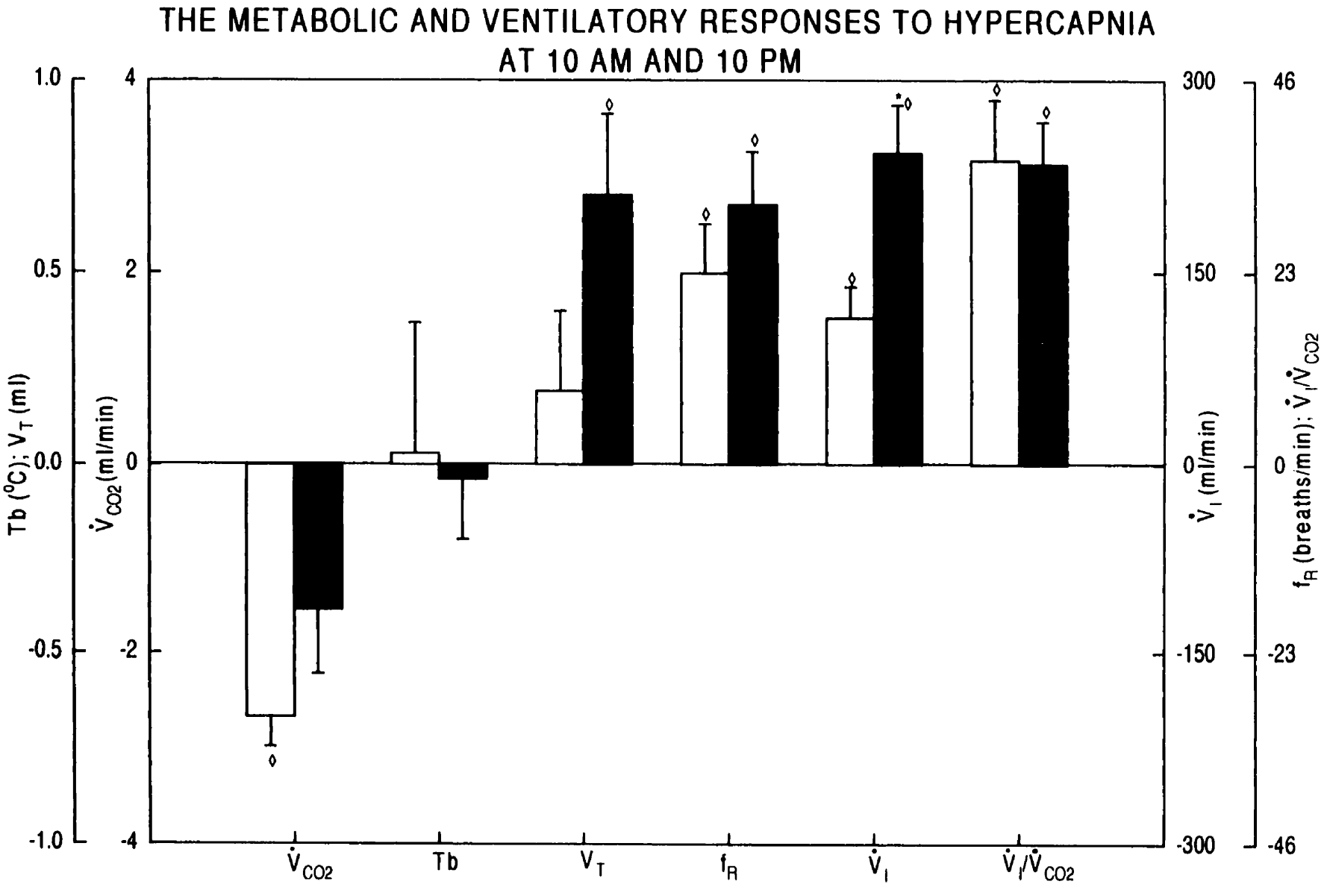

THE METABOLIC AND VENTILATORY RESPONSES TO HYPERCAPNIA
AT 10 AM AND 10 PM
Tb (°C); V_T (ml)
V̇_CO2 (ml/min)
V̇_I (ml/min)
f_R (breaths/min); V̇_I/V̇_CO2
1.0
0.5
0.0
-0.5
-1.0
4
2
0
-2
-4
300
150
0
-150
-300
46
23
0
-23
-46
V̇_CO2
Tb
V_T
f_R
V̇_I
V̇_I/V̇_CO2

TABLE 3.3. Mean (± 1 SE) values of metabolic rate (carbon dioxide production, $\dot{V}_{CO2}$), deep-core body temperature (Tb), tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_I$), and ventilation to carbon dioxide production ratio ($\dot{V}_I/\dot{V}_{CO2}$) in normoxia and in hypercapnia. Measurements were taken from 7 awake adult male rats at 10 am and at 10 pm. * Indicates a significant difference from the corresponding resting normoxic value. ◊ Indicates a significant difference from the corresponding 10 am value.

MEAN VALUES OF METABOLIC AND VENTILATORY VARIABLES BEFORE AND DURING EXPOSURE TO HYPERCAPNIA AT 10 AM AND 10 PM

	HYPERCAPNIC RESPONSES					
	AM			PM		
	Air (a)	3.5% CO_2 (b)	(b)-(a)	Air (c)	3.5% CO_2 (d)	(d)-(c)
$\dot{V}_{CO2}$ (ml/min)	9.3±0.3	6.6±0.3 *	-2.7±0.3	10.8±0.5 ◊	9.3±0.9	-1.6±0.7
Tb (°C)	36.6±0.3	36.7±0.3	0.1±0.3	37.3±0.3 ◊	37.3±0.3	-0.04±0.1
V_T (ml)	4.3±0.2	4.5±0.2 *	0.2±0.2	4.5±0.3	5.3±0.4 *	0.8±0.3
f_R (breaths/min)	93±4	116±8 *	23±6	102±3	133±6 *	31±6
$\dot{V}_I$ (ml/min)	398.8±13.7	513.5±19.5 *	114.7±24.1	453.9±26.8	696.3±52.6 *	242.4±37.9 ◊
$\dot{V}_I/\dot{V}_{CO2}$	42.8±0.90	79.1±7.5 *	36.3±7.3	42.2±2.1	78.0±6.8 *	35.8±5.1

are presented in Figure 3.6. $\dot{V}_I$ was significantly increased in response to hypercapnia at both 10 am and 10 pm (Table 3.3). The 10 pm increase in $\dot{V}_I$ was significantly different from the 10 am response.

The increase in ventilation was the result of statistically significant increases in both f_R and V_T. There was no difference between the 10 am and 10 pm values for either f_R or V_T.

The $\dot{V}_I/\dot{V}_{CO2}$ ratio increased significantly in response to the hypercapnic stimulus (Table 3.3). Although $\dot{V}_I$ at 10 pm was significantly increased compared with that at 10 am, there is no significant difference in the $\dot{V}_I/\dot{V}_{CO2}$ ratio at 10 am and 10 pm (Figure 3.6).

Figure 3.7 presents the results of the day-night differences in breathing pattern and mean inspiratory airflow. Ti was not significantly altered by the hypercapnic stimulus. It remained unchanged during hypercapnic breathing compared with air breathing both at 10 am and 10 pm. At both 10 am and 10 pm Te was significantly decreased in response to the hypercapnic stimulus. However, there was no significant difference between the 10 am and 10 pm decreases in Te in response to hypercapnia (Table 3.4). Therefore, the significant decrease in Ttot in response to the hypercapnic stimulus (both at 10 am and 10 pm) was brought about by the shortening of Te and not by the shortening of Ti. The 10 am decrease in Ttot in response to the hypercapnic stimulus was not signifcantly different from the 10 pm decrease in Ttot in response to the gaseous stimulus. The

FIGURE 3.7. Mean (± 1 SE) values of inspiratory interval (Ti), expiratory interval (Te), total breath duration (Ttot), inspiratory interval to total duration ratio (Ti/Ttot), and mean inspiratory ariflow (V_T/Ti) in response to hypercapnia. Data are expressed as the absolute change from the corresponding normoxic values. Measurements were taken from 7 awake adults rats at 10 am (open bars) and 10 pm (filled bars). * Indicates a significant difference from the corresponding 10 am value. ◊ Indicates a significant difference from the resting normoxic value.

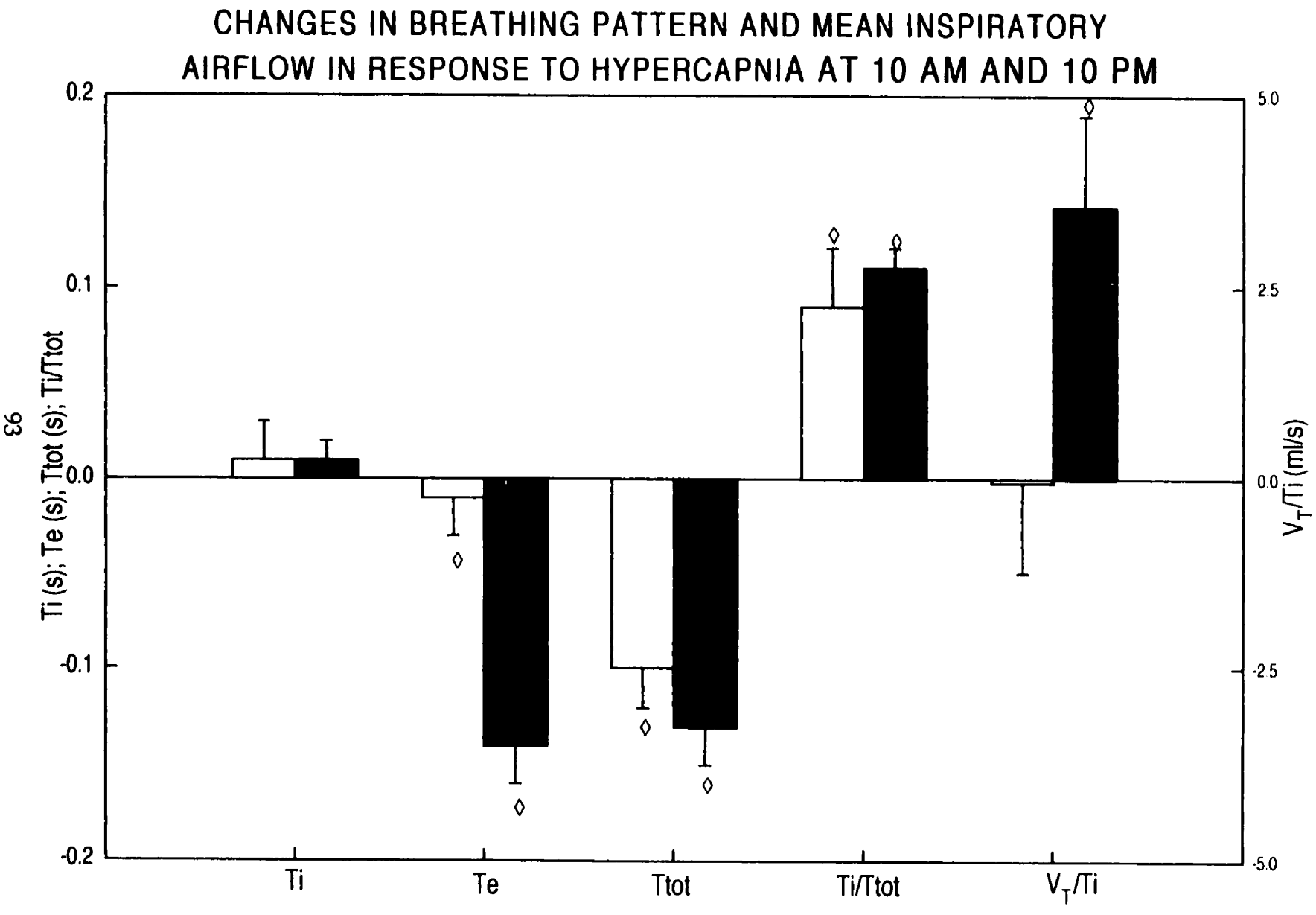

CHANGES IN BREATHING PATTERN AND MEAN INSPIRATORY AIRFLOW IN RESPONSE TO HYPERCAPNIA AT 10 AM AND 10 PM
Ti (s); Te (s); Ttot (s); Ti/Ttot
V_T/Ti (ml/s)
0.2
0.1
0.0
-0.1
-0.2
5.0
2.5
0.0
-2.5
-5.0
Ti
Te
Ttot
Ti/Ttot
V_T/Ti

TABLE 3.4. Mean (± 1 SE) values of inspiratory interval (Ti), expiratory interval (Te), total breath duration (Ttot), inspiratory interval to total breath duration ratio (Ti/Ttot), and mean inspiratory airflow (V_T/Ti) in normoxia and hypoxia. * Indicates a significant difference from the corresponding resting normoxic value. ◊ Indicates a significant difference from the corresponding 10 am value.

MEAN VALUES OF BREATHING PATTERN VARIABLES AND MEAN INSPIRATORY AIRFLOW BEFORE AND DURING EXPOSURE TO HYPERCAPNIA AT 10 AM AND 10 PM

	HYPERCAPNIC RESPONSES					
	AM			PM		
	Air (a)	3.5% CO_2 (b)	(b)-(a)	Air (c)	3.5% CO_2 (d)	(d)-(c)
Ti (s)	0.22±0.02	0.23±0.02	0.01±0.02	0.19±0.01	0.20±0.01	0.01±0.01
Te (s)	0.42±0.03	0.31±0.03 *	-0.11±0.02	0.39±0.02	0.26±0.01 *	-0.14±0.02
Ttot (s)	0.63±0.03	0.53±0.04 *	-0.10±0.02	0.59±0.02	0.46±0.02 *	-0.13±0.02
Ti/Ttot	0.34±0.02	0.43±0.02 *	0.09±0.03	0.33±0.02	0.44±0.01 *	0.11±0.01
V_T/Ti (ml/s)	20.34±0.79	20.29±0.81	-0.05±1.19	23.05±0.87 ◊	26.57±1.87 *	3.52±1.22

Ti/Ttot ratio was significantly increased at both 10 am and 10 pm in response to the gaseous stimulus (Table 3.4). This increase was the result of shortening of Ttot which was due to the reduction of Te, there being no change in Ti. There was no difference in the Ti/Ttot ratio at 10 am compared with that at 10 pm.

Mean inspiratory airflow was significantly elevated by the hypercapnic stimulus at 10 pm but not at 10 am. The V_T/Ti ratio was significantly greater during the hypercapnic stimulus at 10 pm than it was at 10 am.

CHAPTER FOUR

DISCUSSION

BEHAVIOURAL RESPONSES TO HYPOXIA AND HYPERCAPNIA:

Hypoxia: Most rats were transiently alerted by the hypoxic stimulus, and then returned to their previous state. At 10 am animals returned to their sleeping state, and at 10 pm quiet rest.

When the gas flow into the chamber was switched from air to air (control test), the rats did not arouse, so it is unlikely that the experimenter's presence or the sound of altering gas flow through the experimental chamber acted as alerting stimuli. Although the rats may have detected the hypoxic gas mixture, the experimenter could not detect an odour. I conclude that hypoxia itself was the alerting stimulus.

Others have noted that hypoxia acts as a behavioural stimulant. In rats, 10% O_2 causes arousal from slow-wave sleep, and abolishes rapid-eye-movement sleep (Pappenheimer 1977). The peripheral chemoreceptor reflex is essential for these effects (Ryan and Megirian 1982). In both dogs and humans, hypoxia causes arousal from slow-wave and rapid-eye-movement sleep, but only at very low arterial oxygen saturation levels (10-20%), and the peripheral chemorecepetor reflex is again essential in mediating the arousal response (Bowes and Phillipson 1984; Bowes *et al.* 1981). In cats, rabbits, seals and primates stimulation of the peripheral

chemoreceptors by hypoxia and other noxious stimuli cause a general visceral alerting response which may be characterized by hyperventilation, tachycardia, renal and mesenteric vasoconstriction, and vasodilatation in skeletal muscle (Marshall 1986). In anaesthetized cats and dogs, Hugelin *et al.* (1959) demonstrated that the electroencephalogram (EEG) is modified by breathing hypoxia. These findings all indicate that hypoxia is an alerting stimulant.

Hypercapnia: In contrast to the arousal response to hypoxia, the behavioural response to hypercapnia was not immediate. Initially, the rats did not arouse to the stimulus, confirming the results of the air control tests. However, the rats became progressively less active and eventually "froze" in a crouched position. They did not return to either quiet sleep (10 am) or quiet rest (10 pm) until the stimulus had been removed. I conclude that in the awake rat hypercapnia acts as a behavioural stimulant.

Others have also reached this conclusion. In awake humans, CO_2 is known to produce anxiety and can initiate panic in certain individuals (Gorman *et al.* 1989; Papp *et al.* 1993). In sleeping humans, 4-6% CO_2 causes consistent arousal from slow-wave and rapid-eye-movement sleep (Bowes and Phillipson 1984). In rats, 5% CO_2 does not result in arousal from either slow-wave or rapid-eye-movement sleep (Pappenheimer 1977), however, I found 3.5% CO_2 alerts rats during wakefulness during

the morning and evening. From these observations, I conclude that the behavioural response to hypercapnia is not time-of-day dependent, but may be arousal state dependent.

METABOLISM AND VENTILATION DURING NORMOXIA:

Metabolism: Metabolism ($\dot{V}_{CO2}$) and body temperature were different between night and day. These differences represent circadian oscillations which have been previously observed in a number of rodent species (Stupfel *et al.* 1989; Aschoff and Pohl 1970; Rusak and Zucker 1979; Fioretti *et al.* 1974; Honma and Hiroshige 1978).

Ventilation: Minute ventilation was not significantly different between night and day, however there was a 11% increase in ventilation at 10 pm relative to 10 am. The lack of significance in ventilation between the night and day could be due to the large degree of variability in the data, and therefore, the lack of significance could be due to a type II statistical error.

There is a linear relationship between ventilation and metabolism (Wasserman *et al.* 1977). In the steady-state, ventilation is regulated by the chemoreflexes so that the rate of pulmonary excretion of CO_2 matches CO_2 production (Cunningham *et al.* 1986; Phillipson *et. al* 1981; Levine 1977; Mortola and Matsuoka 1993; Wasserman *et al.* 1986;). Therefore, I predicted that ventilation would change in proportion to the circadian

changes in metabolism. When metabolism is elevated then ventilation should be elevated, and conversly, if metabolism is lowered then ventilation should be lowered.

The ventilation to $\dot{V}_{CO2}$ ratio ($\dot{V}_I/\dot{V}_{CO2}$) is a useful indicator of the relationship between metabolism and ventilation. Due to the linear relationship between ventilation and metabolism the $\dot{V}_I/\dot{V}_{CO2}$ ratio should not change as metabolism changes because ventilation varies in direct proportion to it. I found there was no significant difference between the 10 am and 10 pm $\dot{V}_I/\dot{V}_{CO2}$ ratios, therefore supporting the hypothesis.

Saiki and Mortola (1995) found that ventilation and metabolism ($\dot{V}_{O2}$) were significantly higher during the night (7:30 pm) than during the morning (7:30 am) in neonatal rats. They found no significant difference in $\dot{V}_I/\dot{V}_{O2}$ ratios between night and day, and concluded that ventilation changed in proportion to metabolic rate. My results indicate that these phenomena also occur in the adult rat, and therefore do not change with age.

Breathing Pattern: Breathing pattern was different between night and day. Although there was no night-day difference in tidal volume; respiratory frequency was significantly higher at 10 pm than at 10 am. Mean inspiratory airflow (V_T/Ti) may be interpreted as an approximate measure of the central drive to breathe (Lydic and Baghdoyan 1989; Milsom 1988). Because there was no day-night difference in mean

inspiratory airflow rates, I concluded that the central drive to breathe is unchanged between night and day.

METABOLISM AND VENTILATION DURING HYPOXIA:

Metabolism: The reduction of metabolic rate in response to hypoxia has been termed hypometabolism, and is a well documented phenomenon across species of all sizes and ages. Hypoxic hypometabolism is more pronounced in neonatal and juvenile animals than in adults (Mortola and Matsuoka 1993; Saiki *et al.* 1994). The mechanism which mediates the reduction in metabolism during hypoxia is currently not known. The peripheral chemoreceptors do not appear to mediate the metabolic response to hypoxia. In both the cat and rat, Gautier and colleagues (1987, 1993) found that carotid body denervation did not abolish the hypometabolic response to hypoxia, and they concluded that some mechanism other than the carotid body chemoreflex must mediate the response.

In my experiments, metabolism was significantly reduced in response to the hypoxic stimulus both day and night, but the hypometabolic response to hypoxia was the same at both times of the day. During normoxia, metabolism was significantly different between day and night, however during hypoxia, metabolism was the same both day and night. Therefore, I conclude that hypoxia abolished the day-night

difference in metabolism by reducing it to the same absolute levels at 10 am and 10 pm. Saiki and Mortola (1995) found that hypoxia (10% O_2) abolished the day-night difference in metabolism ($\dot{V}_{O2}$) in neonatal rats, and my results support this observation.

Body temperature was not significantly decreased by the hypoxic stimulus during the day, but it was significantly decreased by the hypoxic stimulus at night. This observation confirms the findings of Kwarecki *et al.* (1977). They observed a circadian oscillation in the hypoxic (8% O_2) hypothermic response in adult rats, and found the decrease in body temperature to be significantly larger during the night than during the day. Similarly, Saiki and Mortola (1995) found that, in neonatal rat pups, body temperature decreased less in response to 10% O_2 during the day than during the night.

These observations suggest that the hypoxic hypothermic response follows a circadian rhythm, and the hypoxic hypothermic response is independent of the hypoxic hypometabolic response. As with the hypometabolic response to hypoxia, the mechanism which mediates the hypothermic response to hypoxia does not appear to be mediated via the carotid bodies in either the cat or rat (Gautier and Bonora 1993; Gautier *et al.* 1987).

Ventilation: The ventilatory response to hypoxia was relatively greater at 10 am (43% increase relative to normoxia) than at 10 pm (23%

increase relative to normoxia), but the difference was not significant. In awake humans, it has been observed that there is a circadian rhythm in the ventilatory response to hypoxia which appears independent of arousal state. Raschke and Möller (1989) found that, in awake humans, the ventilatory response to hypoxia during a 24 hour day was highest at 5 pm and lowest at 5 am. These observations in conjunction with mine, suggest that in both the rat and human, there is a time-of-day influence on the hypoxic ventilatory response. The apparent reversal of phase of the hypoxic ventilatory response may be related to the fact that rats are nocturnal and humans are diurnal.

During normoxia the $\dot{V}_I/\dot{V}_{CO2}$ ratio was the same night and day indicating that ventilation changed in proportion to the day-night changes in metabolism. Similarly, there was no difference between the day-night $\dot{V}_I/\dot{V}_{CO2}$ ratio during hypoxia. Ventilation increased in response to the hypoxic stimulus, and the increase was proportional to the hypometabolic response to the stimulus, that is, the ventilatory response to the same hypoxic stimulus varied depending upon the degree of hypometabolism. The more metabolism was reduced in response to hypoxia, the less ventilation increased in response to the stimulus (Saiki *et al.* 1994; Mortola and Matsuoka 1993).

Because the $\dot{V}_I/\dot{V}_{CO2}$ ratio remains unchanged between night and day, it appears that the ventilatory response to the hypoxic stimulus

changes in proportion to metabolic rate. Therefore, it appears that the ventilatory control system can be adjusted to accomodate changes in ambient oxygen partial pressure (hypoxia) while simultaneously adjusting for changes in metabolic rate. In carotid body denervated rats, ventilation changes in proportion to metabolism suggesting that the carotid bodies are not essential for ventilatory-metabolic coupling (Gautier and Bonora 1992; Gautier *et al.* 1987). The mechanisms involved in the control of ventilatory-metabolic coupling are not known. Perhaps the hypoxic and hypercapnic sensitive neurons in the caudal hypothalamus (Waldrop and Porter 1995; Dillon and Waldrop 1993) play a role in ventilatory-metabolic coupling.

Breathing Pattern: Breathing pattern was significantly altered in response to the hypoxic stimulus. Ttot was significantly shortened in response to hypoxia, and respiratory frequency was increased. The reduction in Ttot was due to decreases in both Ti and Te. Tidal volume was not changed in response to hypoxia. Walker *et al.* (1985) found similar changes in breathing pattern in response to hypoxia in adult rats.

There was a significantly greater increase in respiratory frequency in response to hypoxia at 10 am than at 10 pm, despite the fact that Ttot remained statistically unchanged at both times of the day. The lack of day-night significance in Ttot could be due to the large degree of variation in the data, and this lack of significance is probably the consequence of type II

statistical error.

Pappenheimer (1977) found that respiratory frequency increased more in response to hypoxia while the rats were in slow-wave sleep than when awake, but minute ventilation was increased by the same amount during sleep and wakefulness. He concluded that stimulation of breathing was greater during slow-wave sleep than during wakefulness. In my experiments, rats were awake both night and day, thus obviating the affects of arousal state on ventilation. The increase in ventilation and respiratory frequency were greater in response to hypoxia during the morning when the animals were normally asleep, and less during the night when they were normally awake. These observations suggest that the greater hypoxic increase in ventilation and respiratory frequency during the morning may be influenced by arousal state as well as the time-of-day.

Mean inspiratory airflow was significantly increased in response to hypoxia at 10 am, but not at 10 pm suggesting that the hypoxic drive to breathe is elevated during the morning compared with the evening. Ventilation, respiratory frequency and the hypoxic drive to breathe are all elevated during the morning compared with the evening. From these observations, I conclude that the hypoxic ventilatory response is different between day and night, and this difference is not dependent upon arousal state.

METABOLISM AND VENTILATION DURING HYPERCAPNIA:

Metabolism: Saiki and Mortola (1996) found that adult rats increased metabolism ($\dot{V}_{O2}$) in response to hypercapnia (2% and 5% CO_2). In dogs, hypercapnia (5%-8% CO_2) elicits no change in metabolism ($\dot{V}_{O2}$) (Jennings and Laupacis 1982), and in humans, metabolism is increased in response to hypercapnia (Shepard 1955). Unlike the hypometabolic response to hypoxia, the rat's hypercapnic metabolic response is not consistent, and can not be characterized by either an increase or decrease (Gautier 1996, personal communication). I found that most (5 of 7) rats decreased metabolism in response to hypercapnia, however 2 of the 7 increased metabolism.

During the morning, metabolism decreased in response to hypercapnia, but at night it did not. However, these responses were not statistically different. I conclude that, because there is no clear direction to the metabolic response to hypercapnia, a circadian dependence is unlikely.

Hypercapnia has been shown to have no effect on body temperature in adult rats (Saiki and Mortola 1996), and my findings confirm this observation. However, others have noted that body temperature is decreased in response to hypercapnia. Gautier *et al.* (1993) found that body temperature decreased in response to 5% CO_2, despite significant increases in metabolism. It appears that changes in metabolism in response to either hypoxia or hypercapnia occur independently of changes in body

temperature.

Ventilation: Raschke and Möller (1989) found that, in awake humans, the ventilatory response to hypercapnia, like that to hypoxia is highest at 5 pm and lowest at 5 am, and this circadian variation is independent of arousal state. I found that the hypercapnic hyperpnea was significantly higher at 10 pm than 10 am. This response is the reverse of the hyperpnea of hypoxia which was higher at 10 am than 10 pm in rats. It appears that in rats as in humans, the hypercapnic hyperpnea is elevated during the evening compared with the morning suggesting that the hypercapnic ventilatory response is influenced by time-of-day.

When ventilation is expressed relative to $\dot{V}_{CO2}$, the $\dot{V}_I/\dot{V}_{CO2}$ ratio was the same at night and day indicating that changes in ventilation follow the changes in metabolism. During the morning, there was a significant decrease in metabolism in response to hypercapnia, but there was only a minimal decrease in metabolism in response to hypercapnia during the evening. Ventilation increased less during the morning when the degree of hypometabolism was greater, and conversely, ventilation increased proportionately more during the night when the degree of hypometabolism was less. The lack of significance in the $\dot{V}_I/\dot{V}_{CO2}$ ratio between morning and night confirms these observations. From these findings, I concluded that ventilation changes in proportion to metabolism during hypercapnia.

Pattern of Breathing: It has been noted that in rats, tidal volume and respiratory frequency are increased in response to 5% CO_2, and the increase in frequency is due to a decrease in Ttot with the decrease in Ttot resulting from decreases in both Ti and Te (Walker *et al.* 1985; Maskrey 1990). However, Saiki and Mortola (1996) found that in rats, hypercapnia did not change either respiratory frequency or Ttot, but it did increase tidal volume. I found that tidal volume and respiratory frequency were increased in response to hypercapnia with the increase in frequency being due to decreases in Ttot. The decrease in Ttot was the result of a decrease in Te, Ti remained unchanged.

Hypercapnia significantly increased mean inspiratory airflow at 10 pm, but not at 10 am. The increase in mean inspiratory airflow was solely due to increases in tidal volume, as Ti remained unchanged. The increase in mean inspiratory airflow at 10 pm was accompanied by a significant increase in ventilation. Therefore, I conclude that the hypercapnic drive to breathe was greater during the evening than during the morning. During hypoxia the converse is true, ventilation and the drive to breathe were elevated during the morning and not during the evening. These differences suggest that the hypoxic and hypercapnic ventilatory responses may follow independent circadian oscillations. The hypoxic ventilatory response is mediated via the carotid bodies, whereas, the hypercapnic ventilatory response is primarily mediated via the central chemoreceptors.

Therefore, I suggest that the central chemoreceptor sensitivity is greater during the evening thus resulting in a stronger hypercapnic ventilatory response, whereas, carotid body sensitivity is greater during the morning and thus the hypoxic ventilatory response is greater at this time.

OVERALL CONCLUSIONS

1. Both hypoxia and hypercapnia act as behavioural stimulants. Although the effect of hypoxia was transient, the effect of hypercapnia was maintained over the course of the stimulus.

2. Normoxic metabolism and body temperature were significantly higher at 10 pm than 10 am, but ventilation was statistically similar. However, the $\dot{V}_I/\dot{V}_{CO2}$ ratio was the same night and day indicating that ventilation followed the circadian fluctuations in metabolism.

3. There was a day-night difference in the hypoxic hypothermic response, but there was no day-night difference in the hypoxic hypometabolic response suggesting that these responses are independently controlled.

4. Changes in metabolism during hypoxia were followed by changes in ventilation. Changes in ventilation and mean inspiratory airflow were elevated during the morning compared with the evening suggesting that the ventilatory response to hypoxia follows a circadian rhythm.

5. Hypercapnia had no clear effect on metabolism, and it did not affect body temperature.

6. Changes in metabolism during hypercapnia were accompanied by changes in ventilation. These changes in ventilation and mean inspiratory airflow were greater during the evening than during the morning suggesting that the hypercapnic ventilatory response follows a circadian rhythm.

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APPENDIX 1

THE CORRELATION OF NASAL TEMPERATURE WITH RESPIRATORY FREQUENCY, BODY TEMPERATURE, AND AMBIENT TEMPERATURE

RATIONALE

The underlying theory of the barometric technique is based on the correlation of pressure changes with tidal volumes. Drorbaugh and Fenn (1955) were the first to derive a formula for the calculation of tidal volume (V_T) from the observed pressure changes. The pressure changes due to ventilation are the result of both the warming and humidifying of inhaled air (pressure increase) and the subsequent cooling and condensation of expired tidal air (pressure decrease). The original equation (Drorbaugh and Fenn, 1955) for calculating V_T from these pressure changes is based on the assumption that the exhaled air returns from alveolar conditions to chamber conditions before the beginning of the following inhalation. However, both Epstein and Epstein (1978) and Jacky (1980) demonstrated that exhaled air does not return to ambient conditions before the next inhalation, rather it remains at nasal temperatures during the expiratory phase. Jacky (1980) found that if this factor is ignored the Drorbaugh and Fenn (1955) equation underestimates true tidal volumes. Therefore, Jacky (1980) and Epstein and Epstein (1980) derived a formula which corrects for this potential underestimation in V_T

that requires the measurement of both nasal (Tn) and deep-body temperatures (Tb). It is relatively simple to measure core body temperature in the rat by use of chronically implanted radio-transmitters which give a highly accurate value of Tb. However, obtaining Tn in awake and unrestrained animals is very difficult. Therefore, it was the goal of this study to determine whether Tn could be correlated with Tb, and whether respiratory frequency (f_R) or ambient temperature (Ta) had any effect on Tn.

METHODS

Seven (N=7) male Sprague Dawley rats weighing between 250-300g were used for this experiment. The rats were anaesthetized with pentobarbital sodium (40mg/kg) and body temperature was maintained at a specific value by placing the animal on a heating pad. Deep-body temperature (Tb) was measured by placing a thermometer probe (Tele-Thermometer, Yellow Springs Instruments, Inc) 2-2.5 cm into the animals rectum. Nasal temperature (Tn) was obtained by placing a fine thermocouple (Physiotemp, Bat-10) in the expiratory airstream within the nostril (<5mm). Ambient temperature (Ta) was recorded 2 cm from the rat's nose with a similar temperature probe to that used to record Tb. All temperature probes were connected to a chart recorder (MacLab/8) which in turn provided an electronic signal on the computer (Macintosh LC520).

The animal was placed into a small cylinder and both the front and back of the cylinder was loosely covered with parafilm. A humidified gas-line fed either air or 12%O_2 into the cylinder. The gas-line was connected to a three-way valve to allow a specific gas to be delivered to the rat at a specified time.

PROTOCOL

All experiments were performed between 9:00am and 5:00pm. Each animal was weighed, anaesthetized and left for 10 minutes. After the anaesthetic took affect the animal was placed onto a heating pad that was kept at about 40-48°C so that Tb would be maintained at about 37°C. The animal was then placed into the small ventilated cylinder and both the nasal and rectal probes were inserted. The ambient temperature probe was inserted into the cylinder 2 cm from the nose and the ends of the cylinder were loosely sealed with parafilm.

The goal of this study was to determine whether Tn could be correlated with Tb or f_R. Therefore, Tn was recorded over a range of Tb's (33.5-38°C) and at basal ventilatory rates (in air) and at elevated ventilatory rates (in 12%O_2).

The animal's Tb was maintained at approximately 37°C and Tn was recorded for 5 minutes while the animal was breathing humidified room air. After 5 minutes of breathing air, the animal was introduced to the

12%O_2 gas for a 5 minute period or until a constant f_R and Tn signal were observed. After this period, air was reintroduced into the system, and the above protocol was repeated again. Thus, two recordings in air and two recordings while in hypoxia were made at a Tb of approximately 37°C. The animal was then removed from the heating pad and was placed onto a cooling pad. It was left on the cooling pad until Tb decreased to about 33.5-35.5°C. At this point, the cooling pad was removed and the animal was subjected to ambient conditions. Tn was again monitored in air and hypoxia, then the animal was removed from the experimental apparatus and returned to a heating pad and left to recover from the anaesthetic.

DATA ANALYSIS

Twenty breaths were analyzed at the end of each experimental gas trial at both high and low body temperatures. f_R was calculated by dividing the twenty breaths by their total duration, and then multiplying this ratio by 60 seconds so that f_R=breaths/minute. The mean values for Tn, Tb and Ta were recorded during the twenty breath time interval.

Simple regression analyses were performed between Tn and each independent variable (i.e., Tb, Ta, f_R), and subsequently a multiple regression was performed.

RESULTS

Figures A1 to A3 illustrate the three simple regressions performed between nasal temperature and body temperature, respiratory frequency and ambient temperature. In each case, the slope of the regression line was significant ($P>0.05$) in comparison to a slope of zero.

The three simple regression equations are:

$$Tn = -11.6 + 1.2(Tb) \quad (R^2=43.9\%)$$

$$Tn = 33.7 - 0.02(f_R) \quad (R^2 = 3.4\%)$$

$$Tn = 9.2 + 0.90(Ta) \quad (R^2= 30.2\%)$$

The multiple regression equation yields similar results, in that, all three dependent variables contribute significantly ($P<0.05$) to the observed variation in nasal temperature. The regression equation is:

$$Tn = -15.9 + 0.29(Ta) + 1.2(Tb) - 0.04(f_R) \quad (R^2 = 63.4\%).$$

A substantial fraction (36.6%) of the variability in the data was unexplained by this equation, suggesting that one or more additional variables may be important. Tidal volume is a likely candidate.

Figure A1: Nasal Temperature Correlated With Body Temperature

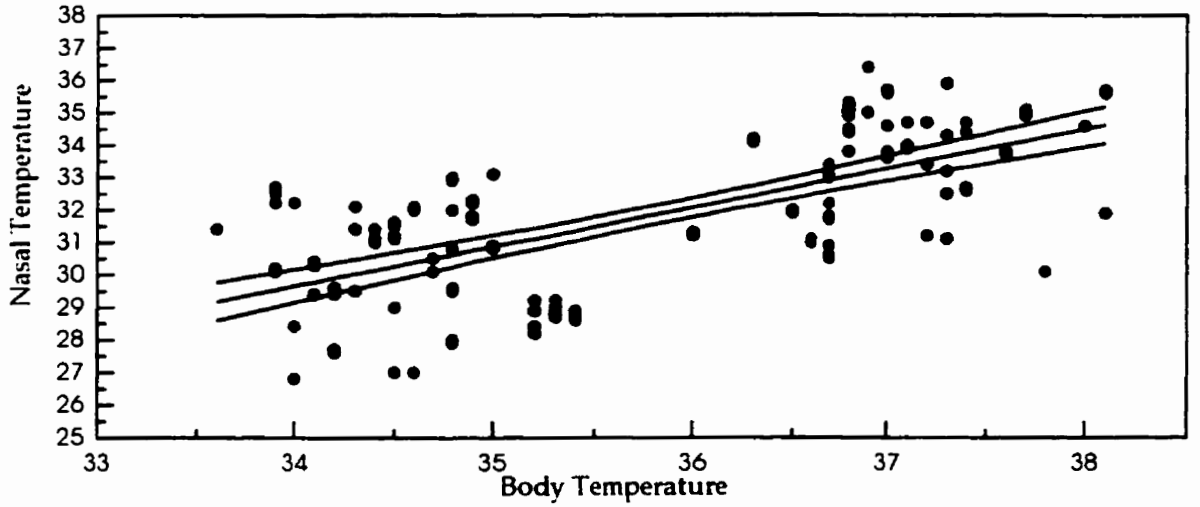


Figure A2: Nasal Temperature Correlated With Respiratory Frequency

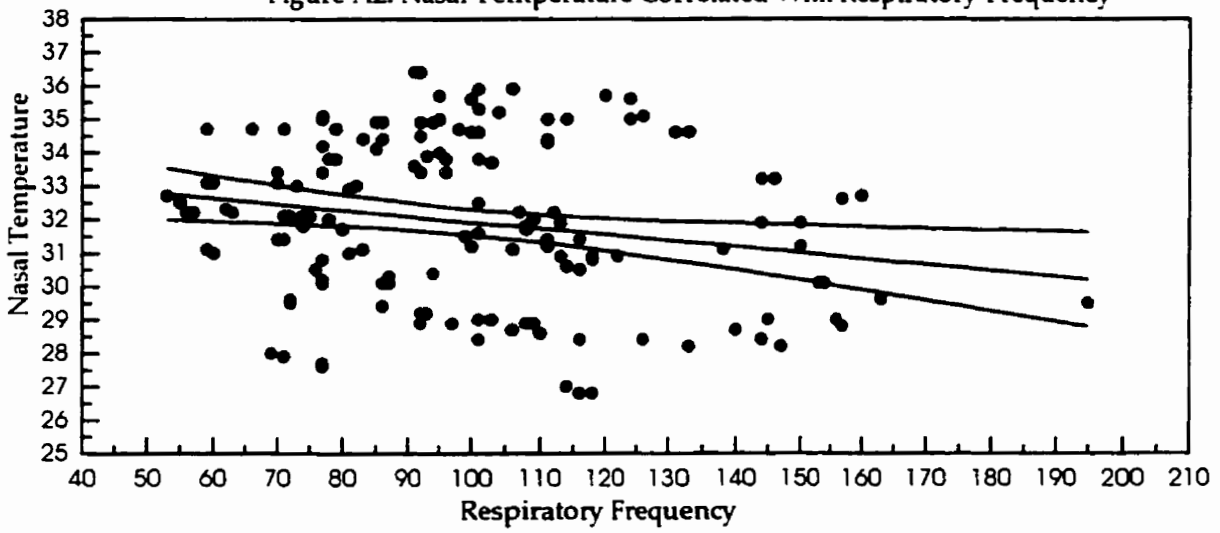
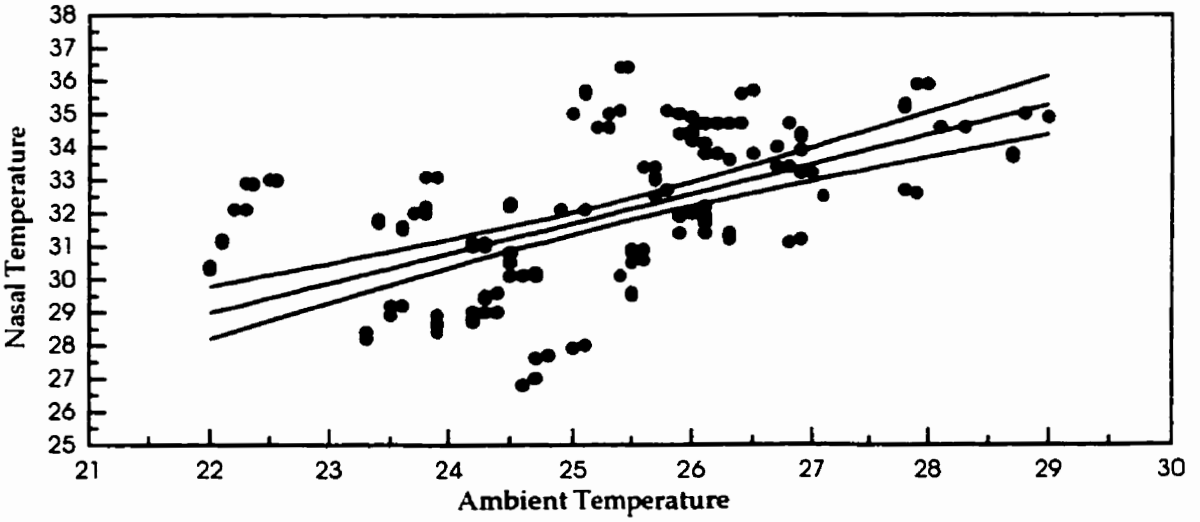


Figure A3: Nasal Temperature Correlated With Ambient Temperature



APPENDIX II

The primary objective of this thesis was to determine day-night differences in ventilation, metabolism, and body temperature during normoxia, hypoxia, and hypercapnia. Experiments were conducted at 10 am (day), and 10 pm (night). These two times of the day were chosen based on the animals usual circadian activity rhythms. Preliminary experiments indicated that at 10 am rats were the least active, and at 10 pm rats were the most active.

The experimental methods and protocol were as follows. Rats (N=8) were placed into the experimental apparatus (as described in the methods) for a two day period. Their activity patterns were determined by attaching the differential pressure transducer and preamplifier to a pen recorder. When rats became active large amplitude pressure changes resulted indicating that the rat was moving around in the experimental chamber; and conversely, when the rats were quiescent there were not pressure changes. This set-up gave a clear indication of the rats overt activity over the two day recording period.

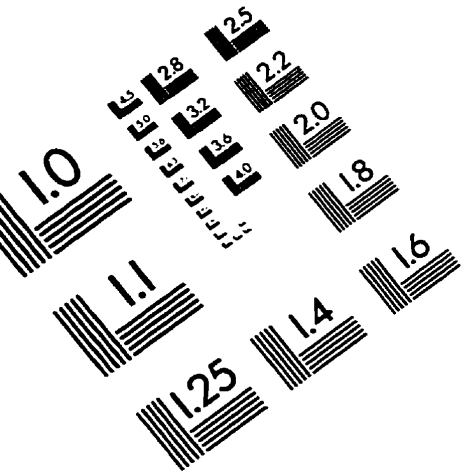
During the light phase (8 am-8pm), rats were generally inactive. I observed that approximately every 20-30 minutes rats would transiently become active for approximately 5 minutes. After this brief bout of activity rats became quiescent again. At 10 am rats were the most quiet, and there were fewer periods of activity during this time (9-10 am).

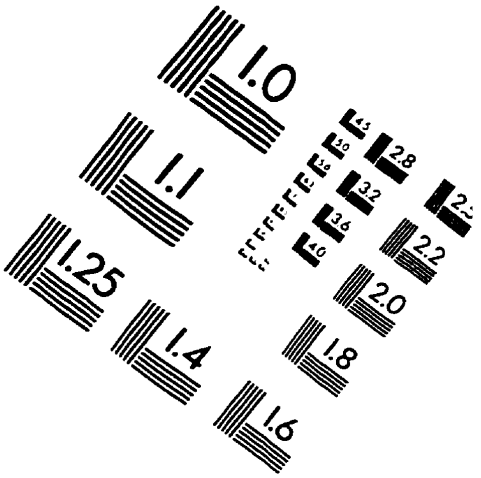
During the dark phase (8pm-8am), rats were active. I observed that activity was the most intense at approximately 10 pm. After this time, activity bouts were

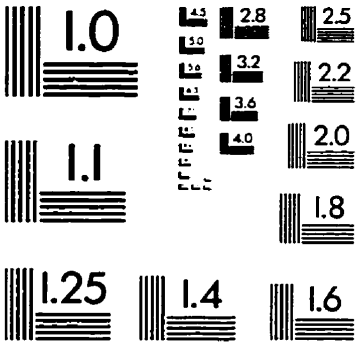
interspersed with 10-15 minute periods of quiescence.

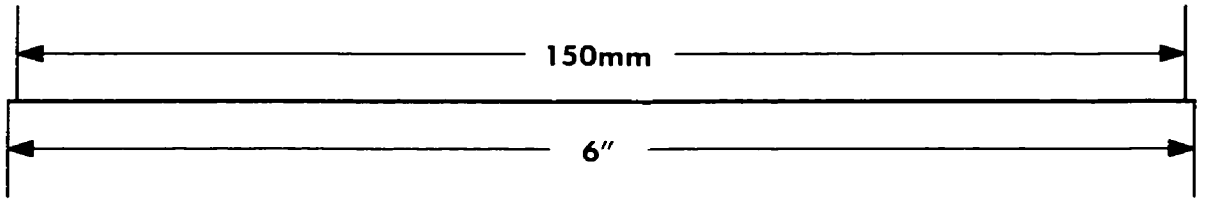
This preliminary experiment indicated that at 10 am rats tended to be the most inactive and at 10 pm the rats tended to be the most active. Therefore, I chose these times because they were the two points during the animals circadian cycle which were the most different, and may represent the peak (10 pm) and trough (10 am) of the circadian activity cycle in the rats.

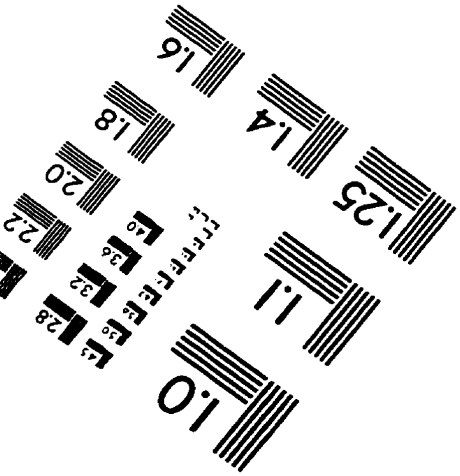
IMAGE EVALUATION
TEST TARGET (QA-3)

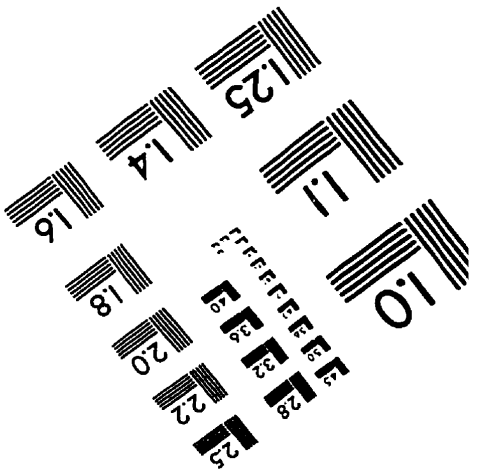












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